Proc. Nat. Acad. Sci. USA
Vol. 68, No. 12, pp. 2932–2936, December 1971

Role of 5S RNA in the Functions of 50S Ribosomal Subunits*

(reconstitution/*B. stearothermophilus*/termination/peptidyl transferase/tRNA binding/G factor)

V. A. ERDMANN†, S. FAHNESTOCK, K. HIGO, AND M. NOMURA‡

Institute for Enzyme Research, and Departments of Biochemistry and Genetics, University of Wisconsin, Madison, Wis. 53706

Communicated by Henry Lardy, September 16, 1971

ABSTRACT 50S ribosomal subunits from *Bacillus stearothermophilus* can be reconstituted from their dissociated components, namely a 5S RNA-free protein fraction, a 5S RNA-free 23S ribosomal RNA fraction, and purified 5S RNA. The biological activity of reconstituted particles in polypeptide synthesis is dependent on the presence of 5S RNA. In the absence of 5S RNA, particles are produced that have greatly reduced activity in (*a*) polypeptide synthesis directed by synthetic, as well as natural, messenger RNA, (*b*) peptidyl transferase assay, (*c*) [^{3}H]UAA binding dependent on peptide chain termination factor R1, (*d*) G factor-dependent[^{3}H]GTP binding, and (*e*) codon-directed tRNA binding assayed in the presence of 30S subunits. Thus, 5S RNA is an essential 50S ribosomal component.

Since 5S RNA was discovered as a component of the large ribosomal subunit (1), many studies have been done on its structure and biosynthesis. The primary sequence of 5S RNA from *Escherichia coli* was first elucidated by Brownlee, Sanger, and Barell (2). Yet, the function of 5S RNA has remained unknown. Several workers devised methods to remove 5S RNA from 50S subunits and found that removal of 5S RNA led to inactivation of 50S subunits (3–5). However, these workers were unable to restore activity to these inactive particles by adding 5S RNA. Thus, it was not possible to decide whether the inactivation was due to the removal of 5S RNA or to some structural disorganization of the particles caused by the treatment used.

We have recently succeeded in reconstituting 50S ribosomal subunits from *Bacillus stearothermophilus* from their dissociated molecular components (6). Using this reconstitution system, we have now shown that 5S RNA is essential for the overall activity of 50S subunits in polypeptide synthesis. This paper describes information on the function of 5S RNA obtained in this system.

MATERIALS AND METHODS

The following buffers are used: *TMA-I;* 0.01 M Tris·HCl (pH 7.4)–0.01 M $MgCl_2$–0.03 M NH_4Cl–6 mM 2-mercaptoethanol. *TMA-II*; Same as TMA-I, except 0.3 mM $MgCl_2$. *TRI;* 0.03 M Tris·HCl (pH 7.4)–0.02 M $MgCl_2$–1 M KCl–6 mM 2-mercaptoethanol. *TRO;* Same as TRI, except no KCl.

B. stearothermophilus 799 was grown at 61°C to early-log phase in medium L (10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, and 1 g of glucose per liter of H_2O). The pH is adjusted to 7.0 with NaOH. Cells were ground with two times their weight of alumina at 0°C and extracted with five times the cell weight of TMA-I buffer containing 2 μg/ml of RNase-free DNase. Alumina, unbroken cells, and cell debris were removed by centrifugation, first at 20,000 × *g* for 20 min, and then 78,000 × *g* for 30 min, to give the crude extract. Crude 70S ribosomes were obtained by centrifugation of the extract at 78,000 × *g* for 10 hr. The 70S ribosomes were then dialyzed against TMA-II overnight at 4°C and the dissociated ribosomal subunits were separated and purified (7). All ribosomes were stored in TMA-I at −80°C.

