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Total Reconstitution of Functionally Active 50S Ribosomal Subunits from *Escherichia coli**

[peptidyltransferase/poly(U) system/natural messenger/chloramphenicol binding/"subtotal" reconstitution]

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ABSTRACT **Total reconstitution of 50S subunits from *E. coli* was achieved by a two-step incubation procedure. In the first step, 23S RNA, 5S RNA, and the total proteins from 50S subunits were incubated for 20 min at 40° in the presence of 4 mM Mg^{++} and 400 mM NH_4Cl. In the second step, the Mg^{++} concentration was raised to 20 mM and the incubation was performed for 90 min at 50°. No requirement for 30S subunits or other components (e.g., polyamine) was found. The reconstituted particle has the same sedimentation coefficient as the native 50S subunit and is highly active in protein synthesis with natural (R17 RNA) and artificial [poly(U)] messengers as well as in tests for peptidyltransferase (fragment assay) and for binding of antibiotics (chloramphenicol).**

The total reconstitution of the 30S ribosomal subunit from *Escherichia coli* (1) has played an important role in elucidation of the structure, function, and assembly of this subunit (for review see ref. 2). A complicated technique for the total reconstitution of the 50S subunit of *E. coli* ribosomes has been described (3), but it could not be verified in other laboratories (2, 4). Therefore, up to now only a partial reconstitution method (5–7) has been available for 50S subunits, which was used successfully for the analysis of some problems, e.g., identification of proteins important for factor binding (8, 9) and of a protein involved in the peptidyltransferase activity (10) or in chloramphenicol binding (11). Nevertheless, it is obvious that this method has a limited application.

50S subunits from the thermophilic organism *Bacillus stearothermophilus* can be reconstituted from dissociated RNA and protein fractions (12). However, most of the biochemical and genetic data on prokaryotic ribosomes have been obtained with *E. coli* ribosomes, and therefore a total reconstitution of the *E. coli* 50S subunit would be much more helpful for detailed studies on the large subunit. In this paper we describe the total reconstitution of the *E. coli* 50S subunit and some functional tests for its biological activity.

MATERIALS AND METHODS

Cells from *E. coli* K12, strain A19, in early logarithmic growth phase were harvested and broken; 50S subunits were isolated as described (13). Activity and purity of the 50S subunits were tested in the poly(U) system, and the intactness of the 23S RNA was checked by RNA gel electrophoresis (see below). Core particles and split protein fractions were prepared as in ref. 10. All split protein fractions were extracted with acetic acid to remove traces of RNA.

Preparation of RNA and Proteins. The RNA was isolated by phenol extraction from 50S subunits containing intact 23S RNA. Redistilled phenol was shaken three times with 0.3 M K phosphate buffer (pH 7.5; the first time with 1 volume and twice with 0.3 volume). Then the phenol phase was shaken three times with 0.3 volume SSC-EDTA (150 mM NaCl, 15 mM Na citrate, and 10 mM EDTA, adjusted to pH 7.5 with NaOH) and stored at −20°. Before use 1/20 volume 1 M Tris-base (unbuffered) was added. To 50S subunits at a concentration of less than 400 A_{260} units/ml, 1/10 volume of 10% sodium dodecyl sulfate, 1/20 volume of 2% bentonite, and 1.2 volume of phenol–SSC–EDTA were added; the mixture was shaken for 8 min and centrifuged for 10 min at 16,000 × *g*. The aqueous phase was mixed with 1.2 volumes of phenol–SSC–EDTA, shaken, and centrifuged; the extraction was repeated a third time. The RNA was precipitated from the aqueous phase at −20° overnight by addition of 2 volumes of cooled (−20°) ethanol (made 20 mM in Tris·HCl, pH 7.8, before use). After centrifugation (45 min at 16,000 × *g*) the RNA was resuspended at a concentration of about 400 A_{260} units/ml in TM buffer [10 mM Tris·HCl (pH 7.6), 10 mM Mg actate, and 0.5 mM EDTA] for reconstitution, or in a buffer [20 mM Tris·HCl (pH 7.6), 50 mM KCl, 1% MeOH] suitable for separation of the 23S and 5S RNA by gel filtration on Sephadex G-100 (column dimensions 2 × 200 cm). Fractions containing 23S RNA or 5S RNA were collected; the RNA was precipitated with ethanol as described above and dissolved in TM buffer to concentrations of about 400 A_{260} units/ml (23S RNA), or 40 A_{260} units/ml (5S RNA).

