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Structural Transitions in Ribonucleic Acid During Ribosome Development

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Amino acid deprivation of a "relaxed" auxotroph of *Escherichia coli* results in the accumulation of protein-deficient, immature ribosomes ("relaxed particles"). The ribonucleic acid (RNA) of these particles was shown to differ from mature ribosomal RNA in both sedimentation characteristics and in elution from columns of methylated albumin-keiselguhr. When relaxed particles were allowed to become converted to mature ribosomes, the unique properties of the RNA were lost, and this RNA became indistinguishable from mature RNA. The conversion of relaxed particles to ribosomes did not involve degradation and resynthesis of RNA. It is concluded that ribosomal RNA undergoes a configurational transition during ribosome development, and that this transition is not the result of changes in the primary structure of the RNA.

Ribosomal ribonucleic acids (rRNA) are synthesized on a deoxyribonucleic acid (DNA) template as intact, macromolecular polyribonucleotides (2, 32). Once formed, the rRNA molecules become integrated with ribosomal proteins, and eventually form mature ribosomes. This integration procedure has been shown to occur in apparently discrete stages (25). These stages, involving protein-deficient "immature" ribosomes, have been studied under various conditions of physiological stress which have provided a useful tool in delineating the molecular events involved in ribosome development.

Thus, there is available a general outline of changes which occur in the ribosome during its development. However, little is known about the structure of rRNA during its transient existence apart from the mature ribosome. Several investigators have studied rRNA by employing a variety of conditions under which "immature" ribonucleoprotein (RNP) particles are formed. Dubin (5) and Dubin and Elkort (6) found abnormal properties for the RNA of "streptomycin particles" and "chloramphenicol particles," and concluded that these properties reflect a secondary structure more highly hydrogen-bonded than that of normal rRNA. Similarly, Kono, Otaka, and Osawa (11) suggested that a change in secondary structure accompanies the shift in sedimentation velocity of chloramphenicol RNA after its conversion to rRNA.

The usefulness of the various "immature ribosome" systems has been demonstrated by recent investigations with the RC^{rel} mutants of *Escherichia coli*. The immature particles which accumulate in this organism during amino acid starvation are indistinguishable from normal ribosome precursors (29), and they are converted directly to mature ribosomes (21, 22, 29, 31) under the proper growth conditions. In the present investigation, we show that the rRNA contained in the immature particles (relaxed particles) is incorporated directly into mature ribosomes without being broken down to the nucleotide level, and that a transition in the structure of this RNA occurs during the development process.

MATERIALS AND METHODS

Bacteria and culture conditions. All experiments were performed with a histidine-arginine-thiamine auxotroph of *E. coli*. The organism, which carries the relaxed allele of the RC gene, was obtained originally from G. S. Stent, and is referred to as HAR (28). For experimental purposes, the bacteria were grown in a mineral salts medium (30) with 0.5% glucose, and supplemented with 50 μg (per ml) of the required amino acids and 0.01% thiamine hydrochloride. The conditions for amino acid starvation, termination of starvation, and isotope labeling have been described in detail (28).

Preparation of extracts. Extracts were prepared by passing a 10 to 20% suspension (wet-weight/volume) of cells through a French pressure cell at 8,000 lb. This corresponds to a total force of 6,250 psi on a hydraulic press. The effluent was centrifuged at 10,000 × *g* in a Sorvall S-34 rotor for 15 min, and then deoxyribonuclease (2 μg/ml) was added to the supernatant fluid. In experiments that involved prolonged handling of the extracts, as in sucrose gradient analy-

sis, the extracts were prepared with Macaloid, a sodium magnesium fluor-litho-silicate (W. M. Stanley, Jr., Ph.D. Thesis, Univ. Wisconsin, Madison, 1963). This was done by suspending the cells in 0.01 M tris(hydroxymethyl)aminomethane (Tris) at *p*H 7.6 and 0.01 M magnesium acetate (TM buffer). A quantity of a Macaloid suspension was added to a final concentration of 1 mg/ml, and then the entire suspension was passed through the pressure cell. The Macaloid was removed from the extract by the 10,000 × *g* centrifugation step. Inclusion of Macaloid during the preparation of the extract caused a five- to sixfold reduction in total ribonuclease activity in the crude extract. Addition of the same quantity of Macaloid after preparation of the extract effected only a 25 to 30% reduction in ribonuclease activity.

Zone centrifugation of crude extracts and the isolation of "relaxed particles" were performed as previously described (28).

Biochemical methods. RNA was extracted from crude extracts or ribosomes by the addition of sodium dodecyl sulfate to a final concentration of 0.5%. This was followed by the addition of an equal volume of TM buffer-saturated phenol. The mixture was held at 0 C for 30 min, with occasional vigorous shaking. The aqueous layer was recovered, and was subjected to a second phenol extraction and to an extraction with chloroform-isoamyl alcohol (24:1, v/v); finally, the nucleic acid was precipitated by adding two volumes of 100% ethyl alcohol. In some cases, RNA was extracted from cells which had been lysed by the lysozyme and freeze-thaw method described by Morris and DeMoss (20). Before use in experiments, each RNA preparation was examined for macromolecular integrity by sucrose gradient centrifugation before and after heating and rapid cooling.

Methylated albumin-kieselguhr (MAK) columns were made exactly as described by Mandel and Hershey (17). From 1 to 2 mg of RNA was loaded onto the columns in a total volume of 25 ml. Elution was accomplished by a linear gradient of NaCl (0.3 to 1.2 M) in sodium phosphate buffer (*p*H 6.7). RNA peaks eluted from the columns are identified in the respective figures by their nominal *S* values. RNA was fractionated in sucrose gradients made up in 0.01 M acetate buffer containing 0.1 M NaCl, at *p*H 5.0. The gradients were centrifuged for 16 hr at 25,000 rev/min at 5 to 7 C in a Spinco SW25.1 rotor.

