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# Role of 5S RNA in assembly and function of the 50S subunit from *Escherichia coli*

**[*in vitro* assembly/peptidyltransferase (EC 2.3.2.12) center/A-site and P-site/elongation factor G dependent GTPase]**

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**ABSTRACT** Total reconstitution experiments performed under various conditions revealed that 5S RNA plays an important role during the last assembly step *in vitro* leading to an active 50S particle. For the preceding steps this RNA species is dispensable. However, 5S RNA can be integrated efficiently during any of the assembly steps *in vitro.*

The 47S particle, reconstituted in two steps and lacking 5S RNA, shows low but significant activity in many functional tests. High activity could be obtained by incubating this particle with 5S RNA alone, demonstrating the importance of the 5S RNA in generating an active ribosomal conformation. In particular, the activity of the peptidyltransferase (peptidyl-tRNA: aminoacyl-tRNA *N*-peptidyltransferase; EC 2.3.2.12) center is drastically influenced by 5S RNA. No significant factor-dependent tRNA binding to the A-site was observed with the 47S particle, in contrast to the corresponding P-site binding. The elongation factor G dependent GTPase activity was not affected by the lack of 5S RNA.

5S RNA is a component of the large subunit from both prokaryotic and eukaryotic ribosomes (for recent reviews see refs. 1 and 2). The primary structure of the 5S RNA from *Escherichia coli* and various other species is known.

50S subunits from which 5S RNA had been removed showed greatly reduced activity (3–5), and a more detailed functional analysis of a totally reconstituted 50S subunit from *Bacillus stearothermophilus* lacking 5S RNA revealed low activities in all ribosomal functions tested (6). No specific correlation of 5S RNA with a particular ribosomal function could therefore be drawn from these experiments. All the particles obtained either by dissociating the 5S RNA from native 50S subunits or by total reconstitution in the absence of 5S RNA lack some ribosomal proteins as well as 5S RNA. Thus, the decrease of activity of these particles cannot be unequivocally assigned to the lack of 5S RNA alone.

Recently, we described a procedure for the total reconstitution of the 50S subunit from *E. coli* (7). Using this system, we describe in this paper the role of 5S RNA during assembly *in vitro* and the various functional activities of a totally reconstituted particle lacking 5S RNA.

## MATERIALS AND METHODS

Cells from *E. coli* K12, strain A19, were harvested in early logarithmic growth phase. 50S subunits were isolated as described (8); preparation of RNA and separation of 23S and 5S RNA followed the procedure of ref. 7. Preparation of total proteins from the 50S subunit (TP50) was as reported recently (Dohme and Nierhaus, manuscript submitted to *J. Mol. Biol.*).

Abbreviations: TP50, total proteins from the 50S subunit; EF-T, elongation factors T (Tu + Ts); EF-G, elongation factor G; ac-, *N*-acetyl-; RI, reconstitution intermediate; $A_{260}$ unit, that amount of material that gives an absorbance of 1 when dissolved in 1 ml of solvent when the light path is 1 cm.

The two-step procedure for total reconstitution followed that of ref. 7, with a slight modification, namely, RNA and TP50 were incubated at 44° for 20 min in the presence of 20 mM Tris·HCl (pH 7.2), 4 mM Mg acetate, 400 mM $NH_4Cl$, and 2 mM 2-mercaptoethanol; after the $Mg^{++}$ concentration was raised to 20 mM the mixture was incubated a second time at 50° for 90 min.

The RNA content of the particles was analyzed by a step-gel electrophoresis [5 and 10% (wt/vol) polyacrylamide], by the method of van Diggelen and Bosch (9). RNA was isolated from the reconstituted particles by phenol treatment (7). It was then heated for 2 min at 70° in the presence of 1% sodium dodecyl sulfate and quickly cooled to 0°. The ionic concentrations were adjusted to those of the electrophoresis buffer, and 1/10 volume of 60% sucrose was added to each sample before it was applied to the gels. After electrophoresis the gels were soaked (15 min) in 12.5% trichloroacetic acid in order to fix the RNA, and were analyzed in a Gilford gel scan spectrophotometer 250. The absorbance at 260 nm was automatically recorded.