50S subunits (900 A_{260}/ml in TMA-I) were treated with an equal volume of 8 M urea–4 M LiCl solution at 0°C for 48 hr, and then centrifuged at low speed. The RNA pellet was washed once at 0°C with a mixture (1:1) of TMA-I and 8 M urea–4 M LiCl solution and then dissolved in water ("RNA 50" fraction). This fraction contains 23S RNA with one protein (L3) still attached* and about 30% of the 5S RNA (6). The supernatant from the second centrifugation was combined with the first, to give the total-protein fraction ("TP50"). This protein fraction contains about 70% of the 5S RNA and lacks most of the L3 protein. For the "standard reconstitution," RNA50 was dialyzed against TRO buffer for 4 hr, and TP50 was dialyzed against TRI buffer for the same length of time at 4°C. Reconstitutions were performed at 60°C for 60 min (6). The reaction mixture contained, in a volume of 8.0 ml, 10 A_{260} units of RNA50, 12.5 A_{260} equivalents of TP50, and a mixture of TRO and TRI buffers, to give a final KCl concentration of 0.3 M. 1 A_{260} equivalent is defined as the amount of protein that can be obtained from 1 A_{260} unit of ribosomes.

RNA50 was freed from 5S RNA by repeated precipitation with 5 M LiCl. RNA50 (100–200 A_{260} units/ml, in water) was mixed with an equal volume of 10 M LiCl at room temperature. The precipitate was collected by centrifugation and then dissolved in water. After another precipitation, 5S RNA-free RNA50 was dialyzed against TRO buffer. 5S RNA was removed from the TP50 fraction by DEAE-cellulose (Sigma) chromatography. TP50 (400 A_{260} equivalents) was dialyzed against the buffer containing 6 M urea–0.01 M Tris (pH 7.8)–0.25 M KCl–1 mM $MgCl_2$–6 mM 2-mercaptoethanol (for 4 hr at 6°C) and then applied to a DEAE-cellulose

* Paper No. 1486 of the Laboratory of Genetics and paper XIII in the series "Structure and Function of Bacterial Ribosomes." Paper XII in this series is by Nashimoto, H., W. Held, E. Kaltschmidt, and M. Nomura, *J. Mol. Biol.*, in press.

† Present address: Max-Planck-Institut fur Molekulare Genetik, 1 Berlin (Dahlem), Ihnestr. 63, Germany.

‡ Author to whom reprint requests should be addressed.

* Erdmann, V. A., S. Fahnestock, and M. Nomura, *Fed. Proc.*. **30**, 1203 Abs (1971).

column (0.7 × 2.5 cm) equilibrated with the same buffer. The column was eluted with 1-ml portions of the same buffer that contained 0.3 M KCl until no more materials that adsorbed at 280 nm were eluted. Proteins eluted were combined and dialyzed for 4 hr at 4°C against TRI buffer. This fraction is called 5S RNA-free protein fraction. 5S RNA was prepared from purified *B. stearothermophilus* 50S subunits by phenol extraction followed by chromatography on Sephadex G-100 (ref. 8, and Erdmann and Cramer, unpublished). Purity of 5S RNA was confirmed by polyacrylamide gel electrophoresis.

The reconstitution with 5S RNA-free RNA and 5S RNA-free protein fractions was performed in the same way as the standard reconstitution, except that 15 A_{260} equivalents of proteins were used instead of 12.5 A_{260} equivalents and 5S RNA was added as indicated. After reconstitution, the samples were centrifuged in a Spinco 65 rotor at 65,000 rpm for 2.5 hr. The pellets were dissolved in TMA-II buffer and the Mg^{++} concentration was then adjusted to 10^{-2} M. Insoluble materials were removed by a low-speed centrifugation, and the samples were assayed for the various biological activities. The methods for these assays are described in the table and figure legends.

In some experiments (Table 1 and 2, and Fig. 2) reconstitution was done with RNA and protein fractions obtained from crude 70S ribosomes. Procedures were the same as used for 50S subunits, except that the reconstituted particles were fractionated by centrifugation on a sucrose gradient in TMA-II buffer. Fractions containing reconstituted "50S" particles or the corresponding particles were pooled and concentrated by centrifugation, and used for further analyses.