Formaldehyde titration of amino groups on RNA was performed as described by Penniston and Doty (26), by use of ^{14}C-labeled formaldehyde in 0.001 M Mg^{++}. Ribonuclease I hydrolysis of RNA was carried out with from 0.05 to 0.2 μg of pancreatic ribonuclease per ml at 37 C in Tris buffer (*p*H 7.4). Protein and RNA were determined by the methods of Lowry et al. (16) and Dische (4), respectively.

Chemicals. L-Methionine-(methyl-^{3}H) was obtained from Tracerlab, Waltham, Mass. All other isotopes were from New England Nuclear Corp., Boston, Mass. Electrophoretically purified deoxyribonuclease and pancreatic ribonuclease were obtained from Worthington Biochemical Corp., Freehold, N.J. Macaloid was a gift from Baroid Division, National Lead Co., Houston, Tex. Tris (Trizma Base) was a product of Sigma Chemical Co., St. Louis, Mo. Grade B Tris, obtained from Calbiochem, caused precipitation of RNP particles from crude extracts and was not suitable for experiments involving the isolation of relaxed particles.

RESULTS

Distinctive properties of RNA from immature particles. These experiments were performed with the amino acid "relaxed" auxotroph, HAR. During amino acid deprivation, the bacteria oversynthesize all classes of RNA (18, 19, 23). The 16*S* and 23*S* rRNA molecules are accumulated in the form of RNP particles with *S* values of 20, 30, and 45 when analyzed in 10^{-2} M Mg^{++} (28, 31). For convenience, the RNA derived from mature ribosomes will be designated as rRNA, and that from relaxed particles as RP-RNA. Sedimentation analysis (Fig. 1) revealed a slight difference between rRNA and RP-RNA. Moreover, when a mixture of rRNA and RP-RNA was chromatographed on MAK columns, the elution profiles were different; RP-RNA did not elute from the column in peaks coincident with those of rRNA (Fig. 2). There was a shift in the "16*S*" peak, so that RP-RNA was eluted early. The "23*S*" peak of RP-RNA was resolved into two peaks; one eluted prior to and one after the "23*S*" peak for rRNA.

The experiments shown in Fig. 1 and 2 were performed with RNA isolated from cells which were starved for 90 min with sufficient ^{14}C-uracil to allow the incorporation to proceed for 60 min (Fig. 3). During the 90-min starvation period, the RNA content of the cells doubled. This is a suffi-

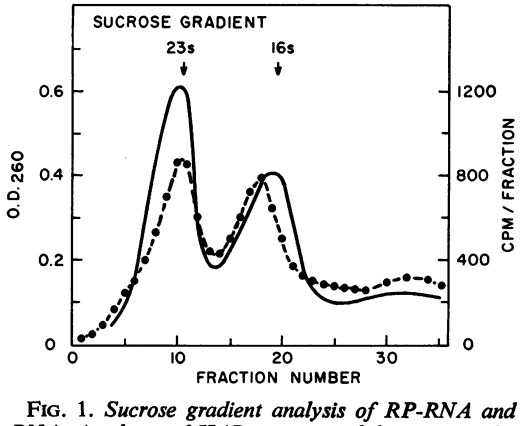

FIG. 1. *Sucrose gradient analysis of RP-RNA and rRNA. A culture of HAR was starved for arginine for 90 min in the presence of* ^{14}*C-uracil. The RNA was prepared from a crude extract and mixed with a fivefold excess of nonlabeled RNA from exponentially growing cells. Solid line, optical density; broken line, counts/minute.*

cient increase of RNA to minimize the preferential labeling of RNA with a rapid turnover rate (e.g., messenger RNA). However, several experiments were performed to verify the apparent difference in elution profile between RP-RNA and rRNA. The validity of the elution profile shown in Fig. 2 for RP-RNA was tested in two ways. First, each peak of RP-RNA ("16S" and both "23S" species) was recovered by selecting peak fractions. Each fraction was mixed with carrier nonlabeled

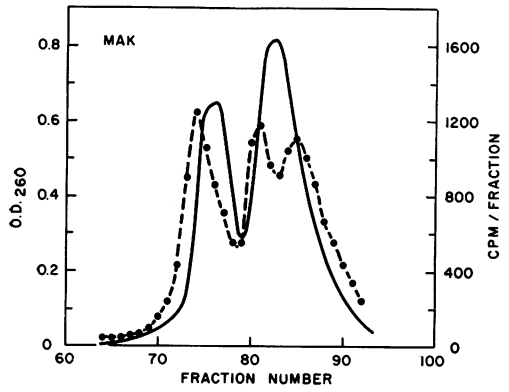

FIG. 2. *MAK analysis of the RNA described in Fig. 1. The nucleic acid was eluted with a 400-ml linear gradient of NaCl (0.3 to 1.2 M). Only the 16S and 23S RNA preparations are shown. Solid line, optical density; broken line, counts/minute.*

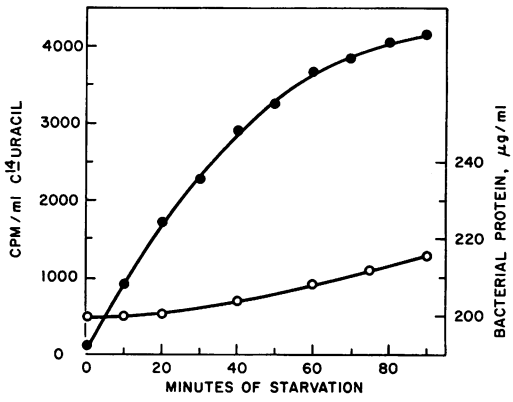

FIG. 3. *Course of ^{14}C-uracil incorporation (●) and protein synthesis (○) in a culture of HAR starved for arginine. Uracil was used at 0.02 µc/ml and 5 µg/ml.*