The total proteins from the 50S subunit (TP 50) were extracted by acetic acid. The 50S suspension (made 0.1 M in magnesium) was stirred for 45 min at 0° in 66% acetic acid (14). After centrifugation at 30,000 × *g* for 30 min the protein supernatant was dialyzed against a 500-fold volume of 0.02% acetic acid for 3 hr, lyophilized, and suspended in glass-distilled water. After addition of 1/100 volume of 1 M Tris-base (unbuffered) the solution was dialyzed against a 500-fold volume of 10 mM Tris·HCl (pH 7.2) for 1 hr. The $A_{260}:A_{280}$ ratio of the protein solution was less than 1.

Abbreviations: SP, split proteins; TP 50, total proteins from 50S subunits; SSC–EDTA, 150 mM NaCl, 15 mM Na citrate, 10 mM EDTA (pH 7.5); TM buffer, 10 mM Tris·HCl (pH 7.6), 10 mM Mg acetate, 0.5 mM EDTA.

*This is paper no. 100 on "Ribosomal proteins." The preceding paper is by K. P. Wong and H. H. Paradies (1974) *FEBS Lett.*, in press.

TABLE 1. *Proteins of cores and RNA preparations*

Cores or RNA	Proteins detected in the cores or in the RNA preparations										
	L2	L3	L4	L13	L15	L17	L20	L21	L22	L23	L29
4.0c	−to±	+	+	+	−to±	+	−to+	+	+	(+)	(+)
5.0c	−	+	(+)	+	−	+	−to±	−	(+)	−	−
6.0c	−	(+)	−	+	−	(+)	−	−	−	−	−
23S Li	−	(+)	−	±	−	±	−	−	±	−	−
23S Ph	−	−	−	−	−	−	−	−	−	−	−

Proteins contained in cores and RNA preparations were determined by two-dimensional electrophoresis (17). 23S Ph is the RNA isolated by phenol extraction; 23S Li by LiCl–urea extraction. The amounts of proteins present in the cores were estimated from two-dimensional electrophoresis plates. +, normal amounts; (+), reduced amounts; ±, present in traces; −, not detectable.

Reconstitution Procedure. The one-step reconstitution followed the 50S partial reconstitution procedure (7) with modifications as described (10). For the two-step reconstitution, 20 μl of TM buffer containing 5 A_{260} units of 23S RNA and 0.02 A_{260} unit of 5S RNA was mixed with 70 μl of 10 mM Tris·HCl (pH 7.2) containing 7–10 equivalent units of protein (1 equivalent unit of protein is the amount of protein extracted from 1 A_{260} unit of 50S subunits). Ten microliters of a mixture containing 110 mM Tris·HCl (pH 7.2), 20 mM Mg acetate, 4.0 M NH_4Cl, and 20 mM 2-mercaptoethanol were added. The final concentrations of the reconstitution mixture were therefore 20 mM Tris·HCl (pH 7.2), 4 mM Mg acetate, 400 mM NH_4Cl, and 2 mM 2-mercaptoethanol. After incubation at 40° for 20 min, the Mg^{++} concentration was raised to 20 mM by addition of 4 μl of 400 mM Mg acetate, and the second incubation (90 min at 50°) followed. Fifty microliters of the reconstitution mixture were tested in the fragment assay for peptidyltransferase activity and 40 μl in the poly(U) system.

