Ribosomal Ribonucleic Acid Synthesis in *Bacillus subtilis*

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The mode of biosynthesis of the 16S and 23S ribosomal ribonucleic acids (rRNA) was studied in *Bacillus subtilis* 168thy⁻. Three criteria were used to define the characteristics of the rRNA species: (i) the time required at 37°C to synthesize 16S and 23S rRNA chains *de novo* in growing cultures; (ii) the degree of reactivity of the 3'-terminal groups of the rRNA molecules with periodate and [carbonyl-¹⁴C]-isonicotinic acid hydrazide; and (iii) the reactivity of the 5'-terminal regions of the rRNA molecules with the bacterial exonuclease purified by Riley (1969). The 16S and 23S chains of *B. subtilis* were synthesized at rates of 22 ± 2 and 21 ± 2 nucleotides added/s. The periodate-[¹⁴C]isonicotinic acid hydrazide and the exonuclease techniques for estimating apparent chain lengths of RNA indicated that the chain length of the 23S rRNA was 1.8 times that of the 16S fraction. The apparent chain lengths of each rRNA species were: 16S rRNA, 1650 ± 50 nucleotide residues; 23S rRNA, 3050 ± 90 nucleotide residues. It appears that, the 16S and 23S rRNA molecules in *B. subtilis* are synthesized in the expected manner, by simple polymerization of the final products on independent cistrons.

Earlier hybridization studies have indicated that, in *Escherichia coli*, the 16S and 23S RNA species extracted from the ribosomal subunits have nucleotide sequences sufficiently in common for competition to occur when these fractions are bound with homologous DNA (Attardi, Huang & Kabat, 1965; Avery & Midgley, 1968; Mangiarotti, Apirion, Schlessinger & Silengo, 1968; Avery, Midgley & Pigott, 1969; Pace, Peterson & Pace, 1970). No such sharing of similar nucleotide sequences has been found on hybridizing the corresponding rRNA species from the organism *Bacillus subtilis* (Doi & Igarashi, 1966; Oishi & Sueoka, 1966; Mangiarotti *et al.* 1968; Smith, Dubnau, Morell & Marmur, 1968; Avery & Midgley, 1969). A question arising from these findings is whether they are the results of a basic difference in the way in which rRNA is transcribed in these bacteria.

A considerable body of evidence has accumulated that suggests that, in *E. coli*, the 23S rRNA chains may be formed by the dimerization of smaller precursors (McCarthy & Britten, 1962; Midgley, 1965*a*; McIlreavy & Midgley, 1967; Midgley & McIlreavy, 1967; Fellner & Sanger, 1968; Mangiarotti *et al.*

1968; Adesnik & Levinthal, 1969; Riley, 1969). This problem can be approached through the study of the kinetics of labelling of the rRNA in growing bacterial cells by exogenous labelled nucleic acid precursors. The time required for synthesis of a 16S or 23S rRNA chain is a diagnostic feature of the transcriptional events on the DNA cistrons. Some estimates in *E. coli* (Mangiarotti *et al.* 1968; Adesnik & Levinthal, 1969) have indicated that the same time is needed for the synthesis of 16S and 23S rRNA, whereas other studies (Bremer & Yuan, 1968) suggest a linear relationship between RNA chain length and the time for its synthesis. However, except for the indirect estimations by Zimmermann & Levinthal (1967), no equivalent studies have been carried out on the relative rates of synthesis of 16S and 23S rRNA in *B. subtilis*.

In this paper, we have measured the rates of transcription of 16S and 23S rRNA molecules in exponentially growing cultures of *B. subtilis*. In addition, the apparent chain lengths of the 16S and 23S rRNA molecules in these species have been measured by the periodate-[¹⁴C]isonicotinic acid hydrazide technique of Midgley (1965*b*) and the exonuclease method of Riley (1969).

MATERIALS AND METHODS

Organisms. The organism used was *Bacillus subtilis* 168^{thy-}. This strain required thymine as an exogenous supplement.