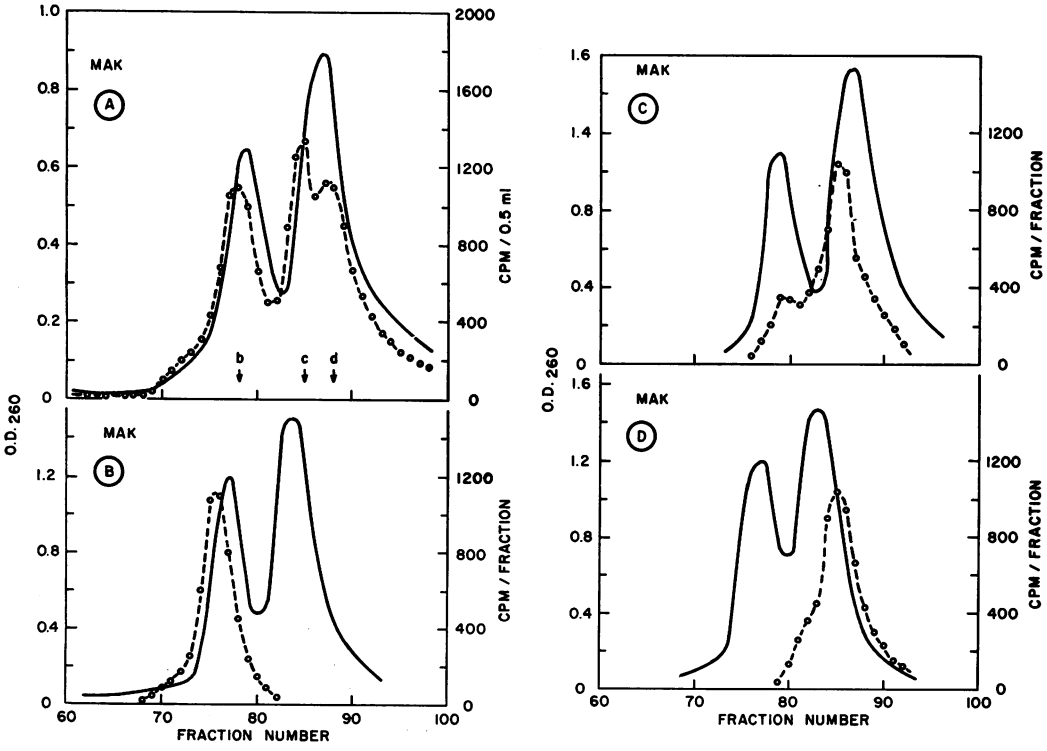

FIG. 4. *Separation and rechromatography of RP-RNA (broken lines) and rRNA (solid lines) on MAK. Fractions indicated by the arrows (b, c, d) in A were diluted and chromatographed on fresh columns with carrier rRNA, as shown in B, C, and D, respectively.*

rRNA and rechromatographed on a fresh column. Each fraction selected from the column in Fig. 4A rechromatographed precisely as it did initially (Fig. 4B–D).

The second test for authenticity of the elution profile of RP-RNA was performed by recovering RNA fractions after zone centrifugation in sucrose gradients. The 23*S* and 16*S* fractions were taken from the gradient (Fig. 5A), mixed with nonlabeled rRNA, and chromatographed on MAK. As shown in Fig. 5B and C, the RP-RNA fractionated on the sucrose gradient was eluted in the same manner as it was prior to fractionation (compare Fig. 3). Thus, the 23*S* RP-RNA is resolved into two fractions by MAK chromatography, a phenomenon which does not occur with the rRNA isolated from mature ribosomes.

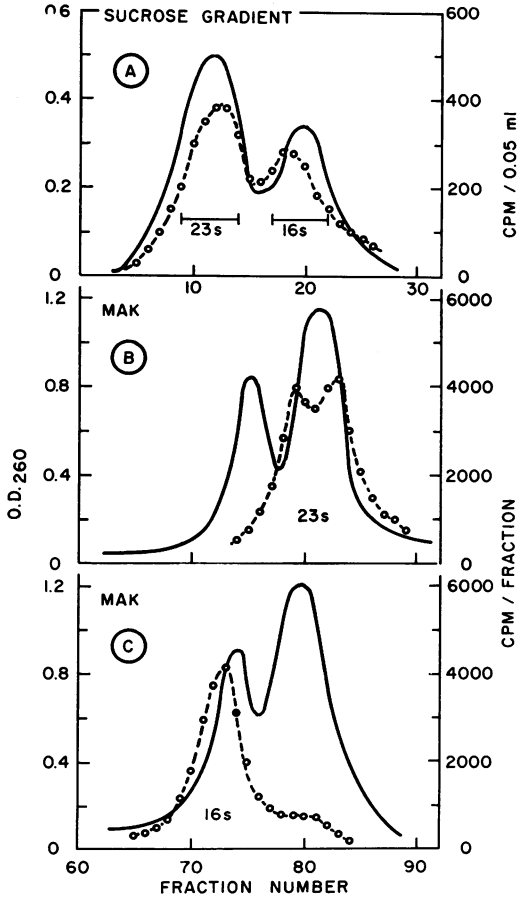

FIG. 5. *MAK chromatography of the fractions obtained by separation on sucrose gradients. The 23S and 16S fractions in A were mixed with carrier rRNA and chromatographed in B and C, respectively. The RNA was labeled and prepared as in Fig. 1.*

Conversion of RP-RNA to rRNA. The differences observed between rRNA and RP-RNA may indicate that a transition in the structure of RP-RNA occurs during its incorporation into mature 70*S* ribosomes. Lindigkeit and Handshack (15) have shown similar MAK profiles for the RNA isolated from "precursor ribosomes." These precursor particles were obtained from normal cells after chromatography of crude extracts on diethylaminoethyl cellulose. There is evidence that relaxed particles are converted *in toto* to mature ribosomes, with no apparent breakdown of the RP-RNA or dissociation of RNA and protein (29), and that RP-RNA is converted to rRNA upon restoration of the amino acid (22, 31). These same studies showed that relaxed particles were metabolically indistinguishable from normal precursor RNP particles (29).