The binding of C-A-C-C-A-ac[$^3$H]Leu (acLeu, *N*-acetylleucine) to various particles was measured under conditions of equilibrium dialysis, as described (10). The two 50-$\mu$l chambers of a cell were separated by a cellulose acetate filter (AC 64) from Schleicher & Schüll, Dassel, Germany. Into one chamber were injected 2.5 $A_{260}$ units of particles in 30 $\mu$l, and into the other, 30 $\mu$l containing 1.2 pM of C-A-C-C-A-ac[$^3$H]Leu, equivalent to about 30,000 cpm. (An $A_{260}$ unit is that amount of material that gives an absorbance of 1 when dissolved in 1 ml of solvent when the light path is 1 cm.) The buffer consisted of 30 mM Tris·HCl (pH 7.2), 17 mM Mg acetate, 20 mM $NH_4Cl$, and 150 mM KCl, in 50% ethanol. After 26 hr at 4°, duplicate 10-$\mu$l aliquots were removed from each chamber and assayed for radioactivity. Each dialysis assay was performed in duplicate.

The references to other methods used are given in Table 2. Elongation factor G (EF-G) was a kind gift from Dr. C. v. Meyenburg, University Institute for Microbiology, Copenhagen; the preparation of elongation factors Tu + Ts (EF-T) was kindly provided by Dr. N. Ulbrich, Max-Planck-Institut, Berlin.

## RESULTS AND DISCUSSION

### 5S RNA and assembly *in vitro*

Recently we have shown that the assembly *in vitro* of *E. coli* 50S subunit proceeds via discrete intermediates (RI particles) which can be schematically represented as follows:

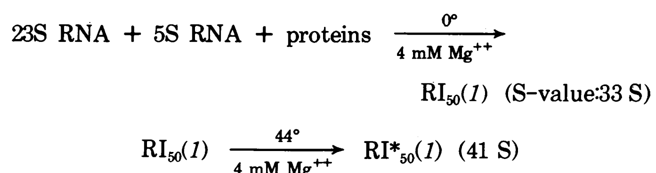
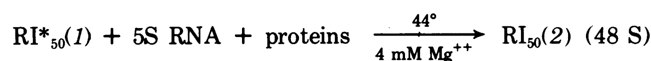


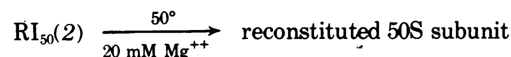
Table 1. Activities of particles that integrate the 5S RNA at various assembly stages

| Exp. no. | First incubation (23S + TP50): 5S RNA | First incubation (23S + TP50): Temp., mM $Mg^{++}$ | Second incubation: 5S RNA | Second incubation: TP-50 | Second incubation: Temp., mM $Mg^{++}$ | Poly(U) system (% of native 50S) |
|---|---|---|---|---|---|---|
| 1 | + | 0°, 4 | – | – | – | <1 |
| | | | + | + | 50°, 20 | 5 |
| | | [$RI_{50}(1)$ particle] | – | – | Two-step | 14 |
| | | | + | – | Two-step | 15 |
| | | | – | + | Two-step | 37 |
| | | | + | + | Two-step | 34 |
| 2 | + | 44°, 4 | – | – | – | <1 |
| | | [$RI_{50}(2)$ particle] | – | – | 50°, 20 | 66 |
| | | | + | + | 50°, 20 | 52 |
| | | | + | + | Two-step | 68 |
| 3 | + | Two-step (reconst. 50S particle) | – | – | – | 76 |
| 4 | – | 0°, 4 | – | – | – | <1 |
| | | (31S particle) | + | + | 50°, 20 | 14 |
| | | | + | – | Two-step | 8 |
| | | | – | + | Two-step | 13 |
| | | | + | + | Two-step | 57 |
| 5 | – | 44°, 4 | – | – | – | <1 |
| | | (42S particle) | – | – | 50°, 20 | 13 |
| | | | + | – | 50°, 20 | 44 |
| | | | – | + | 50°, 20 | 17 |
| | | | + | + | 50°, 20 | 71 |
| | | | + | + | Two-step | 72 |
| 6 | – | Two-step | – | – | – | 20 |
| | | (47S particle) | – | – | 50°, 20 | 19 |
| | | | + | – | 50°, 20 | 46 |
| | | | – | + | 50°, 20 | 26 |
| | | | + | + | 50°, 20 | 76 |
| | | | + | + | Two-step | 77 |
| | – | Two-step | – | – | – | 18 |
| | – | Two-step | – | – | – | +5S 19 |
| Controls | | Native 50S | | | | 100 (19400 cpm) |
| | | 30S alone | | | | 433 cpm |