TABLE 1. *Biological activity of [−5S] particles**

	Poly(U) (Phe)	f2 RNA (Val)	Peptidyl transferase	G-factor (GTP binding)	R1-factor (UAA binding)
Rec. "50S"	1020	911	3969	1349	2274
[+5S]	656	883	2972	1901	1261
[−5S]	4	194	430	330	129
50S	3202	916	4241†	4028	4100
Blank	(247)	(422)	(43)	(237)	(120)

The same reconstituted particles described in Fig. 2 were assayed for various biological activities. Aliquots containing 1.0 A_{260} unit of reconstituted particles were used for the assays. Poly(U)-directed [^{14}C]-phenylalanine incorporation and f2 RNA-directed [^{14}C]-valine incorporation were measured in the presence of 1.0 A_{260} units of 30S subunits. Peptidyl transferase activity was assayed in the absence of 30S subunits by the formation of [^{35}S]fMet-puromycin from puromycin and fMet-tRNA. The reaction was as described in the legend to Fig. 1, except that the fragment was replaced by whole fMet-tRNA (50,000 cpm, 14 Ci/mmol), and all volumes were 50% less. After 10 min at 0°C, the reaction was terminated with 10 μl of 10 N KOH and samples were incubated for 10 min at 37°C to hydrolyze fMet-methyl ester, which is formed in this assay (27, 29). 1 ml of 1 M sodium phosphate buffer (pH 7.0) was then added and the product was extracted with ethyl acetate. The formation of a stable complex of [^{3}H]GTP and G-factor with the 50S subunit in the presence of fusidic acid was determined by the method of Bodley (12, 14). The incubation mixture contained, in 50 μl: 0.01 M Tris·HCl(pH 7.4), 0.03 M NH_4Cl, 6 mM 2-mercaptoethanol, 25 pmol [^{3}H]GTP (5.6 Ci/mmol), 3 mM fusidic acid, 2.5 μg of purified G-factor (a gift from Dr. P. Leder), and 1.0 A_{260} unit of reconstituted particles. G-factor was added last to initiate the reaction, which was allowed to proceed for 5 min at 0°C. Samples were then diluted with 3 ml of TMA-I buffer, containing 10 μM fusidic acid, and passed immediately through a Millipore filter. The amount of ^{3}H on the filter was then measured. Ability of the reconstituted particles to interact with the release factors and the termination codons was measured according to Scolnick and Caskey (19). Both the [^{3}H]UAA triplet and the *E. coli* R1 release factor were generous gifts from Dr. C. T. Caskey. The 0.05-ml reaction mixtures contained, besides the reagents listed by Scolnick and Caskey (17), 1 A_{260} unit of 50S subunits or reconstituted particles and 0.5 A_{260} unit of *E. coli* 30S subunits. Blank values in the absence of 50S or reconstituted particles are subtracted in all cases.

* cpm/A_{260} unit; † cpm/0.1 A_{260} unit.

TABLE 2. *Phe-tRNA-binding activity of reconstituted particles*

	Phe-tRNA bound (cpm)		
	+Poly(U)	−Poly(U)	Δ
Expt. 1: Millipore filter assay			
[+5S RNA]	2960	371	2589
[−5S RNA]	2282	312	1970
50S	3798	591	3207
Blank, 30S only	2230	206	2024
Expt. 2: RNase assay			
[+5S RNA]	2306	189	2117
[−5S RNA]	617	169	448
50S	3130	224	2906
Blank, 30S only	281	152	129

Both [+5S RNA] and [−5S RNA] particles were prepared as described in the legend to Fig. 2. The Millipore filter assay was done according to Nirenberg and Leder (18). Each sample (50 μl) contained 0.1 M Tris·HCl (pH 7.4), 0.024 M $MgCl_2$, 0.05 M KCl, 0.012 M NH_4Cl, 2 mM 2-mercaptoethanol, 0.04 mg poly(U), [^{14}C]Phe-tRNA (New England Nuclear Corp., 9000 cpm, 385 Ci/mol, 32 μg of tRNA), 0.5 A_{260} unit of *E. coli* 30S subunits, and 1.0 A_{260} unit of 50S subunits or reconstituted particles. Incubation was at 30°C for 20 min. The RNase assay was according to Pestka (22). Incubation was as described above for the Millipore filter assay. After 20 min at 30°C, 14 ng of pancreatic RNase was added in 5 μl, and a second incubation was performed for 7 min at 30°C. Cold 5% Cl_3CCOOH (2 ml) was then added and the amount of acid-precipitable ^{14}C was measured.