Although existing evidence supports the conclusion that RP-RNA molecules are precursors to the rRNA molecules of mature ribosomes, we attempted a more rigorous examination of this problem. In the following experiments, we tested the stability of RP-RNA during the conversion of relaxed particles to ribosomes by employing a method devised by Dubin and Elkort (7). In addition, the transition of RP-RNA to rRNA has been examined during the conversion of relaxed particles to ribosomes.

That RP-RNA is converted directly to rRNA without disruption of the macromolecule is demonstrated in Table 1. In this experiment, a culture of HAR was starved for arginine for 90 min in the presence of ^{14}C-uracil. During this period, L-methionine-(methyl-^{3}H) was introduced to label the methylated bases of the RP-RNA. After 90 min, one portion of the culture was harvested and the RNA was extracted. The second portion was washed free of the radioactive materials, and the cells were suspended in complete medium containing (per ml) 25 μg each of L-methionine and uracil. This culture was allowed

TABLE 1. *^{3}H-^{14}C ratios in 16S and 23S RNA*[a]

Culture no.	Labeling period	Sample period	^{3}H-^{14}C ratio
1	Growth	Growth	0.132
2	Starvation	Starvation	0.078
3	Starvation	Recovery	0.070

[a] Cells were labeled at the indicated times with L-methionine-methyl-^{3}H and ^{14}C-uracil. One culture (no. 3), which was labeled during starvation, was washed free of the isotopes and resuspended in complete medium containing nonradioactive methionine and uracil. The sample was taken after 60 min in recovery medium.

to grow, and samples were removed after 30 and 60 min. At 60 min, there is nearly complete conversion of relaxed particles to ribosomes (29). The RNA extracted from these samples was analyzed by MAK chromatography. Table 1 shows that the ratio of methyl groups (^3H) to RNA (^{14}C) formed during starvation does not change appreciably during conversion of relaxed particles to ribosomes. Since RNA is methylated at the polymer level (8, 9), RP-RNA could not have been degraded nor the radioactive methylated bases incorporated into newly synthesized rRNA. In agreement with Borek and Ryan (3), we conclude that RP-RNA is stable during the conversion process. By comparing the RNA species synthesized during the growth and starvation periods, it can be seen that RP-RNA is methylated to about 60% of the level of rRNA (Table 1).

Having shown that RP-RNA becomes incorporated into mature ribosomes without undergoing degradation, we examined the transition in the properties of RP-RNA during its conversion to rRNA. This was accomplished by starving cells for arginine for 90 min and labeling the RP-RNA with ^{14}C-uracil. After 90 min, the cells were washed free of the uracil and suspended in complete medium containing 25 μg of ^{12}C-uracil per ml. Samples were removed at the time starvation was terminated and at intervals during recovery. The RNA from these cells was chromatographed on MAK, and Fig. 6 shows the elution profiles which were obtained. The data show a gradual conversion of the RP-RNA elution profile to one coincident with rRNA after 60 min in the recovery medium.

The results of the experiments in Table 1 and Fig. 6 show that RP-RNA, possessing unique properties, is a precursor to rRNA, and that there is a transition in the properties of the nucleic acid upon conversion of immature particles to mature ribosomes.

Effect of hydrogen-bond disruption on the properties of RP-RNA and rRNA. Dubin and Elkort (6) suggested that the abnormal properties of chloramphenicol RNA were due to changes in secondary structure. If RP-RNA and rRNA