Other Methods. The fragment assay followed the procedure of ref. 10, the poly(U) system that of ref. 15, and the R17 system that of ref. 16. Two-dimensional electrophoresis of the proteins was performed according to ref. 17, and RNA gel electrophoresis according to ref. 18, except that the gels were run for 2 hr at 0.5 mA per gel, and then for 2 hr at 1 mA per gel. Tritium-labeled 50S subunits were a generous gift from Dr. R. Brimacombe, Max-Planck-Institut in Berlin-Dahlem.

RESULTS AND DISCUSSION

50S derived cores can be prepared by treatment with high salt concentration. They contain 23S RNA, 5S RNA, and many of the 50S proteins. They are inactive in peptide bond formation but regain their activity after incubation with the complementary split proteins (7, 10). Furthermore, incubation of 50S subunits with very high LiCl concentrations (e.g., 4 M LiCl) leads to cores containing 23S RNA and only a few proteins (13). Surprisingly, even this core, with a highly reduced protein content, could be reconstituted to a fully active particle under the standard conditions for partial reconstitution (see below). We call the reconstitution of these cores "subtotal" reconstitution, as they have lost most but not all of the 50S proteins. We studied the subtotal reconstitution, hoping that this would lead to the total reconstitution. However, the solution to the problem was not a successive addition of proteins, i.e., first the reconstitution of a core-like particle and then its complementation to a 50S subunit. Instead, the *total* reconstitution of the *E. coli* 50S subunit was achieved by a two-step reconstitution changing the ionic conditions and the incubation temperatures.

Subtotal Reconstitution (i.e., reconstitution starting with core particles). When 50S subunits are incubated with increasing LiCl concentrations, a stepwise dissociation of the proteins occurs (13). 5S RNA is not found in the residual core at LiCl concentrations of higher than 1.5–2 M (19, 20). The 4.0c core (50S subunits incubated with 4 M LiCl) contains only eight proteins (L3, L4, L13, L17, L21, L22, L23, and L29), with, in addition, variable amounts of L20 and/or traces of L2 and L15 (see Table 1). In the 5.0c core the number of proteins is reduced to five (L3, L4, L13, L17, and L22); and only three proteins (L3, L13, and L17) are present in the 6.0c core.

All the cores prepared at lithium concentrations up to 4.0 M can be reconstituted to a particle that is fully active in the fragment assay and in the poly(U) system (see for example the 4.0c + SP4.0 + 5 S reconstitution in Table 2, Exp. 1). Even the 5.0c core can be reconstituted to a particle with significant activity in both systems, but the 6.0c core cannot. The loss of activity depends on the cores themselves, as the corresponding split protein fractions SP5.0 and SP6.0 are fully active after reconstitution with the 4.0c core (Table 2, Exp. 1). At least one (perhaps more) of the proteins L4, L21, L22, L23, and L29, which are missing from the 6.0c core but present on the 4.0c core, may be important for the finding that the 6.0c core shows no activity after reconstitution. The reconstitution in this case was performed under conditions optimized for the partial reconstitution of the 50S subunit (7).

The subtotal reconstitution of the 4.0c core appears nearly independent of the addition of 5S RNA (Table 2, Exp. 2). The reason is the following: After incubation of the 50S subunits in the presence of 4 M LiCl, the cores were pelleted (5 hr at 176,000 × *g*). Under these high salt conditions most of the 5S RNA is pelleted with the 4.0c core. On the other hand, the RNA present in the split protein fraction was removed by the acetic acid method. Thus, the 4.0c core fraction was the source of the 5S RNA. The 5S RNA can be separated from the 4.0c cores by gel filtration (Sepharose 4B), and in this case the activity of the 4.0c core (4.0c Seph) after subtotal reconstitution is fully dependent on the addition of 5S RNA (Table 2, Exp. 2). It is clear that the incorporation of 5S RNA is not a critical step during reconstitution of the 50S subunit from *E. coli*.