RESULTS

Reconstitution of 50S subunits active in polypeptide synthesis

We have shown (6) that purified radioactive 5S RNA added to the reconstitution system is incorporated into the reconstituted "50S" particles. The calculation showed that the reconstituted particles contain about one molecule of 5S RNA per molecule of 23S RNA. It was found that 5S RNA is present both in the RNA fraction ("RNA50" fraction) and in the protein fraction ("TP50" fraction). We described preliminary experiments (6) in which 5S RNA was partially removed from the fractions and some stimulation of the reconstitution by added 5S RNA was observed. We have now been able to devise a method by which the reconstitution is entirely dependent on 5S RNA.

5S RNA was removed from the protein fraction by DEAE-cellulose chromatography. The RNA fraction was also freed

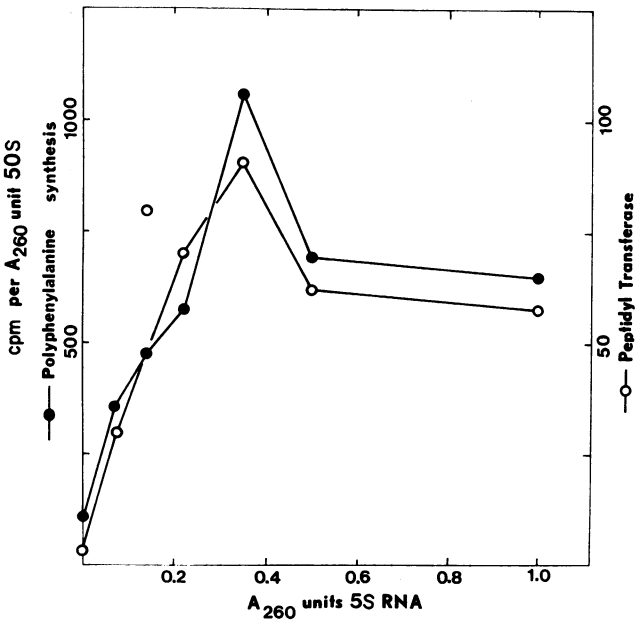

FIG. 1. Reconstitution of 50S particle activities with 5S RNA-free RNA and 5S RNA-free protein fractions. 5S RNA-free RNA and 5S RNA-free proteins were prepared from purified 50S subunits. 10 A_{260} units of 5S RNA-free RNA, 15 A_{260} equivalents of 5S RNA-free protein, and various amounts of purified 5S RNA, as indicated, were used for the reconstitution. The theoretical molar equivalent value of 5S RNA is 0.35 A_{260} units per 10 A_{260} units of 23S RNA. Reconstituted "50S" particles (0.5 A_{260} units per assay) were analyzed for poly(U)-dependent polyphenylalanine synthesis (7) in the presence of 0.5 A_{260} units of *E. coli* 30S subunits, and for the peptidyl transferase assay. The peptidyl transferase assay (10) was done as follows: Before the addition of methanol the reaction mixture (0.15 ml) contained 0.06 M Tris·HCl (pH 8.1 at 0°C), 0.4 M KCl, 0.02 M $MgCl_2$, 6 mM 2-mercaptoethanol, [^{35}S]fMet-fragment (4300 cpm, 3 Ci/mmol), and 0.5 A_{260} units of reconstituted particles. The reaction was initiated with 75 μl of methanol, which contained 1 mM puromycin. After 30 min at 0°C, the reaction was terminated with 0.05 ml of 0.1 M $BeCl_2$, and 0.1 ml of 0.3 M sodium acetate (pH 5.5) saturated with $MgSO_4$ was added. The mixture was extracted with 1.5 ml of ethyl acetate at room temperature and 1 ml of the organic phase containing fMet-puromycin was counted. Both fMet-RNA and fMet-hexanucleotide fragment were prepared from purified $tRNA_{Met,f}$ obtained from Oak Ridge National Laboratories, as described (26, 27). The fragment was a gift from Dr. V. Lieck.

from 5S RNA by repeated precipitation with 5 M LiCl. Reconstitution was then performed using the 5S RNA-free TP50 and 5S RNA-free RNA 50, with and without further addition of 5S RNA. As shown in Fig. 1, biological activity of reconstituted particles assayed by poly(U)-dependent synthesis of polyphenylalanine was proportional to the amount of 5S RNA added, up to a molar equivalent of 1:1 23S RNA to 5S RNA. An increase in 5S RNA concentration beyond the equivalence point caused some drop in activity. In the absence of 5S RNA, no significant activity was observed. We conclude that 5S RNA is an essential 50S ribosomal component.