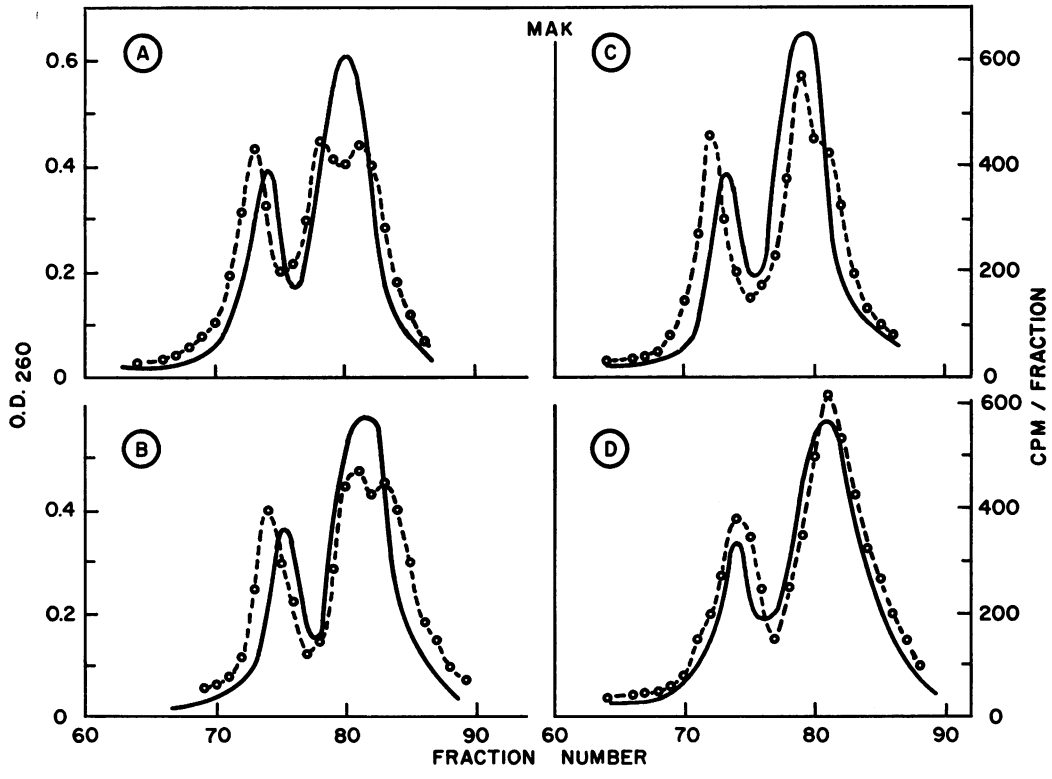

FIG. 6. *MAK analysis of RP-RNA and rRNA during recovery from starvation. A culture of HAR was starved for 90 min and labeled with ^{14}C-uracil. The cells were removed from the medium by filtration and suspended in fresh, complete medium containing* L*-arginine and ^{12}C-uracil. Samples were taken just after resuspension (A), at 20 min (B), at 40 min (C), and at 60 min (D) during the ensuing recovery period. The RNA was prepared as described in Fig. 1.*

differ only in their secondary or tertiary structure and not in the sequence of nucleotides, it should be possible to subject both molecules to conditions which would render them identical in overall configuration. This can be accomplished by denaturing the molecules and then allowing them to form hydrogen-bonded regions at random. In this way, both types of RNA should acquire a spectrum of random configurations, all satisfying a low-energy state. These populations of RP-RNA and rRNA should now be indistinguishable from each other. This possibility was examined by "melting out" the secondary structure at 70 C for 5 min, followed by rapid cooling. The mixture of RP-RNA and rRNA was divided and chromatographed on MAK and centrifuged in a sucrose gradient with an excess of nonheated rRNA. Figure 7 shows two significant results. First, it is shown that rRNA acquires a different elution profile after heating and cooling. This is not the result of thermally induced hydrolysis of phosphate-ester bonds in RNA, since the RNA sediments at 23*S* and 16*S* (Fig. 8). Second, Fig. 7 shows that rRNA and RP-RNA are indistinguishable in their elution profiles. This is also shown by sedimentation analysis (Fig. 8); both molecules sediment in sucrose at the same rate (compare with Fig. 1). Apparently, both RP-RNA and rRNA acquired the same distribution of configurations when the molecules were permitted to randomize their secondary and tertiary structures. Experiments with 7 M urea and dimethyl sulfoxide gave results similar to those obtained by thermal denaturation, in that both molecular species of ribosomal RNA were indistinguishable in their sedimentation and chromatographic properties after the "native" secondary structure has been destroyed.

Measurement of the extent of secondary structures. From the suggestions of others (6, 11) and the results of the hydrogen-bond disruption experiments, it is reasonable to assume that RP-RNA and rRNA differ in secondary or tertiary configurations. This is supported by experiments showing no detectable difference in the ribonuclease T1 digest "maps" of RP-RNA and rRNA (Sypherd, *unpublished data*). We have attempted to assess the significance of secondary interactions in producing the unique MAK elution profiles shown in Fig. 2.

The secondary structures of RP-RNA and rRNA were compared by examining their susceptibility to hydrolysis by pancreatic ribonuclease. Figure 9 shows the extent of ribonuclease digestion of a mixture of RP-RNA (labeled with ^{14}C-uracil) and rRNA (labeled with ^{3}H-uracil). It can be seen that hydrolysis is described by a biphasic curve representing two different rates. It is

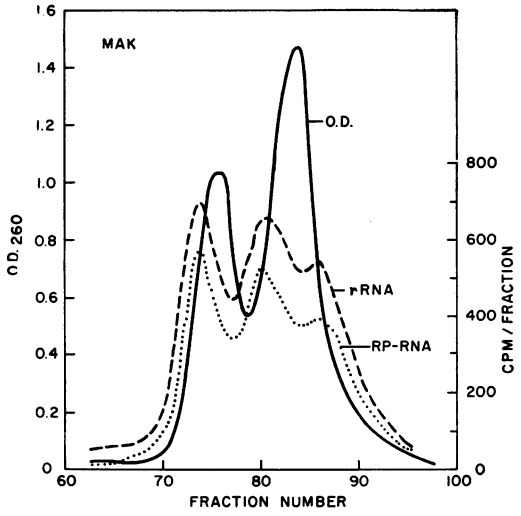

FIG. 7. *Elution from MAK of RNA after heating and quick cooling. A mixture of RP-RNA and rRNA, labeled with ^{14}C-uracil and ^{3}H-uracil, respectively, was heated at 70 C for 5 min, and then placed in ice. A five-fold excess of nonradioactive rRNA was added, and the mixture was chromatographed on MAK.*

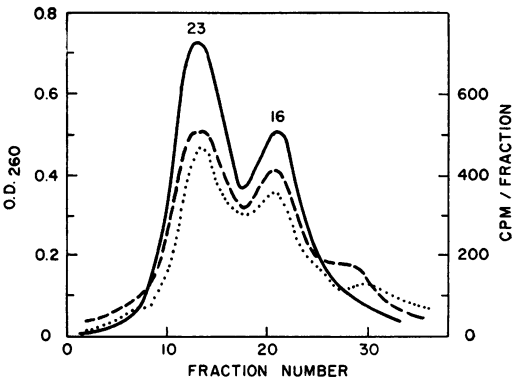

FIG. 8. *Sucrose-gradient analysis of RP-RNA and rRNA after heating and rapid cooling. The RNA fractions were prepared as described in Fig. 7.*

assumed that the plateau represents the hydrogen-bonded duplexes, since these regions would be relatively resistant to enzyme attack (24, 33). Table 2 summarizes the results of similar experiments carried out under different conditions of ionic strength and enzyme concentrations. In all cases, there was no apparent difference in the amount of ribonuclease-resistant material in either species of RNA. The method is sensitive enough to demonstrate relative stabilities of the duplex structures as the ionic environment is changed.