Total Reconstitution (i.e., reconstitution starting with protein-free RNA). Proteins and RNA were prepared in two different ways: First, proteins and RNA were isolated by the LiCl–urea method, and second, RNA was extracted with phenol and the proteins (TP 50) with acetic acid. 23S RNA and 5S RNA were separated from the phenol-extracted RNA

TABLE 2. *Activities of one-step reconstitution particles*

Exp. no.	One-step reconstitution	Peptidyltransferase activity (%)	Poly(U)-dependent poly(Phe) synthesis (%)
1	4.0c + 5 S	0.1	0.1
	SP4.0 + 5 S	0	0
	4.0c + SP4.0 + 5 S	70	85
	5.0c + SP5.0 + 5 S	23	29
	6.0c + SP6.0 + 5 S	0	0.3
	4.0c + SP5.0 + 5 S	77	76
	4.0c + SP6.0 + 5 S	66	75
	Control	50 S 100 (6.262 cpm)	30 S + 50 S 100 (44.361 cpm)
2	4.0 + SP4.0 + 5 S	37	88
	4.0c + SP4.0	23	75
	4.0c Seph + SP4.0	2	1.7
	4.0c Seph + SP4.0 + 5 S	27	78
	Control	50 S 100 (4.321 cpm)	30 S + 50 S 100 (17.337 cpm)
3	23S Li + TP 50 Li + 5 S	1.4	29
	23S Li + TP 50 Li	1.7	28
	23S Li + TP 50 HOAc + 5 S	1.9	26
	23S Li + TP 50 HOAc	1.7	27
	23S Ph + TP 50 Li + 5 S	3.6	26
	23S Ph + TP 50 Li	3.1	17
	23S Ph + TP 50 HOAc + 5 S	5.2	32
	23S Ph + TP 50 HOAc	1.5	0.3
	Control	50 S 100 (3.323 cpm)	30 S + 50 S 100 (51.634 cpm)
4	23S Ph + 4.0c HOAc + SP4.0 + 5 S	1.6	19
	23S Ph + 4.0c HOAc + 5 S → + SP4.0	1.4	17
	Control	50 S 100 (4.610 cpm)	30 S + 50 S 100 (37.725 cpm)

In the poly(U) assay, 1 A_{260} unit of 30S subunits was added to 2 A_{260} units of reconstituted particles or 50S subunits. Background values [fragment assay: control without particles; poly(U) assay: 30S subunits only] were subtracted before the percentage values were calculated. 4.0c Seph is the 4.0c core from Exp. 1 passed through a Sepharose 4B column [2 × 50 cm; buffer: 20 mM Tris·HCl (pH 7.8), 200 mM NH_4Cl, 20 mM Mg acetate, and 2 mM 2-mercaptoethanol]. The loss of activity of the 4.0c + SP4.0 + 5S particle in Exp. 2 as compared to the equivalent particle in Exp. 1 may be explained by the fact that the 4.0c cores of Exp. 2 were frozen and thawed several times. Exp. 3: 23S Ph and 23S Li are RNA preparations isolated by the phenol and LiCl–urea method, respectively. Exp. 4: 4.0c HOAc are the acetic-acid-extracted proteins from the 4.0c core. 23S Ph + 4.0c HOAc + 5S → + SP4.0 means that 23S Ph + 4.0c HOAc + 5S were incubated for 30 min at 50°; after addition of the split proteins SP4.0 the incubation was continued for 60 min at 50° without a change in the ionic conditions.

by Sephadex G-100 gel filtration. During all steps of the RNA isolation the pH was maintained at 7.0–7.5.