The activities were measured under saturating phenylalanine concentrations (70 $\mu$M). After the first incubation the particles were pelleted through a sucrose cushion: 25 ml of the reconstitution mixture was layered over 10 ml of 40% sucrose containing the same ionic concentrations (Beckman SW 27 rotor, 18 hr at 80,000 $\times g$). The pellets were resuspended in a buffer containing 10 mM Tris·HCl (pH 7.4) and 4 mM Mg acetate and subjected to a second incubation as indicated. 44°, 4 means the first and 50°, 20 the second step of the two-step procedure (see *Materials and Methods*). During all incubations the $NH_4Cl$ concentration was 400 mM. 0.04 $A_{260}$ unit of 5S RNA and/or TP50 derived from 1.4 $A_{260}$ units of 50S subunits was added per one $A_{260}$ unit of 23S RNA or particle, where indicated. When 5S RNA was added to the poly(U) system, the activity of the 47S particle, reconstituted in two steps and lacking 5S RNA, was not affected (Table 1, controls), indicating that 5S RNA was not integrated under the conditions of the poly(U) system. Therefore it follows that the components used for the poly(U) system [e.g., tRNA (*E. coli*) or S-150 enzymes] do not have to be freed from traces of 5S RNA. An additional control experiment showed that assays carried out with tRNA and S-150 enzymes both freed of 5S RNA gave similar residual activities of 20% for the 47S particle.



$$RI^*_{50}(1) + 5S\ RNA + proteins \xrightarrow[4\ mM\ Mg^{++}]{44°} RI_{50}(2)\ (48\ S)$$



$$RI_{50}(2) \xrightarrow[20\ mM\ Mg^{++}]{50°} reconstituted\ 50S\ subunit$$

The optimal formation of the intermediate $RI^*_{50}(1)$ occurs during the first-step incubation of the two-step procedure, whereas the conversion of the $RI_{50}(2)$ particle to an active 50S subunit occurs in the second-step incubation (Dohme and Nierhaus, manuscript submitted to *J. Mol. Biol.*).

The successful incorporation of 5S RNA into the reconstituted 50S subunit can be assessed by measuring the activity of the particle in the poly(U) system, since in the absence of 5S RNA, particles with low activities are obtained. In order to identify the precise assembly step during which 5S RNA was integrated, we performed the following experiment: $RI_{50}(1)$ and $RI_{50}(2)$ particles were isolated. These particles were themselves not active at all in the poly(U) system. We then determined whether the addition of 5S RNA to these particles during a second incubation was required to induce activity in the poly(U) system (Table 1, Exps. 1 and 2). It is clear from these data that both particles already contained a full complement of 5S RNA, since a later addition of this RNA species did not increase the activity of the reconstituted 50S subunit. The $RI_{50}(1)$ particle needs a two-step incubation with the addition of proteins to gain full activity, whereas a second-step incubation alone (90 min at 20 mM $Mg^{++}$ and 50°) is sufficient for the $RI_{50}(2)$ particle, without addition of any 50S component.

The finding that the $RI_{50}(1)$ particle already contains 5S RNA is in agreement with previous results on the assembly *in vivo*, where the first precursor $p_1$50S was found to contain stoichiometric amounts of this RNA species (11, 12).

Reconstitution experiments were performed without 5S RNA under analogous conditions to those used for the intermediates and the reconstituted 50S subunit (Table 1, Exps. 4–6). Fig. 1 illustrates the sedimentation properties of the resulting particles. After incubation at 4 mM $Mg^{++}$ and 0°, a 31S particle is found (Fig. 1A). At the end of the first-step incubation (20 min at 4 mM $Mg^{++}$ and 44°), the 31S particle is converted to a 42S particle (Fig. 1B), which shows a further increase in S-value to 47S after the second-step incubation (90 min at 20 mM $Mg^{++}$ and 50°; Fig. 1C).