Bacterial growth. *B. subtilis* 168^{thy-} was maintained on slopes containing (per litre of medium) 8 g of tryptone (Oxoid Ltd., London E.C.4, U.K.), 5 g of NaCl, 1 g of casein hydrolysate (Oxoid Ltd.), 1 g of yeast extract (Difco Laboratories, Detroit, Mich., U.S.A.) and 0.25 g of thymine. Cultures were grown at 37°C in glucose-salts medium (Roberts, Cowie, Abelson, Bolton & Britten, 1957), supplemented with 1 g of casein hydrolysate, 0.1 g of yeast extract and 0.05 g of thymine/litre. The doubling time was about 40 min at 37°C.

Preparation of DNA. Cultures of *B. subtilis* 168^{thy-} were grown for at least four generations in the presence of [2-¹⁴C]thymidine, to label the DNA specifically. The cells were harvested and broken and the labelled DNA was isolated as described by Pigott & Midgley (1968) and Avery & Midgley (1969).

Preparation of ribosomal RNA. Cultures of *B. subtilis* 168^{thy-} were grown in the presence of [5-³H]uracil over several generations to randomly label the stable RNA. Labelled samples of cells were harvested, broken and deproteinized as described by Pigott & Midgley (1968). The RNA pellet from the final ethanolic precipitation was extracted twice with ice-cold 2 M-NaCl. This was necessary to remove any polysaccharide remaining in the preparations, which interfered grossly with the separation of the rRNA species on sucrose density gradients, unless removed at this stage. The final preparation consisted only of mRNA, 16S and 23S rRNA and their larger precursors (Midgley, 1965a; Volkin & Riggsby, 1969). The samples were taken up in a small volume of 0.01 M-sodium acetate-acetic acid buffer, pH 4.6, and the 16S and 23S rRNA species were resolved on sucrose density gradients as described by Avery & Midgley (1969). The radioactivity in each fraction was determined by the dilution of a portion to 8 ml, followed by the addition of 10 ml of Insta-gel (Packard Instrument Co. Inc., La Grange, Ill., U.S.A.). Radioactivity was then measured by scintillation counting (Avery & Midgley, 1969).

Unlabelled rRNA from *B. subtilis* 168^{thy-} was prepared as described by Pigott & Midgley (1968), and was then subjected to chromatography on methylated bovine serum albumin-coated kieselguhr (Mandell & Hershey, 1960). The column was prepared as described by Avery *et al.* (1969) and the RNA samples were fractionated. Those including 23S rRNA were pooled, dialysed into 0.6 M-NaCl-0.02 M-sodium phosphate buffer, pH 6.7, and were then chromatographed again. Finally, another cycle of collection, dialysis and column fractionation was carried out. The elution profile of the RNA in the final chromatogram is shown in Fig. 1. The 23S rRNA was essentially free from 16S material after this treatment.

Incorporation of radioactive precursors into nucleic acids. To allow the cultures to settle into a steady rate of growth throughout the experiments, a small amount of unlabelled uracil was added as a routine, such that it was nearly all incorporated over 10 min. After this time, [5-³H]uracil was injected with sufficient unlabelled carrier to ensure its continuous uptake throughout the experiment. The incorporation of the label into nucleic acids was followed

Fig. 1. Chromatography of purified *B. subtilis* 23S rRNA on column of kieselguhr coated with methylated bovine serum albumin. For details, see the text.

by injection of 2 ml portions into 4 ml of ice-cold 10% (w/v) trichloroacetic acid. After the mixtures had stood at 0°C for 30 min, the samples were collected by filtration on Oxoid cellulose acetate membrane filters, with three washes of 5 ml of ice-cold 10% trichloroacetic acid. The filters were then dried and their radioactivities counted as described by Avery & Midgley (1969).

DNA-RNA hybridization. The technique was essentially that described by Pigott & Midgley (1968).

RNA chain-length estimations by the isonicotinic acid hydrazide method. The method of oxidation of RNA by periodate, and the attachment of [carbonyl-¹⁴C]isonicotinic acid hydrazide to the 3'-terminal groups of the oxidized RNA chains was as described by Midgley & McIlreavy (1967). The radioactivity in the derivatives was measured by trichloroacetic acid precipitation as described above.