All possible combinations of the RNA and protein fractions were first tested under conditions of partial reconstitution (one-step reconstitution, see Table 2, Exp. 3). All combinations showed significant activity in the poly(U) system (up to 30% of the 50S control) in the presence of 5S RNA. The activity was only dependent on added 5S RNA when phenolized 23S RNA was combined with acetic-acid-extracted proteins. This demonstrates that both the RNA and the protein fraction isolated by the LiCl–urea treatment contain 5S RNA, in agreement with a previous report (12). Furthermore, the LiCl-23S RNA contained protein L3 and traces of L13, L17, and L22, as was shown by two-dimensional gel electrophoresis. These proteins were always found, and some preparations of LiCl-23S RNA contained even more proteins. Therefore, reconstitution with this RNA preparation is—in a strict sense—not a total reconstitution. On the other hand, the phenolized RNA was protein-free, as judged by two-dimensional electrophoresis. In the following reconstitutions we preferentially used phenolized RNA and acetic-acid-extracted proteins, as this system is better defined than the combinations containing either or both LiCl–urea-treated components.

In spite of the poly(U) activity of the particles obtained after total reconstitution (one-step), no activity could be detected in the fragment assay (Table 2, Exp. 3). One possible explanation may be the following: The fragment assay requires addition of 33% alcohol. This condition induces an unfolding of the ribosome [e.g., the S-value of the 50S subunit is reduced to 38S (21)]. Gel electrophoresis of the RNA from the reconstituted particles revealed that the 23S RNA is degraded to large fragments, whereas naked 23S RNA, incubated under reconstitution conditions, shows much less degradation (Fig. 1). Thus, the poor activity in the fragment assay could be due to the effect of methanol on the particles containing degraded RNA. A detailed analysis (Dohme and Nierhaus, manuscript in preparation) revealed that a change in the concentrations of the compounds present in the reconstitution mixture could not improve the fragment assay activity.

Since the 4.0c could be easily reconstituted to a fully active particle, we tried a step-wise reconstitution: 23S → 4.0c →

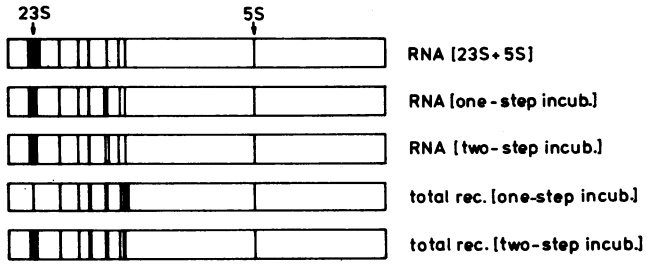

FIG. 1. RNA patterns of reconstituted particles, cores, and phenolized RNA obtained by gel electrophoresis. One-step and two-step incubation refers to the ionic conditions of the one-step and two-step reconstitution, respectively. For details see *Materials and Methods*.

50S under the conditions of the partial reconstitution (Table 2, Exp. 4). Again, the activity of the reconstituted particles was much lower in the fragment assay than in the poly(U) assay.

In the next series of experiments we tested the possibility that the first step in the assembly needs optimal ionic conditions that differ from those required in the later steps (which may be similar to the subtotal reconstitution). In the first set, the Mg^{++} dependence of the activity at different temperatures was measured. 23S RNA, 5S RNA, and total proteins were incubated together for 30 min; then the ionic conditions were adjusted to those of the partial reconstitution and incubation was continued for 90 min at 50° (Fig. 2). With this procedure we now found significant activity in the fragment assay. The interpolation curve of the maxima from the curves at different temperatures has a maximum at about 4 mM Mg^{++} at a temperature between 37° and 42°. Therefore, we chose for the first