The 31S particle requires both a two-step incubation and the addition of 5S RNA and proteins in order to form a particle that is active in the poly(U) system (Table 1, Exp. 4). This is a similar requirement to that of the $RI_{50}(1)$ particle (compare with Exp. 1). Furthermore, the conformational change of the $RI_{50}(1)$ intermediate to the $RI^*_{50}(1)$ particle can occur in the absence of 5S RNA. Without 5S RNA but in the presence of all other 50S components, the first-step incubation causes conversion of the 31S particle to a 42S particle (Fig. 1A and B), a process equivalent to the conversion found in the presence of 5S RNA. The 42S particle lacking 5S RNA only needs the second-step incubation and the addition of 5S RNA to be converted to a 50S particle with good activity [Table 1, Exp. 5; compare with the $RI_{50}(2)$ particle, Exp. 2].

Similarly, the assembly step $RI^*_{50}(1) \rightarrow RI_{50}(2)$ seems to be independent of 5S RNA. This is indicated by the result obtained

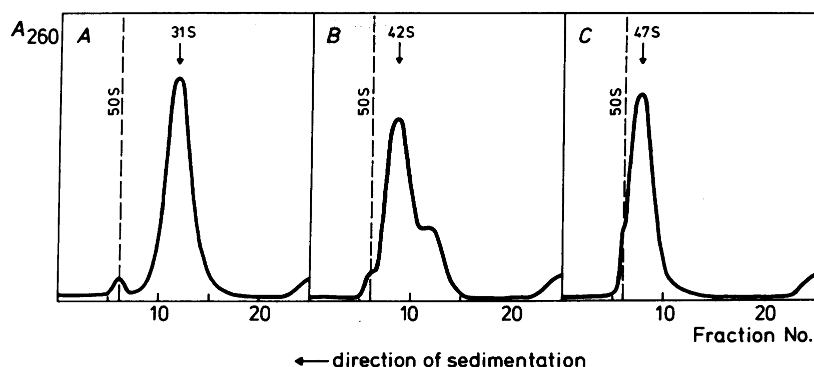



FIG. 1. Sucrose gradient profiles of particles assembled without 5S RNA. TP50 and 23S RNA were incubated together, and 40 μl containing 1 $A_{260}$ unit were applied to a 5–20% sucrose gradient (ionic conditions identical to those of the respective incubation mixture; SW 40 rotor, 5 hr at about 150,000 × *g*). Incubation conditions: (A) 0° and 4 mM $Mg^{++}$; (B) 20 min at 4 mM $Mg^{++}$ and 44°; (C) standard two-step incubation. Other components were those of the standard two-step procedure (see *Materials and Methods*). Each tube in addition contained 0.1 $A_{260}$ unit of 50S [$^3$H]subunits as a marker (see small $A_{260}$ peak at the 50S position in A and B). The calculation of the S values followed the method of McEwen (13).

with the 47S particle (two-step reconstituted without 5S RNA). This particle has an S-value similar to that of the $RI_{50}(2)$ particle (48 S) and can be converted to a highly active particle by a repetition of the second-step incubation in the presence of 5S RNA (Table 1, Exp. 6). The addition of 5S RNA alone to the 47S particle is not sufficient, as the second-step incubation was found to be indispensable (see controls in Table 1). This may indicate that the incorporation of 5S RNA at this stage is accompanied by a structural rearrangement. We conclude that 5S RNA is not required for the first three steps of assembly *in vitro* but plays an important role during the conversion of the $RI_{50}(2)$ particle to a 50S particle. In spite of this specific involvement of 5S RNA in the course of assembly, this RNA species can be integrated at each step. This suggests that the attachment site for 5S RNA on the reconstituted particle is easily accessible throughout the assembly process *in vitro*.