RNA chain-length estimations by the bacterial exonuclease method. Bacterial extracellular exonuclease, obtained from cultures of *B. subtilis*, was the kind gift, as a purified preparation, from Dr W. T. Riley, Sir William Dunn School of Pathology, University of Oxford. Purified labelled rRNA preparations were diluted in 0.2 M-glycine-HCl buffer, pH 9, containing 3 mM-CaCl₂. To 10 ml of the RNA solution, 0.1 ml of the enzyme preparation was added, and incubation was carried out at 37°C for 10 min, when not more than 35-40% of the input RNA had been digested. The progress of the enzyme action was monitored by precipitating portions of the incubation mixture with ice-cold 10% trichloroacetic acid, filtering, drying and counting the radioactivity as described above. In agreement with the results of Riley (1969), the kinetics of enzymic digestion of the RNA molecules was linear over this range.

Radiochemicals. [carbonyl-¹⁴C]isonicotinic acid hydrazide (specific radioactivity 9.6 mCi/mmol), [5-³H]uracil (specific radioactivity 1000 mCi/mmol) and [2-¹⁴C]thymidine (specific radioactivity 54.5 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

RESULTS

In considering the kinetics of labelling of bacterial RNA by an exogenous precursor, let us for the moment disregard the existence of the intracellular

nucleotide pools. Thus, if an exogenous source of a radioactively labelled nucleic acid precursor (e.g. [5-³H]uracil) is added to steadily growing and dividing bacterial cultures, the precursor first passes through a pool of incomplete RNA chains before entering the fraction of completed molecules. If the entry of labelled precursor into completed chains of RNA is monitored over a suitable time-interval, the kinetic curve obtained shows a rapid change of slope from the origin to a constant rate of incorporation of radioactivity at later times. When this point has been reached, all pre-existing unlabelled material has been 'washed out' of the pool of incomplete chains, and this pool is now saturated with materials having the same specific radioactivity as the exogenous precursor. In certain circumstances, the time-interval between the addition of the exogenous labelled precursor and the first attainment of the steady rate of incorporation of radioactivity into the completed RNA molecules is a measure of the time required for the synthesis of a complete chain.

This latter statement is only correct if there is no further delay in the appearance of label in the completed RNA chains from the earlier passage of the precursor through the intracellular nucleotide pools, or from the effects of recycling pre-existing unlabelled breakdown products from unstable RNA fractions. McCarthy & Britten (1962), in a detailed study of the relationships between the labelling of nucleotide pools and RNA in bacteria, found no evidence of such a delay. They concluded that compartmentalization occurred, allowing uracil from the medium to enter the RNA rapidly by bypassing the nucleotide pools. Buchwald & Britten (1963) extended these findings and calculated the fraction of exogenous precursors such as adenine, guanine, cytosine or uracil that entered the RNA directly. With uracil, roughly 50% of the base entered RNA through the bypass.

Several authors (e.g. Bolton & McCarthy, 1962; McCarthy & Bolton, 1964; Pigott & Midgley, 1968; Midgley, 1969) extended these findings in a study of the kinetics of labelling of the mRNA and rRNA fractions in bacteria. Studies by DNA-RNA hybridization have shown that the incorporation of exogenous precursors into both RNA fractions gives a linear kinetic plot that cuts the origin at zero time (Bolton & McCarthy, 1962; Pigott & Midgley, 1968). Thus, an undue influence of the intracellular nucleotide pools and the instability of mRNA can be excluded, at least on the early kinetics of labelling the rRNA fraction.

In the experiments described below, bacteria were grown in the presence of high concentrations of [5-³H]uracil, so that the radioactivity was incorporated linearly into rRNA from zero time with virtually no kinetic delay. From the argu-

Fig. 2. Time-course of labelling the RNA of growing cultures of *B. subtilis* with [5-³H]uracil. For details, see the text.

ments presented above, any extra delay that occurs in the entry of radioactivity into completed rRNA chains can be considered to be due entirely to the time required for the completion of these polynucleotides.

The kinetic analysis of RNA labelling was done in the way described by Mangiarotti *et al.* (1968).