Biological activities of [−5S RNA] particles

The above results show that the particles reconstituted in the absence of 5S RNA ([−5S RNA] particles) were not active in a poly(U)-dependent synthesis of polyphenylalanine. We have also assayed the activity of [−5S] particles in cell-free protein synthesis directed by RNA from phage f2. The particles were devoid of any significant activity (Table 1). It was then of interest to examine some known specific functions of the 50S subunit that are required for protein synthesis to determine which of them are impaired. We have tested (*a*) the activity of peptidyl transferase, (*b*) the ability of the particles to bind the G-factor and GTP, (*c*) the ability of the particles to bind the chain termination codon (triplet), UAA, in the presence of a soluble chain termination factor, R1, and (*d*) the ability of the particles to participate in the poly(U)-dependent nonenzymatic binding of Phe-tRNA. Like protein synthesis assayed with poly(U) and f2 RNA, all of these activities are drastically reduced in the [−5S RNA] particles as compared to [+5S RNA] particles (particles reconstituted in the presence of 5S RNA).

The enzyme that catalyzes the formation of peptide bonds during protein synthesis, peptidyl transferase, is an integral part of the 50S subunit (9, 10). Its activity can be assayed independently of other ribosomal functions by the formation of fMet-puromycin from puromycin and fMet-tRNA (11) or from the terminal hexanucleotide fragment of fMet-tRNA (9). This reaction does not require the presence of the 30S subunit or any soluble factors, provided it is performed in the presence of alcohol (e.g., 33% methanol). Table 1 and Fig. 1 show that peptidyl transferase activity is greatly reduced in [−5S RNA] particles (to 14% of the activity of [+5S RNA] particles).

The 50S subunit also has the ability to bind G factor and GTP (12), presumably as a part of the translocation function. This binding appears to take place at a site that is independent of peptidyl transferase (13). In the presence of fusidic acid, the 50S–G factor–GTP complex is stabilized, and it can be assayed by its binding to a nitrocellulose-filter (12, 14). Table 1 shows that this activity is also greatly reduced in [−5S RNA] particles (to 17% of the [+5S RNA] activity).

Polypeptide chain termination in response to the proper codon (e.g., UAA) requires the participation of a soluble protein factor (R1 or R2), which interacts with the 50S subunit (15, 16). The ability to interact with the termination factor can be determined by the formation of a complex consisting of the radioactive trinucleotide UAA, R1 Factor, and 30S and 50S ribosomal subunits (17). The results of this assay, also shown in Table 1, indicate that this activity is, for the most part, absent from [−5S RNA] particles (10% of the [+5S RNA] activity).

Another aspect of the biological activity of the 50S subunit is its ability to participate with the 30S subunit in the codon-directed binding of aminoacyl-tRNA, although 50S subunits alone do not show any such binding activity. When tRNA binding is assayed by the nitrocellulose filter technique of Nirenberg and Leder (18), the participation of 50S subunits is manifest as a stimulation of the binding observed with 30S subunits alone (19, 20). The degree of stimulation is usually about 2-fold with Phe-tRNA (19, 20), and much higher with some other aminoacyl-tRNAs (21). The participation of the 50S subunit in the tRNA binding can be assayed more specifically by the method of Pestka (22), which is based on the fact that aminoacyl-tRNA bound to 30S subunits is sensitive to low concentrations of RNase, while the presence of 50S subunits protects bound aminoacyl-tRNA from degradation. In this assay, Phe-tRNA precipitable by cold