## Analysis of the 47S particle lacking 5S RNA

Aliquots of a large-scale preparation of 47S particles (600 $A_{260}$ units) were submitted to both structural and functional tests. The protein content was determined by two-dimensional electrophoresis (14). Four proteins (L5, L16, L18, and L25) were found in reduced amounts, whereas all the other proteins were present in normal amounts. In order to detect any residual 5S RNA, we examined the RNA by gel electrophoresis (Fig. 2). The RNA content of 16 $A_{260}$ units of native 50S subunits was compared with that of the same quantity of 47S particles. No significant absorbance due to 5S RNA was found in the 47S sample, whereas the native 50S subunits showed a distinct 5S RNA peak (Fig. 2B). A significant 5S RNA peak could be seen even with an input of 0.2 $A_{260}$ unit of 50S subunits (absorbance profile not shown). We conclude that the 47S particle used for the following tests contained less than 1% 5S RNA as compared to native 50S subunits. When incubated with 5S RNA alone this particle became highly active (Table 1, Exp. 6), indicating that the low activity of the 47S particle is due to the lack of 5S RNA and not to the reduced amounts of some proteins.

The totally reconstituted 50S particle forms 70S couples with the same efficiency as native 50S subunits (Fig. 3A and B). However, the formation of couples is greatly reduced under our experimental conditions when 47S particles are substituted for 50S subunits (Fig. 3C), and a heavy shoulder of about 62S is seen instead of a 70S peak.

Other functional tests are compiled in Table 2. The totally reconstituted 50S particle containing 5S RNA was highly active in all tests (75–120%, as compared to native 50S subunits) except in the chloramphenicol binding assay, where about 50% activity was found.

The general importance of 5S RNA in maintaining an active 50S conformation is indicated by the reduction of various ribosomal activities of the 47S particle. (*i*) As already described, the capacity to form 70S ribosomes with native 30S subunits is greatly reduced (Fig. 3), in contrast to a previous report (5). (*ii*) The 47S particle shows low but significant activity in protein-synthesizing systems using both natural (R17) and artificial [poly(U)] mRNA (Table 2, Exps. 1 and 2, respectively). Little activity was found in the puromycin reaction. This reaction was performed after initiation-factor-dependent binding of acPhe-tRNA to 70S ribosomes, or with C-A-C-C-A-acLeu using the large subunit only (Table 2, Exps. 6 and 7, respectively). In both cases a significant activity was found (8 and 14%, respectively). However, substrates known to bind to the peptidyltransferase (peptidyl-tRNA:aminoacyl-tRNA *N*-peptidyltransferase; EC 2.3.2.12) center, i.e., chloramphenicol (A-site region) and the fragment C-A-C-C-A-ac Leu (P-site region) did not bind to the 47S particle in significant amounts under the conditions used, whereas some binding of the fragment (15%) was detected in the presence of chloramphenicol. This drug markedly stimulates the binding of substrates to the P-site region of the peptidyltransferase center (ref. 21 and Table 2, Exp. 9). Thus, 5S RNA strongly influences the activity of the peptidyltransferase center.

Some detailed information on the role of 5S RNA with respect to ribosomal function can be obtained from the experiments presented in Table 2. Nonenzymatic binding of tRNA was tested by the RNase assay (16), in which [$^{14}$C]Phe-tRNA in the ternary complex 30S·poly(U)·[$^{14}$C]Phe-tRNA is protected against RNase attack in the presence of functional 50S subunits. In this test system the 47S particle showed some effect (17%; Table 2, Exp. 3). To differentiate between binding to the A-site (entry site of aminoacyl-tRNA) and the P-site (binding site of peptidyl-tRNA) tRNA was bound enzymatically to the A-site or to the P-site, respectively. No significant EF-T-dependent binding of tRNA to the A-site of the 47S particle was observed, whereas the initiation-factor-dependent binding to the P-site of the 47S particle was rather efficient (24%, compare Exps. 4 and 5 in Table 2). This finding implies a more direct involvement of 5S RNA in the binding of tRNA to the A-site, whereas the P-site binding, like other functions tested, is affected indirectly. Our experiments are in full agreement with the hypothesis (22) and with evidence (23, 24) indicating that the