In brief, the labelling patterns for total rRNA (i.e. complete and incomplete chains) were compared with the corresponding curves for completed molecules of 16S and 23S RNA, as isolated by sucrose-density-gradient sedimentation. The extra delay in the labelling of the completed rRNA molecules could be accounted for entirely by the presence of a pre-existing pool of unfinished RNA precursors. By suitable kinetic analysis (Mangiarotti *et al.* 1968) the time needed to synthesize both 16S and 23S rRNA was estimated. To avoid complications arising from expansion of the total system over the period of labelling, the experiments were carried out over times that were short compared with the cell-division time.

Kinetics of incorporation of exogenous labelled uracil into the nucleic acids of B. subtilis. To steadily growing cultures of *B. subtilis*, a small amount of unlabelled uracil was added, followed after 5 min by [5-³H]uracil. Samples of the cells were removed at intervals and were assayed for acid-precipitable radioactivity. This gave the time-course of incorporation of label into the cellular nucleic acids. The curve obtained (Fig. 2) when extrapolated passed

through the origin, indicating a kinetic delay of not more than 10s. This result agreed with that of Midgley (1969), who also showed, by DNA-RNA hybridization, that both the mRNA and rRNA

fractions were labelled by exogenous uracil without delay.

Analysis of labelled RNA in B. subtilis by sucrose-density-gradient sedimentation. Simultaneously, in

Fig. 3. Sucrose-density-gradient sedimentation of NaCl-insoluble ^3H -labelled RNA from *B. subtilis*. RNA was from cultures labelled with $[5\text{-}^3\text{H}]\text{uracil}$ for (a) 0.25 min, (b) 0.5 min, (c) 0.75 min, (d) 1.0 min, (e) 1.5 min, (f) 2.0 min, (g) 4.0 min, (h) 7.0 min, (i) 15.0 min. For details, see the text. $\circ$, E_{260} ; $\bullet$, ^3H radioactivity in RNA.

the previous experiment, samples of the labelled culture were poured on crushed ice, and the total ^3H -labelled sodium chloride-insoluble RNA was extracted (see the Materials and Methods section). A series of sucrose density gradients was prepared, and the mixtures of labelled and unlabelled RNA were displayed by centrifugation (Fig. 3). It is clear, from the E_{260} profile, that the gradients separated 1 weight unit of 16S rRNA from 2 weight units of 23S rRNA. This indicated that the rRNA had been extracted without detectable degradation (Midgley, 1965a). At the earliest times of labelling, acid-precipitable radioactivity was distributed throughout the gradients between the 23S peak and the top, but by 45s, two distinct labelled peaks had appeared in the 16S and 23S regions. Initially, the apparent specific radioactivity of the 16S region

was much greater than that of the 23S region, but equality was reached at later times. This initial difference may be explained in part by the presence of labelled precursors of 23S rRNA in the 16S regions, in part by the shorter time required to complete 16S molecules, and in part by a greater contamination of some areas of the gradients by labelled mRNA than in others.

Correction of the gradients for mRNA contamination. As mRNA is also labelled heavily when the cells are exposed to labelled precursors for a short period, its contribution to the radioactivity in each fraction must be estimated and corrected for. Samples of labelled RNA from each fraction containing the 23S or 16S E_{260} peaks were hybridized with denatured DNA as described by Pigott & Midgley (1968). DNA/RNA weight ratios of 5:1 were used, the

Table 1. *Estimated mRNA contamination of labelled B. subtilis 23S and 16S rRNA fractions in sucrose density gradients of NaCl-insoluble rapidly labelled RNA*

mRNA contamination was obtained as a mean value of the percentage of hybridized radioactivity (c.p.m.) in the labelled RNA in all fractions under the 16S and 23S rRNA peaks in the gradients. The values have been corrected for rRNA hybridization at DNA/RNA ratios 5:1 (i.e. about 2% of the hybridized radioactivity; Avery & Midgley, 1969), and for the efficiency of the hybridization process (85%).