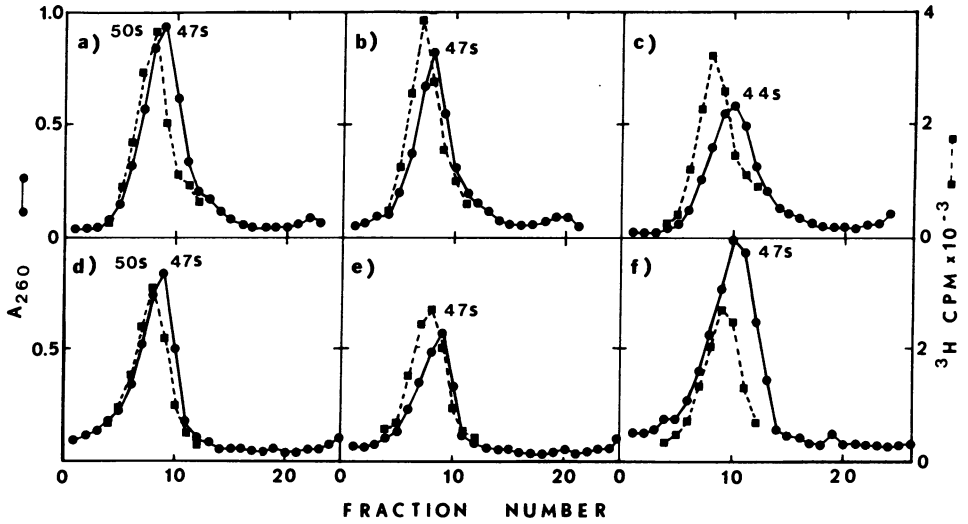

FIG. 2. Sucrose density gradient analysis of reconstituted particles. RNA and protein fractions were prepared from 70S ribosomes and reconstitution was with 232 A_{260} units of 5S RNA-free RNA and 410 A_{260} equivalents of 5S RNA-free proteins, with (*b* and *e*) and without (*c* and *f*) 9 A_{260} units of 5S RNA in 180 ml. The standard reconstitution was also done with protein and RNA not depleted of 5S RNA (*a* and *d*). The sedimentation of reconstructed particles (A_{260}) is compared to the sedimenation of intact, reference 50S particles (^{3}H). Particles corresponding to reconstituted "50S" were isolated by sucrose density gradient centrifugation. These preparations were analyzed for biological activities (see Table 1) and for sedimentation characteristics. Aliquots were mixed with radioactive ^{3}H-labeled 50S subunits from *E. coli*. The Mg^{++} concentration of the samples was then adjusted to 3×10^{-4} M (*a*, *b*, and *c*) or 10^{-2} M (*d*, *e*, and *f*). Samples were placed on linear 5–20% sucrose gradients in TMA-II buffer (*a*, *b*, and *c*) or TMA-I buffer (*d*, *e*, and *f*), and centrifuged in a SW50 rotor for 90 min at 50,000 rpm. 3-drop fractions were collected from the bottom, and both A_{260} and ^{3}H were measured in appropriate ways.

Cl_3CCOOH is determined after RNase treatment, providing a measure of the amount of Phe-tRNA bound to 30S–50S couples, i.e., 70S ribosomes.

Results of both types of assay are shown in Table 2. With the Millipore filter assay, it was observed that [−5S RNA] particles do not stimulate Phe-tRNA binding directed by poly(U). With the second (RNase) assay any activity of [−5S RNA] particles would be much more clear, since the blank value (binding to 30S only) was greatly reduced. In this assay [−5S RNA] particles show about 20% of the activity of [+5S RNA] particles.

In order to examine the possibility that [−5S RNA] particles are able to join the 30S–Phe-tRNA–poly(U) complex but may fail to protect the bound tRNA from RNase due to some structural difference, various samples described in Fig. 2 were examined by sucrose gradient centrifugation (5–20% gradient in the same buffer used for the binding assay, 50,000 rpm for 70 min in SW50 rotor). Phe-tRNA bound in the presence of [−5S RNA] particles plus 30S subunits sediments at about 30S, like that bound in the presence of 30S subunits alone. In contrast, Phe-tRNA bound in the presence of [+5S RNA] particles plus 30S subunits sediments at about 70S. This assay shows no effect of [−5S RNA] particles on the binding of Phe-tRNA to 30S subunits. Thus, our observation differs from that of Siddiqui and Hosokawa (5), who found that EDTA-treated 50S subunits, from which 5S RNA had been removed, affected the binding of Phe-tRNA to 30S subunits but did not afford protection from RNase.

Other properties of [−5S RNA] particles

As expected, [−5S RNA] particles do not contain any significant amount of 5S RNA (less than 5% of the amount in [+5S RNA] particles). This was confirmed by polyacrylamide gel electrophoresis of RNA prepared from the [−5S RNA] particles.