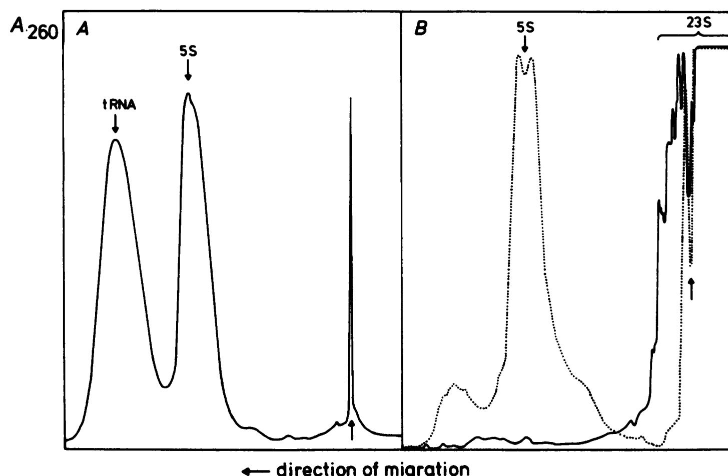



FIG. 2. $A_{260}$ profiles of RNA gels. The interface between the 10% (left) and 5% (right) acrylamide gel is indicated by an arrow. (A) Control gel with 0.5 $A_{260}$ unit of tRNA and 0.5 $A_{260}$ unit of 5S RNA. (B) Gels of 16 $A_{260}$ units of phenol-extracted RNA from native 50S subunits (----) and 47S particles lacking 5S RNA (—); the $A_{260}$ profiles of both gels are presented together.

T-Ψ-C-G loop of tRNAs interacts with 5S RNA. The T-Ψ-C-G sequence is found in all tRNAs except eukaryotic initiator tRNAs and tRNAs needed in cell wall synthesis (for review see ref. 2), suggesting that the proposed interaction is specific for tRNA bound to the A-site.

Surprisingly, the 47S particle was nearly as active as the native 50S subunit in the EF-G-dependent GTPase reaction (Table 2, Exp. 10), and the kinetics of this GTPase activity were similar both for the native 50S subunit and the 47S particle (Fig. 4). It follows that 5S RNA is neither directly nor indirectly involved

Table 2. Activities of reconstituted particles compared to native 50S subunits

| Exp. no. | System | Ribosomal function tested | Controls (cpm) | | | Rec. 50S(+5S) (% of native 50S) | Rec. 47S(−5S) (% of native 50S) | Ref. |
|---|---|---|---|---|---|---|---|---|
| | | | Background | | Native 50S | | | |
| 1 | R17 system | Natural mRNA-dependent protein systhesis | (30S) | 179 | 10150 | 79.5 | 23.4 | 15 |
| 2 | Poly(U) system | Poly(U)-dependent poly(Phe) synthesis | (30S) | 157 | 20890 | 97.9 | 20.5 | 12 |
| 3 | [$^{14}$C]Phe-tRNA binding (RNase assay) | Nonenzymatic binding to ribosome, association of subunits | (30S) | 256 | 4258 | 74.3 | 17.4 | 16 |
| 4 | EF-T-dependent [$^{14}$C]Phe-tRNA binding | tRNA binding to the A-site | (30S) | 2405 | 15530 | 91.2 | 4.3 | 17 |
| 5 | IF-dependent ac[$^{14}$C]Phe-tRNA binding | tRNA binding to the P-site | (30S) | 2522 | 8796 | 88.4 | 23.8 | 18 |
| 6 | PM reaction with IF-dependent bound ac[$^{14}$C]Phe-tRNA | P-site binding and peptidyltransferase activity | (30S) | 1310 | 26230 | 79.6 | 8.0 | 18 |
| 7 | PM reaction with C-A-C-C-A-ac[$^{3}$H]Leu | Peptidyltransferase activity | (—Particle) | 305 | 25571 | 83.1 | 10.0 | 19 |
| 8 | [$^{14}$C]CAM binding (equilibrium dialysis) | CAM binds to the peptidyltransferase center (A-site) | (—Particle) | 61 | 994 | 48.5 | 2.4 | 10 |
| 9 | C-A-C-C-A-ac[$^{3}$H]Leu binding (equilibrium dialysis) | Fragment binds to the peptidyltransferase center (P-site) | (—Particle) | 11 | 738 | 123 | 0 | This paper |
| | (+ CAM) | | | (24) | (1512) | (129) | (14.5) | This paper |
| 10 | EF-G-dependent GTPase | | (30S) | 1174 | 21983 | 102 | 82.1 | 20 |