Another measure of the extent of intramolec-

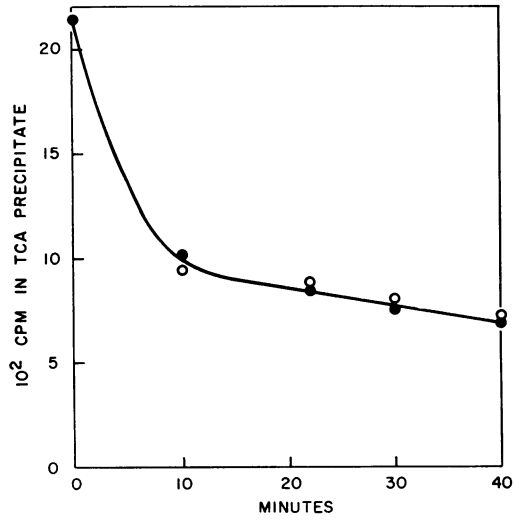

FIG. 9. *Course of ribonuclease digestion of RP-RNA (○) and rRNA (●). The RNA preparations were incubated in 0.01 M Tris buffer (pH 7.4) containing 0.005 M Mg^{++}, 0.05 M KCl, and 0.05 μg (per ml) of ribonuclease I. The total RNA concentration was 435 μg/ml.*

ular hydrogen bonding may be obtained by determining the number of bases which are not engaged in base-pairing, since this number will vary inversely with the extent of hydrogen bonding. The number of nonpaired bases was estimated by titrating the free amino groups with ^{14}C-labeled formaldehyde (26). RP-RNA and rRNA bound formaldehyde to the same extent (Fig. 10), indicating that both species of nucleic acid have about the same number of bases hydrogen-bonded in maintaining double-helical and tertiary configurations. In experiments not reported here, RP-RNA and rRNA exhibited the same amount of hyperchromicity when heated from 20 to 90 C. We conclude from these experiments that there is no detectable difference between RP-RNA and rRNA in the number of hydrogen-bonded regions.

DISCUSSION

The accumulation of immature ribosome particles in an amino acid-deprived "relaxed" auxotroph provided an excellent opportunity to study the structure of rRNA as it exists prior to incorporation into the mature particle. Sedimentation analyses revealed only the small differences between rRNA and RP-RNA shown in Fig. 1, i.e., displacement of the 16S peak. However, the MAK analyses demonstrate even more pronounced differences between these two species of RNA. It should be noted that Turnock and

TABLE 2. *Effect of ribonuclease treatment of RP-RNA and rRNA*

Sample	KCl concn	Mg^{++} concn	Ribonuclease	Per cent resistant[a]
	M	M	μg/ml	
RP-RNA	0.05	0.005	0.05	42.2
rRNA	0.05	0.005	0.05	41.7
RP-RNA	0.05	0.005	0.2	20.3
rRNA	0.05	0.005	0.2	19.1
RP-RNA	0	0.005	0.05	33.6
rRNA	0	0.005	0.05	34.7
RP-RNA	0	0	0.05	26.8
rRNA	0	0	0.05	26.2
RP-RNA (heated)	0.05	0.005	0.05	38.1
rRNA (heated)	0.05	0.005	0.05	36.3

[a] Amount precipitable by trichloroacetic acid after 30 min at 37 C divided by the amount at zero-time. The initial radioactivity (counts/min) in each reaction was: RP-RNA (^{14}C), 17,000; rRNA (^{3}H), 23,000. Samples (0.1 ml) were taken in duplicate at intervals and pipetted into 5% trichloroacetic acid. A correction was applied for the decreased self-adsorption resulting from smaller amounts of precipitate being applied to the membrane filters as the reaction progressed.

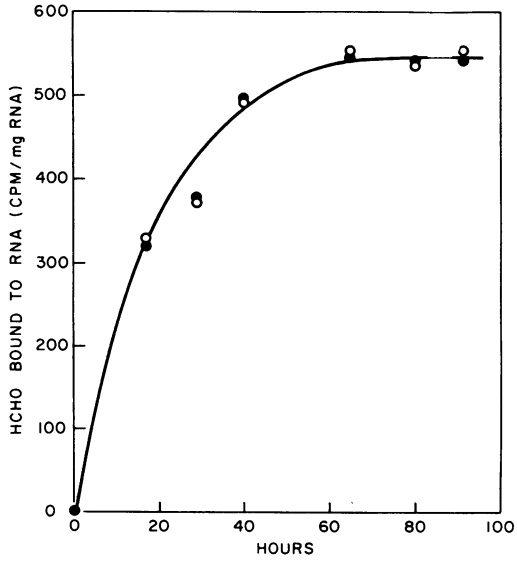

FIG. 10. *Titration of amino groups of RNA with ^{14}C-formaldehyde. The RNA was mixed with 0.3% formaldehyde in 0.01 M triethanolamine buffer (pH 7.2) containing 0.001 M Mg^{++}. Samples were taken at intervals and precipated with 5% trichloroacetic acid; the precipitates were counted on membrane filters. RP-RNA concentration (○), 1.66 mg/ml; rRNA concentration (●), 1.46 mg/ml.*