Time of labelling with [5- ³ H]uracil (min)	Mean mRNA contamination of rRNA peaks (% of radioactivity present)	
	16S	23S
0.25	10.9	14.3
0.50	13.4	19.6
0.75	14.9	17.9
1.0	11.8	16.3
1.5	12.3	10.1
2.0	16.1	11.0
4.0	11.4	7.9
7.0	7.4	8.6
15.0	6.9	3.4

amount of RNA varying between 10 and 20 μ g. With these amounts, essentially all of the mRNA and only a very small proportion of rRNA will hybridize (Midgley, 1969). The hybridization values for labelled mRNA were corrected downwards for the small rRNA contribution and upwards for the incompleteness of the hybridization process (Pigott & Midgley, 1968). The values, expressed as an average of the percentages of hybridizable labelled RNA in the fractions under each rRNA E_{260} peak, are shown in Table 1. As expected, the contribution of the labelled mRNA to the total radioactivity in each fraction falls with time, and the mRNA contamination in both peaks is less than 10% of the label after 2 min.

Kinetics of labelling of completed chains of 23S rRNA in B. subtilis. If the radioactivity under the 23S rRNA peak is corrected for mRNA contamination (from Table 1) for each of the samples displayed on density gradients in Fig. 3, it is possible to plot the flow of labelled exogenous precursor into completed 23S chains as a function of time. The kinetic curve resulting is shown in Fig. 4. As predicted (Mangiarotti *et al.* 1968) a two-phase labelling curve was obtained, indicating the passage of the labelled uracil through a pool of uncompleted chains before its emergence in the pool of finished 23S molecules. The extrapolation of the major linear portion of the curve gives an intercept on the time axis of 1.2 min. On the basis of earlier arguments (Mangiarotti *et al.* 1968) the time for synthesis of

Fig. 4. Kinetics of labelling of completed chains of 23S rRNA in *B. subtilis*. For details, see the text.

a 23S rRNA chain in *B. subtilis* cultures growing under the conditions described is twice this time, i.e. 2.4 min. If the molecular weight of this RNA species is 1.1×10^6 (Stanley & Bock, 1965) and the chain length corresponds to 3100 nucleotide residues, this RNA fraction must be polymerized at a rate of 22 nucleotides added/s at 37°C.

Estimation of the contamination of the 16S regions of sucrose density gradients of rapidly labelled B. subtilis RNA with mRNA and precursors of 23S rRNA. First, as shown in Table 1, the contamination of the 16S region with labelled mRNA was corrected for as described above. However, a further correction is required in this case, since unfinished 23S rRNA precursors also may contaminate this fraction, especially at early times. In *B. subtilis*, the two rRNA species do not compete for the same hybridization sites on homologous DNA (Doi & Igarashi, 1966; Oishi & Sueoka, 1966; Mangiarotti *et al.* 1968; Smith *et al.* 1968; Avery & Midgley, 1969). Thus, the contribution of labelled precursors of 23S rRNA to the 16S regions in density gradients may be estimated by competition between the labelled fractions and unlabelled *B. subtilis* 23S rRNA.

Hybridization experiments were carried out on fractions in the 16S region of the gradients. The ratio of DNA to labelled RNA in each experiment was 660:1 (w/w). The amount of RNA used in each experiment was 0.5 μ g. This ratio is sufficient to ensure that essentially all of the 16S RNA will hybridize under the experimental conditions (Avery & Midgley, 1969). Then 250 μ g of unlabelled 23S rRNA was also added to each hybridization system. This large excess of unlabelled competitor prevented completely the effective hybridization of labelled 23S precursors present in each mixture. The

Table 2. Contamination of the 16S regions of sucrose density gradients of *B. subtilis* rapidly labelled RNA by precursors of 23S rRNA

The contamination of the 16S rRNA peak with 23S rRNA precursors was calculated as a mean value of the percentage of labelled RNA in each fraction prevented from hybridizing with DNA by excess of unlabelled *B. subtilis* 23S rRNA. The values obtained were normalized upwards to account for the 85% efficiency of hybridization.

Time of labelling with [5- ³ H]uracil (min)	Mean contamination of 16S rRNA peak with labelled precursors of 23S rRNA (% of radioactivity present)
0.25	25.0
0.50	36.6
0.75	38.8
1.00	23.7
1.50	40.9
2.0	42.9
4.0	20.3
• 7.0	5.3
15.0	0.7

average difference between the amounts of hybridization of labelled RNA in the presence or the absence of unlabelled competitor gives an estimate of the contamination by 23S precursors. For each RNA specimen isolated, the estimated degree of contamination by 23S precursors in the 16S region is shown in Table 2.