The sedimentation properties of [−5S RNA] particles were also studied. In the presence of 0.01 M Mg^{++}, [−5S RNA] particles sedimented with *s* values (47S) identical to the control [+5S RNA] particles or the reconstituted particles prepared in the standard way (Fig. 2*d–f*). In the presence of 0.3 mM Mg^{++}, however, [−5S RNA] particles sedimented at a definitely slower rate (44 S) than did the control [+5S RNA] particles (Fig. 2*a–c*). It appears that [−5S RNA] particles have a "loose" structure at low concentrations of Mg^{++}, but assume a more compact structure in high concentrations of Mg^{++}.†

The protein content of [−5S RNA] and [+5S RNA] particles, purified by sucrose gradient centrifugation, was analyzed by two-dimensional polyacrylamide gel electrophoresis (28). It was found that [−5S RNA] particles lack abou 4 out of 39 possible 50S ribosomal protein spots that are present in [+5S RNA] particles (unpublished experiments).

The question was then asked whether the absence of some proteins in the [−5S RNA] particles is related to the inactivity of the particles. It was found that restoration of activity to the inactive [−5S RNA] particles depends on the presence of the missing proteins, in addition to 5S RNA. Thus, some or all of the proteins that are absent in [−5S RNA] particles are important for the activity of 50S subunits in polypeptide synthesis. The details of these experiments will be reported elsewhere.

DISCUSSION

The results of the experiments described in this paper clearly establish the essential role of 5S RNA in the assembly of

† Reconstituted "50S" particles generally sediment slightly slower (47S) than do intact 50S markers. Assays for the activity of fractions from sucrose gradients showed that functionally active particles also sediment at 47S.

active 50S ribosomal subunits. [−5S RNA] Particles showed greatly reduced activities in (*a*) poly(U)-dependent synthesis of polyphenylalanine, (*b*) f2 RNA-directed incorporation of [^{14}C]valine, (*c*) peptidyl transferase assay, (*d*) R1 factor-dependent binding of [^{3}H]UAA, (*e*) G factor-dependent binding of [^{3}H]GTP, and (*f*) participation in codon-directed tRNA binding. As mentioned above, [−5S RNA] particles are deficient in some proteins important in these functions. Thus, 5S RNA has a structural role, in the sense that some functionally important proteins fail to join the particle in the absence of 5S RNA. Whether 5S RNA has any direct role in these functions in the completed ribosome structure must await further investigations.

5S RNA from *E. coli* has a sequence (—CGAAC—, 43rd to 47th residue) that is complementary to the —GTψC$^{A}_{G}$ sequence found in all tRNAs sequenced thus far (23). Furthermore, the region of 5S RNA containing this sequence (—CGAAC—) appears to be the most exposed single-stranded region of the molecule (24). Thus, it was hypothesized that 5S RNA has a function in the binding of tRNA by virtue of this complementary base pairing (24). Present results indicate that [−5S RNA] particles are about 20% as active in non-enzymatic binding of Phe-tRNA as are [+5S RNA] particles. Reduction of activity to this low amount in the absence of 5S RNA does not contradict the above hypothesis. It cannot be interpreted as supporting evidence, however, since all of the functions we have examined are similarly affected. It is likely that the overall structure of the ribosome is altered, either by the absence of 5S RNA or the absence of those proteins that fail to bind without 5S RNA. The above hypothesis could be tested more specifically by the use of 5S RNA that is chemically modified in the appropriate region in this reconstitution system.

There is a report that 5S RNA stimulates the incorporation of amino acids directed by phage RNA but not by poly(U), suggesting a specific function of 5S RNA that is related to natural messenger RNA (25). It is difficult to explain this observation. Our results clearly show that 5S RNA is essential for activity in protein synthesis directed by synthetic, as well as by natural, messenger RNA.

NOTE ADDED IN PROOF

[−5S RNA] particles have consistently demonstrated some activity (up to 25%) in many of the assays described above. Though these activities are much lower than those of [+5S RNA] particles, they are probably significant. They are too large to be accounted for by the presence of small amounts of residual 5S RNA. Particles have been obtained in other experiments that have less than 1% of the amount of 5S RNA present in [+5S RNA] particles or 50S ribosomes, as demonstrated by polyacrylamide gel electrophoresis. The activity of these [−5S RNA] particles, relative to that of [+5S RNA] particles, in various assays is as follows: (*a*) peptidyl transferase, 7%; (*b*) G factor-dependent GTP binding, 25%; (*c*) Phe-tRNA binding (RNase assay), 20%.