The background values in each test were obtained with 30S subunits only (30S) or without ribosomal particles (−particles). The background was subtracted before the % activities of the reconstituted (Rec.) particles were calculated. All data are average values from two determinations. In Exp. 1 the RNA from phage R17 was used as natural mRNA. (+CAM) in Exp. 9 means that the C-A-C-C-A-ac[$^{3}$H]Leu-binding was measured in the presence of chloramphenicol (0.3 mM). The references for the various test systems are given in the last column. PM, puromycin; IF, crude initiation factors.

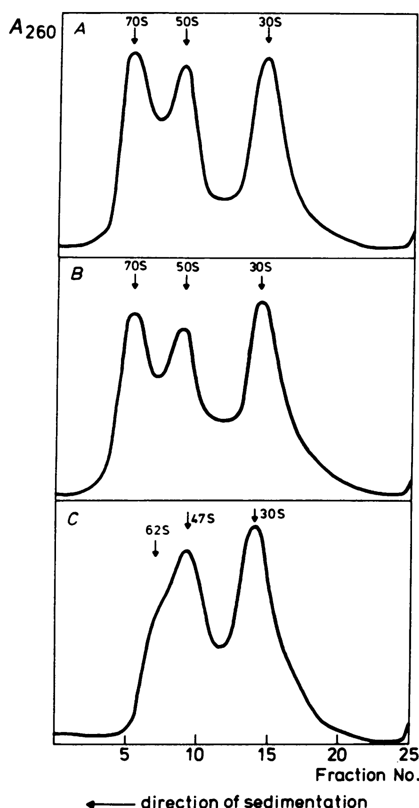



FIG. 3. Association of 30S subunits with 50S subunits or reconstituted particles. 1 $A_{260}$ unit of 30S subunits was mixed with 1 $A_{260}$ unit of 50S subunits (A) or reconstituted 50S subunits (B) or 47S (−5S) particles (C) in the presence of 10 mM Tris·HCl (pH 7.6), 10 mM Mg acetate, 60 mM $NH_4Cl$, and 6 mM 2-mercaptoethanol. After incubation at 37° for 10 min the mixture was applied to a sucrose gradient (10–30%) containing the same ionic concentrations. Centrifugation was performed in a Beckman SW 40 rotor for 21 hr at about 40,000 × *g*.

in this GTPase activity. A factor-independent GTPase activity of a 5S RNA-protein complex containing L5, L18, and L25 was found (25, 26). Furthermore, analysis of ribonucleoprotein fragments derived from 50S subunits after RNAse digestion revealed that protein L18 is involved in this factor-independent activity, whereas intact 5S RNA was not required (27). However, it is not clear whether this activity is related to the factor-dependent GTPase activity. EF-Tu seems to carry its own GTPase center (28), and evidence for a similar center on EF-G was recently reported (29). It remains open whether the center of the EF-G-dependent GTPase activity is located on ribosomal protein or on the factor or on both. However, the involvement of 5S RNA in this activity can be specifically excluded in view of the experiments reported here.

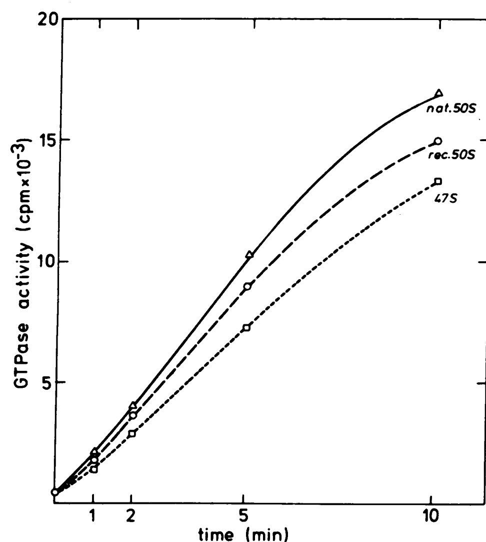



FIG. 4. Kinetics of EF-G-dependent GTPase activity using 30S subunits and native 50S subunits (nat. 50S), reconstituted 50S particles (rec. 50S) containing 5S RNA, or 47S particles (47S) lacking 5S RNA. The assay was performed as described (20).