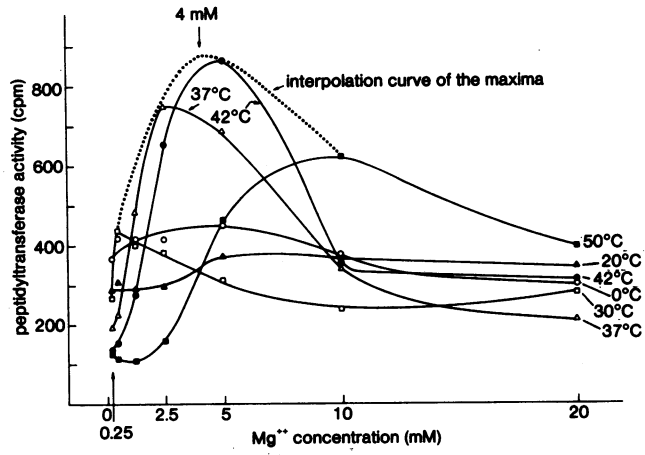

FIG. 2. Peptidyltransferase activities (fragment assay) after two-step reconstitutions. The first incubation was performed in the presence of various Mg^{++} concentrations (0.25–20 mM) at different temperatures for 30 min. After adjustment to 20 mM Mg^{++}, the second incubation followed for 60 min at 50°. The peptidyltransferase activity is plotted against the Mg^{++} concentration in the first incubation at various temperatures (solid curves). The maxima of the different curves were connected by an interpolation curve (dotted curve). The background of the fragment assay system in this experiment was 102 cpm (control without particles). Equivalent curves were obtained by measuring the poly(U)-dependent poly(Phe) synthesis of the reconstituted particles.

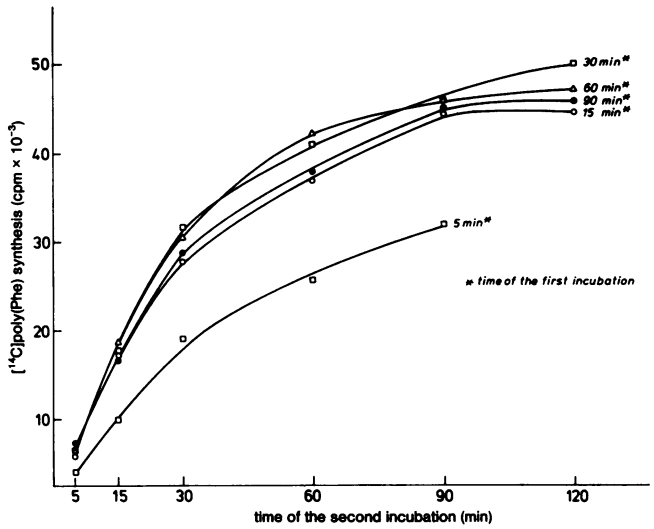

FIG. 3. Determination of suitable times for the first and second incubations. The activities in the poly(U) system after two-step reconstitutions were measured. Kinetics with respect to the second incubation were taken for various times of the first incubation. Equivalent curves were obtained by measuring the peptidyltransferase activity of the reconstituted particles.

step an incubation at 4 mM Mg^{++} and 40°. The optimization curve of NH_4^+ shows an optimum at about 400 mM, the same concentration as for the second step (data not shown). In a similar way the optima for the second incubation were tested and found to be identical to the conditions of the partial reconstitution [400 mM NH_4Cl, 20 mM Mg acetate (7); data not shown].

By use of various fixed times for the first incubation step, the kinetics of the second step were determined and the result is shown in Fig. 3. It is clear that for the first incubation step 15 min is sufficient, whereas a 90-min incubation is required for the second step. Therefore, we chose incubation times of 20 min at 40° for the first step and 90 min at 50° for the second. It has been suggested that polyamines may be important for assembly of the 50S subunit *in vitro* from *E. coli* (22). The