We thank Dr. J. C. Garver for the use of his pilot plant. This work was supported in part by the College of Agriculture and Life Sciences, University of Wisconsin, and by grants from the National Institute of General Medical Sciences (GM-15422) and National Science Foundation (GB-6594). Operation of the Biochemistry Department's pilot plant is supported by a grant (FR00226) from the National Institutes of Health. V.A.E. and S.F. were supported by fellowships from the National Institutes of Health.

1. Rosset, R., R. Monier, and J. Julian, *Bull. Soc. Chim. Biol.* **46,** 87 (1964).
2. Brownlee, G. G., F. Sanger, and B. G. Barrell, *Nature,* **215,** 735 (1967).
3. Aubert, M., R. Monier, M. Reynier, and J. F. Scott, *Proc. Fourth FEBS Meeting,* **3,** 151 (1967).
4. Sarkar, N., and D. G. Comb, *J. Mol. Biol.* **39,** 31 (1969).
5. Siddiqui, M. A. Q., and K. Hosokawa, *Biochem. Biophys. Res. Commun.,* **36,** 711 (1969).
6. Nomura, M., and V. A. Erdmann, *Nature,* **228,** 744 (1970).
7. Traub, P., and M. Nomura, *J. Mol. Biol.,* **34,** 575 (1969).
8. Reynier, M., M. Aubert, and R. Monier, *Bull. Soc. Chim. Biol.,* **49,** 1205 (1967).
9. Monro, R. E., *J. Mol. Biol.,* **26,** 147 (1967).
10. Maden, B. E. H., and R. E. Monro, *Eur. J. Biochem.,* **6,** 309 (1968).
11. Monro, R. E., J. Cerna, and K. A. Marcker, *Proc. Nat. Acad. Sci. USA,* **61,** 1042 (1968).
12. Bodley, J. W., and L. Lin, *Nature,* **227,** 60 (1970).
13. Modolell, J., D. Vazquez, and R. E. Monro, *Nature New Biol.* **230,** 109 (1971).
14. Bodley, J. W., F. J. Zieve, L. Lin, and S. T. Zieve, *Biochem. Biophys. Res. Commun.,* **37,** 437 (1969).
15. Caskey, T., E. Scolnick, R. Tompkins, J. Goldstein, and G. Milman, *Cold Spring Harbor Symp. Quant. Biol.,* **34,** 479 (1969).
16. Capecchi, M. R., and H. A. Klein, *Cold Spring Harbor Symp. Quant. Biol.,* **34,** 469 (1969).
17. Scolnick, E. M., and C. T. Caskey, *Proc. Nat. Acad. Sci. USA,* **64,** 1235 (1969).
18. Nirenberg, M. W., and P. Leder, *Science,* **145,** 1399 (1964).
19. Suzuka, I., H. Kaji, and A. Kaji, *Proc. Nat. Acad. Sci. USA* **55,** 1483 (1966).
20. Pestka, S., and M. Nirenberg, *J. Mol. Biol.,* **21,** 145 (1966).
21. Nomura, M., and C. V. Lowry, *Proc. Nat. Acad. Sci. USA,* **58,** 946 (1967).
22. Pestka, S., *J. Biol. Chem.,* **243,** 4038 (1968).
23. Zachau, H. G., *Angew. Chem., Int. Ed. Engl.,* **8,** 711 (1969).
24. Jordan, B. R., *J. Mol. Biol.,* **55,** 423 (1971).
25. Kirtikar, D. M. W., and A. Kaji, *J. Biol. Chem.,* **243,** 5345 (1968).
26. Fahnestock, S., and A. Rich, Nature, New Biol., **229,** 8 (1971).
27. Fahnestock, S., H. Neumann, V. Shashoua, and A. Rich, *Biochemistry,* **9,** 2477 (1970).
28. Kaltschmidt, E., and H. G. Wittmann, *Anal. Biochem.,* **36,** 401 (1970).
29. Miskin, R., A. Zamir, and D. Elson, *J. Mol. Biol.,* **54,** 355 (1970).