At early times in labelling, the contamination of the 16S region is considerable, but has decreased to a negligible amount by 15 min. This finding is consistent with the passage of a radioactive precursor through a finite pool of incomplete chains of 23S rRNA into the finished 23S rRNA product.

Time-course of incorporation of labelled uracil into completed chains of B. subtilis 16S rRNA. As described for the 23S rRNA fraction, the time-course of appearance of labelled uracil in completed 16S rRNA chains could be followed, after correction for mRNA and 23S rRNA precursor contaminations. Fig. 5 shows the flow of radioactivity into completed 16S rRNA molecules with time. Just as for 23S rRNA labelling, a biphasic curve was obtained. Extrapolation of the linear portion of the curve to an intercept on the abscissa gave a time-value of 0.65 min. From application of the kinetic equations derived earlier (Mangiarotti *et al.* 1968) the time of synthesis of a 16S chain is 1.3 min in these conditions. If the molecular weight of 16S RNA is about half that of 23S rRNA (Stanley & Bock, 1965), this indicates that the polymerization rate of this species is 21 nucleotides added/s.

Fig. 5. Kinetics of labelling of completed chains of 16S rRNA in *B. subtilis*. For details, see the text.

Apparent chain lengths of 16S and 23S rRNA from B. subtilis. (a) Periodate-[¹⁴C]isonicotinic acid hydrazide method. Cultures of *B. subtilis* were harvested and crushed, and the rRNA was extracted non-enzymically as described by Midgley (1965a,b). The rRNA was then oxidized by sodium periodate (0.1M) and reacted with [carbonyl-¹⁴C]isonicotinic acid hydrazide as described by Midgley (1965b) and McIlreavy & Midgley (1967). The labelled rRNA derivatives were then subjected to sucrose-density-gradient sedimentation (Fig. 6). The specific radioactivity of the 23S rRNA-isonicotinoyl hydrazide of *B. subtilis* was almost half that of the corresponding 16S derivative. A calculation of the apparent chain lengths of each rRNA species, assuming that 1 mol of isonicotinic acid hydrazide reacts with 1 mol of oxidized rRNA (Hunt, 1965; Midgley, 1966), gave values of 1650 ± 50 and 3050 ± 90 nucleotide residues for *B. subtilis* 16S and 23S rRNA.

(b) Extracellular bacterial exonuclease method. Cultures of *B. subtilis* were grown for several generations in the presence of [5-³H]uracil, to randomly label the nucleic acids. The RNA was extracted and the labelled rRNA was purified by repeated extraction with ice-cold 2M-sodium chloride containing 10% (v/v) propan-2-ol (Riley, 1969). The 16S and 23S rRNA species were then separated in the usual way by sucrose-density-gradient sedimentation. The quality of the specimens was such that less than 3% of each fraction could be considered as a cross-contaminant from the other.

A sample (0.5–1.0 ml) of the pooled rRNA-containing fractions (0.06 mg of rRNA in each case) was diluted to 10 ml in glycine-HCl buffer, pH 9.0, containing 3 mM-CaCl₂. The solution was pre-warmed to 37°C for 5 min, and 0.1 ml of a purified preparation of *B. subtilis* extracellular exonuclease

Fig. 6. Sucrose-density-gradient sedimentation of 16S and 23S rRNA-[^{14}C]isonicotinoyl hydrazones from *B. subtilis*. For details, see the text. $\circ$, E_{260} ; $\bullet$, ^{14}C radioactivity (c.p.m./fraction); $\square$, $10^{-3} \times ^{14}\text{C}$ c.p.m./ml per E_{260} unit. Fractions were 0.26 ml in volume.