Wild (31) reported a second, or slow, "23S" peak after chromatographing RP-RNA on MAK. They concluded, however, that this peak consisted of messenger RNA accumulated during starvation. The results obtained in the present investigation, particularly those with sucrose gradient-fractionated RP-RNA, argue against their conclusion. It has been suggested (17, 27) that MAK separates nucleic acids by at least three parameters: molecular weight, base composition, and extent of hydrogen bonding. The elution-profile differences between RP-RNA and rRNA cannot be explained by differences in molecular weight or base composition (19, 27). Moreover, both species of RNA are similar in their content of hydrogen-bonded duplexes (Fig. 8 and 9). The data suggest a qualitative difference in the overall configuration of the nucleic acids, since destruction of the native secondary and tertiary structures by heat, urea, or dimethyl sulfoxide yielded molecules which were indistinguishable in their sedimentation and chromatographic characteristics. It is inferred then that the RP-RNA and rRNA differ not in the number of double helices, but in the arrangement of these helices in the tertiary structure.

Although the evidence presented here suggests a configurational difference between RP-RNA and rRNA, there is the possibility that a difference in primary structure also exists. Even if there are no differences between rRNA and RP-RNA in the major purine and pyrimidine bases (19), some minor primary-structure feature could effect transitions in secondary structure. Dubin and Elkort (6) and Dubin and Gunlap (Biochim. Biophys. Acta, *in press*) attributed the unique properties of chloramphenicol RNA and RP-RNA to the fact that these molecules have not been methylated to normal levels, resulting in a larger number of hydrogen-bonded regions. From our own unpublished experiments, we know that RP-RNA formed during lysine or arginine deprivation is methylated to 50 to 60% of the level of rRNA. These RP-RNA preparations cannot be distinguished from the RP-RNA formed during methionine starvation, even though the latter is not methylated. There are approximately 20 methylated bases in the average rRNA molecule. If these bases are responsible for the structural transition reported here, it is the methylation of the last eight to ten which effect the transition. Presently, the data are insufficient to conclude that a deficiency in methylation is the factor responsible for the unique properties of RNA from immature ribosomes. Moreover, although changes may occur in the primary structure of RNA during ribosome development, the results of denaturation-renaturation studies make this unlikely as an explanation for the properties of RP-RNA. As a result, we favor a model which involves only transitions in the arrangement of the hydrogen-bonded double helices. This model can be developed in the following way. A molecule the size of either species of rRNA (16S or 23S) may exist in many configurations which would represent low-energy states for the molecule. However, biological considerations place a restriction on the overall structure, favoring one or a few of the possible configurations. In the cell, these restrictions are effected by the stepwise addition of ribosomal protein to the rRNA, and a particular configuration becomes most probable. The formation of hydrogen bonds and the establishment of hydrophobic interactions stabilize this structure. For a given stage in ribosome development, therefore, the configurations of the RNA in the particle would be a reflection not only of its primary structure, but also of the extent to which protein addition had restricted the formation of secondary and, finally, tertiary structure. The isolation of an intermediate in ribosome formation (e.g., relaxed particles) would then yield RNA molecules which have a selected configuration and which differ from those found in mature ribosomes.

The extent to which this particular model is plausible depends upon one major assumption: that RNA can be isolated from cells by a method which does not produce helix-coil transitions. The extraction procedure employed in the present work uses phenol and sodium dodecyl sulfate at 0 C, and, as yet, there has been no direct test of what effect this has on the secondary structure of nucleic acids. Several investigators have shown that nonaqueous solvents disrupt hydrogen-bonded helices in DNA (10, 12), as evidenced by a lowering of the thermal transition temperature. Indeed, Lindahl and Adams (13) and Lindahl et al. (14) assumed that phenol treatment of transfer RNA denatures its extensive secondary structure. However, there is some evidence that molecules recovered by phenol extraction have not been completely randomized as a result of extensive destruction of original secondary structures. For example, Boedtker (1) found that tobacco mosaic virus RNA assumed a different configuration after remaining in solution for several days. Stanley (Ph.D. Thesis, Univ. Wisconsin, Madison, 1963) reported changes in sedimentation coefficients and viscosity of *E. coli* RNA as a result of "aging." These changes were not the result of intramolecular scissions, but of conformational changes. Thus, as Stanley pointed out, RNA molecules in solution may become a random population with respect to secondary structure, as a result of thermal agitation.

In our experiments, we demonstrated that *E. coli* rRNA acquired a different MAK elution profile after heating to 70 C for 5 min. This elution profile alteration was not accompanied by a loss of molecular integrity, since there was little change in the ratio of 23*S* to 16*S* rRNA after heating when the sample was analyzed on a sucrose gradient. Our interpretation of this phenomenon is that heating destroyed the configuration which existed in the freshly isolated RNA, and, upon cooling, the molecules reformed an array of different configurations. From these considerations, we conclude that rRNA possesses a unique secondary and tertiary structure in the ribosome, and that much (or all) of this characteristic structure is present in newly isolated molecules of rRNA. Similarly, it can be argued that RP-RNA is extracted from relaxed particles with its unique structure unchanged. Thus, we conclude that the data presented here provide evidence for transitions in the configuration of rRNA during the process of ribosome development.