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1. Monier, R. (1974) in *Ribosomes*, eds. Nomura, M., Tissières, A. & Lengyel, P. (Cold Spring Harbor Monograph Series, Cold Spring Harbor, N.Y.), pp. 141–168.
2. Erdmann, V. A. (1976) in *Progress in Nucleic Acid Research and Molecular Biology*, ed. Cohn, W., (Academic Press, New York), Vol. 18, in press.
3. Aubert, M., Monier, R., Reynier, M. & Scott, J. F. (1967) *Proceedings of the Fourth FEBS Meeting* **3**, 151–168.
4. Sarkar, N. & Comb, D. G. (1969) *J. Mol. Biol.* **39**, 31–44.
5. Siddiqui, M.A.Q. & Hosokawa, K. (1969) *Biochem. Biophys. Res. Commun.* **36**, 711–720.
6. Erdmann, V. A., Fahnestock, S., Higo, K. & Nomura, M. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 2932–2936.
7. Nierhaus, K. H. & Dohme, F. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4713–4717.
8. Schreiner, G. & Nierhaus, K. H. (1973) *J. Mol. Biol.* **81**, 71–82.
9. van Diggelen, O. P. & Bosch, L. (1973) *Eur. J. Biochem.* **39**, 499–510.
10. Nierhaus, D. & Nierhaus, K. H. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2224–2228.
11. Hayes, F. & Hayes, D. H. (1971) *Biochimie* **53**, 369–382.
12. Nierhaus, K. H., Bordasch, K. & Homann, H. E. (1973) *J. Mol. Biol.* **74**, 587–597.
13. McEwen, C. R. (1967) *Anal. Biochem.* **20**, 114–149.
14. Kaltschmidt, E. & Wittmann, H. G. (1970) *Anal. Biochem.* **36**, 401–412.
15. Funatsu, G., Nierhaus, K. H. & Wittmann-Liebold, B. (1972) *J. Mol. Biol.* **64**, 201–209.
16. Pestka, S. (1968) *J. Biol. Chem.* **243**, 4038–4044.
17. Ballesta, J. P. G. (1974) in *Methods in Enzymology* (Academic Press, New York and London), Vol. XXX, part F, pp. 232–235.
18. Zagorska, L., Dondon, J., Lelong, J. G., Gros, F. & Grunberg-Manago, M. (1971) *Biochimie* **53**, 63–70.
19. Nierhaus, K. H. & Montejo, V. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1931–1935.
20. Parmeggiani, A., Singer, C. & Gottschalk, E. M. (1971) in *Methods in Enzymology* (Academic Press, New York and London), Vol. XX, part C, pp. 291–302.
21. Celma, M. L., Monro, R. E. & Vazquez, D. (1970) *FEBS Lett.* **6**, 273–277.
22. Forget, B. G. & Weissman, S. M. (1967) *Science* **158**, 1695–1699.
23. Erdmann, V. A., Sprinzl, M. & Pongs, O. (1973) *Biochem. Biophys. Res. Commun.* **54**, 942–948.
24. Erdmann, V. A., Sprinzl, M., Richter, D. & Lorenz, S. (1974) *Acta Biol. Med. Ger.* **33**, 605–608.
25. Horne, J. R. & Erdmann, V. A. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2870–2873.
26. Gaunt-Klöpfer, M. & Erdmann, V. A. (1975) *Biochim. Biophys. Acta* **390**, 226–230.
27. Roth, H. E. & Nierhaus, K. H. (1975) *J. Mol. Biol.* **94**, 111–121.
28. Wolf, H., Chinali, G. & Parmeggiani, A. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4910–4914.
29. Marsh, R. C., Chinali, G. & Parmeggiani, A. (1975) *J. Biol. Chem.* **250**, 8344–8352.