Fig. 7. Kinetics of exonuclease digestion of ^3H -labelled 16S and 23S rRNA from *B. subtilis* at 37°C . For details, see the text. $\circ$, 23S rRNA; $\bullet$, 16S rRNA.

was added. At intervals, portions (0.5 ml) were withdrawn and squirted into 4 ml of ice-cold 10% trichloroacetic acid, and the acid-precipitable radioactivity was measured. The results for *B.*

subtilis 16S and 23S rRNA are shown in Fig. 7. In agreement with the estimations by the periodate-[^{14}C]isonicotinic acid hydrazide technique, it appears that there are only about half as many sites open to attack by the enzyme in a given weight of *B. subtilis* 23S rRNA as in the same weight of 16S rRNA. However, some caution should be exercised in the interpretation of these results, as hidden end-groups may be missed in some or all of the fractions by either method (Riley, 1969). Nevertheless, it appears that these values are probably a correct estimate of the number of end-groups per rRNA molecule, as Riley (1970) has shown that *E. coli* 16S and 23S rRNA (which has two reactive termini/molecule) is degraded at twice the rate found for the RNA of the R17 bacteriophage. As the viral RNA has a particle weight very close to that of bacterial 23S rRNA (Mitra, Enger & Kaesberg, 1963; Gesteland & Boedtker, 1964) and has one 5'-terminal region per molecule (Sinha, Enger & Kaesberg, 1965) it would seem that the estimations of the apparent particle weight of the bacterial rRNA molecules made in the present paper are valid.

DISCUSSION

In prokaryotes, it is accepted that the rRNA chains transcribed on a small group of specific DNA cistrons fall into two size groups, the 16S and 23S fractions, with molecular weights of 5.5×10^5 and 1.1×10^6 respectively (Loening, 1968). A general view on the molecular characteristics of 16S and 23S rRNA is that they are transcribed on

distinct, multiple cistrons, to form precursor polynucleotides that are slightly larger than the rRNA molecules extracted from mature ribosomes (Hecht & Woese, 1968; Adesnik & Levinthal, 1969).

In organisms of the genus *Bacillus*, all the available evidence has indicated that the two rRNA species are synthesized on different groups of multiple DNA cistrons, producing precursors larger, in each case, than the final products (Hecht & Woese, 1968). The arrangement of the cistrons suggests that there are nine or ten tandem groups, each possessing a contiguous 16S and 23S gene, and separated from each other by other DNA regions (Colli & Oishi, 1969; Purdom, Bishop & Birnstiel, 1970). The nucleotide sequences of the two isolated rRNA species are sufficiently different for them to hybridize, without cross-competition, to their respective DNA sites (Yankofsky & Spiegelman, 1963; Oishi & Sueoka, 1966; Smith *et al.* 1968; Avery & Midgley, 1969). Finally, as expected from the above evidence, twice as much homologous DNA will accept 23S rRNA as a hybrid as will accept 16S rRNA (Oishi & Sueoka, 1966; Smith *et al.* 1968; Avery & Midgley, 1969).

All the results for *B. subtilis* in this paper support the model for the synthesis of rRNA described above. The rRNA chains, in each case, appear to be synthesized at the same rate (22 nucleotides added/s at 37°C) and possess the expected number of 3'-terminal and 5'-terminal groups per molecule. The evidence suggests that neither chain is subject to hidden scissions that might alter its molecular characteristics and stability after its extraction from mature ribosomal particles. From studies *in vitro* on the formation of rRNA from nucleoside triphosphates by DNA-dependent RNA polymerase, Pettijohn, Stonington & Kossman (1970) showed that the tandem cistrons of 16S and 23S rRNA are transcribed to form an intact species of mol.wt. 1.7×10^6 . Possibly, *in vivo*, the non-appearance of this putative precursor of 16S and 23S rRNA is due to endonucleolytic scissions before its transcription is completed. Our results support both this possibility and the alternative suggestion that the 16S and 23S rRNA species may be produced independently by transcription of their respective cistrons. However, our findings do not support the idea that the large precursor found *in vitro* can be completed before its scission, as otherwise the kinetic delay in the labelling of the completed 16S rRNA chains would be larger and would approximate to that of 23S rRNA.

In any event, the labelling patterns for the rRNA species of *B. subtilis* are basically different from those for the corresponding molecules in *E. coli* (see, e.g., Mangiarotti *et al.* 1968; Adesnik & Levinthal, 1969; R. J. Avery & J. E. M. Midgley, unpublished work). This is further evidence of

differences in the mode of biosynthesis of rRNA in these two organisms.

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