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LITERATURE CITED

1. BOEDTKER, H. 1960. Configurational properties of tobacco mosaic virus ribonucleic acid. J. Mol. Biol. **2**:171–188.
2. BOEDTKER, H., W. MÖLLER, AND E. KLEMPERER. 1962. On the validity of the evidence for subunits of high molecular weight ribonucleic acids. Nature **194**:444–446.
3. BOREK, E., AND A. RYAN. 1958. Studies on a mutant of *Escherichia coli* with unbalanced ribonucleic acid synthesis. II. The concomitance of ribonucleic acid synthesis with resumed protein synthesis. J. Bacteriol. **75**:72–76.
4. DISCHE, Z. 1955. Color reactions of nucleic acid components, p. 285. *In* E. Chargaff and J. N. Davidson [ed.]. The nucleic acids, vol. 1. Academic Press, Inc., New York.
5. DUBIN, D. T. 1964. Some effects of streptomycin on RNA metabolism in *Escherichia coli*. J. Mol. Biol. **8**:749–767.
6. DUBIN, D. T., AND A. T. ELKORT. 1964. Some abnormal properties of chloramphenicol RNA. J. Mol. Biol. **10**:508–518.
7. DUBIN, D. T., AND A. T. ELKORT. 1965. A direct demonstration of the metabolic turnover of chloramphenicol RNA. Biochim. Biophys. Acta **103**:355–358.
8. FLEISSNER, E., AND E. BOREK. 1963. Studies on the enzymatic methylation of soluble RNA. 1. Methylation of the S-RNA polymer. Biochemistry **2**:1093–1100.
9. GORDON, J., AND H. G. BOMAN. 1964. Studies on microbial RNA. II. Transfer of methyl groups from methionine to the RNA of a ribonucleoprotein particle. J. Mol. Biol. **9**:638–653.
10. HERSKOVITS, T. T., S. J. SINGER, AND E. P. GEIDUSCHEK. 1961. Non-aqueous solutions of DNA. Denaturation in methanol and ethanol. Arch. Biochem. Biophys. **94**:99–114.
11. KONO, M., E. OTAKA, AND S. OSAWA. 1964. Changes in sedimentation properties of ribosomal ribonucleic acids during the course of ribosome formation in *Escherichia coli*. Biochim. Biophys. Acta **91**:612–618.
12. LEVINE, L., J. A. GORDON, AND W. P. JENCKS. 1963. The relationship of structure to the effectiveness of denaturing agents for deoxyribonucleic acid. Biochemistry **2**:168.
13. LINDAHL, T., AND A. ADAMS. 1966. Native and and renatured transfer ribonucleic acid. Science **152**:512–514.
14. LINDAHL, T., A. ADAMS, AND J. R. FRESCO. 1966. Renaturation of transfer ribonucleic acids through site binding of magnesium. Proc. Natl. Acad. Sci. U.S. **55**:941–948.
15. LINDIGKEIT, R., AND W. HANDSCHACK. 1965. Some properties of ribonucleic acid obtained from ribosomal precursors or *Escherichia coli*. Biochim. Biophys. Acta **103**:241–251.
16. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
17. MANDEL, J. D., AND A. D. HERSHEY. 1960. A fractionating column for analysis of nucleic acids. Anal. Biochem. **1**:66–77.
18. MANDEL, L. R., AND E. BOREK. 1962. Ribonucleic acid synthesis under relaxed control. Biochem. Biophys. Res. Commun. **9**:11–17.
19. MANDEL, L. R., AND E. BOREK. 1963. The nature of the RNA synthesized during conditions of unbalanced growth in *E. coli* K_{12}W-6. Biochemistry **2**:560–566.
20. MORRIS, D. W., AND J. A. DEMOSS. 1965. Role of aminoacyl-transfer ribonucleic acid in the regulation of ribonucleic acid synthesis in *Escherichia coli*. J. Bacteriol. **90**:1624–1631.
21. NAKADA, D. 1965. Formation of ribosomes by a "relaxed" mutant of *Escherichia coli*. J. Mol. Biol. **12**:695–725.
22. NAKADA, D., I. A. C. ANDERSON, AND B. MAGASANIK. 1964. Fate of ribosomal RNA produced by a "relaxed" mutant of *Escherichia coli*. J. Mol. Biol. **9**:472–488.
23. NEIDHARDT, F. C., AND L. EIDLIC. 1963. Characterization of the RNA formed under conditions of relaxed amino acid control in *Escherichia coli*. Biochim. Biophys. Acta **68**:380–388.
24. NISHIMURA, S., AND G. D. NOVELLI. 1963. Resistance of S-RNA to ribonucleases in the presence of magnesium ion. Biochem. Biophys. Res. Commun. **11**:161–165.
25. OSAWA, S. 1965. Biosynthesis of ribosomes in

bacterial cells, p. 161–188. *In* J. N. Davidson and W. E. Cohn [ed.]. Progress in nucleic acid research and molecular biology, vol. 4. Academic Press, Inc., New York.

26. PENNISTON, J. T., AND P. DOTY. 1963. Reaction of formaldehyde with soluble ribonucleic acid. Biopolymers **1**:145–151.
27. SUEOKA, N., AND T. Y. CHENG. 1962. Fractionation of nucleic acids with the methylated albumin column. J. Mol. Biol. **4**:161–172.
28. SYPHERD, P. S. 1965. Accumulation of ribonucleoprotein particles in a relaxed mutant of *Escherichia coli*. J. Bacteriol. **90**:403–410.
29. SYPHERD, P. S. 1965. Formation of ribosomes from precursor ribonucleoprotein particles. J. Bacteriol. **94**:411–417.
30. SYPHERD, P. S., AND N. STRAUSS. 1963. Chloramphenicol-promoted repression of β-galactosidase synthesis in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **49**:400–407.
31. TURNOCK, G., AND D. G. WILD. 1965. The synthesis of ribosomes by a mutant of *Escherichia coli*. Biochem. J. **95**:597–607.
32. YANKOFSKY, S. A., AND S. SPIEGELMAN. 1963. Distinct cistrons for the two ribosomal RNA components. Proc. Natl. Acad. Sci. U.S. **49**: 538–544.
33. ZUBAY, G., AND R. MARCIELLO. 1963. A chemical method for studying detailed secondary structure of RNA. Biochem. Biophys. Res. Commun. **11**:79–82.