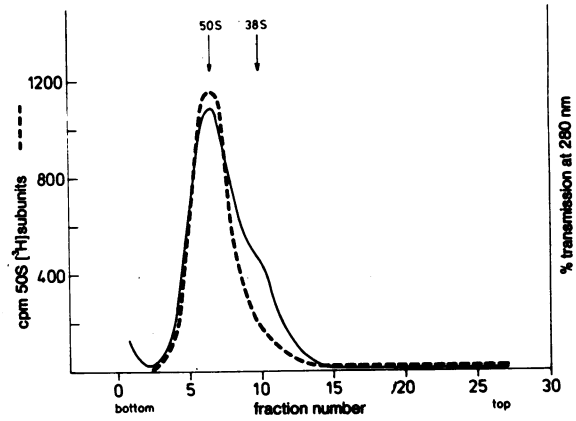

FIG. 4. A_{260} profile of 3 A_{260} units of two-step reconstituted particles (solid curve) in the presence of ^3H-labeled 50S subunits (0.1 A_{260} unit, dashed curve) after centrifugation in an SW 40 rotor (7 hr at 160,000 × *g*). The gradient [4–20% sucrose in 20 mM Tris·HCl (pH 7.3), 400 mM NH_4Cl, and 20 mM Mg acetate] was fractionated (18 drops per fraction). The S-value of the shoulder was calculated according to McEwen (23).

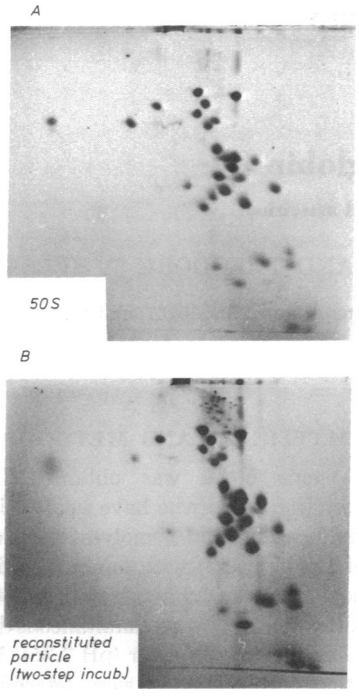

FIG. 5. Protein patterns of 50S subunits (A) and two-step reconstituted particles (B) after two-dimensional electrophoresis.

reconstitution of active 50S subunits described in this paper required neither polyamines nor the presence of 30S subunits.

Under optimal conditions a large-scale reconstitution was performed. The particles were pelleted and subjected to some structural and functional tests. The S-value of the reconstituted particles was compared to native 50S subunits (Fig. 4). The bulk of the reconstituted particles migrated with the 50S subunits; in addition, a shoulder sedimenting at about 38S was detected. Preliminary experiments suggest that the 38S material is an intermediate during the course of assembly. All 50S proteins were found in the particles (Fig. 5). Most of the 23S RNA remains intact during the two-step reconstitution in contrast to the one-step reconstitution (Fig. 1). This finding may account for the considerable increase of the fragment assay activity [compare fragment assay activity after one-step (Table 2, Exp. 3) and two-step incubation (Table 3)]. The reconstituted particles (two-step incubation) show 40–60% activity in the fragment assay, 80–110% in the poly(U) assay, and 50–80% in the R17 system as compared to native 50S subunits (Table 3). These values are higher than those obtained after total reconstitution of 50S subunits from *B. stearothermophilus* (12).

We found the following points to be important for a successful reconstitution: (*i*) Ribosomes should be derived from cells in early logarithmic growth phase. (*ii*) Proteins should be dissolved and stored before use in the presence of low concentrations of monovalent ions. (*iii*) RNA should be isolated from 50S subunits containing intact 23S RNA. (*iv*) Reconstitutions should be performed in two steps: the first one at 40° for 20 min in presence of 4 mM Mg^{++} and 400 mM NH_4^+ and the second at 50° for 90 min in presence of 20 mM Mg^{++} and 400 mM NH_4^+.

TABLE 3. *Activities of two-step reconstituted particles*

	Chloramphenicol binding (pmole/nmol of particle)	Peptidyltransferase (fragment assay, %)	Poly(U)-dependent [^{14}C]poly(Phe) synthesis (%)	R17 system (%)
23 S + 5 S	0	0	0	—
TP 50	26	0	0	—
23 S + 5 S + TP 50 (two-step reconstitution)	183	48	109	55
Native 50 S	456	100 (3.377 cpm)	100 (61.757 cpm)	100 (28.660 cpm)

Background values were subtracted before the percentage values of the fragment assay, of the poly(U) system, and of the R17 system were calculated. 23S and 5S are the 23S RNA and 5S RNA, respectively, and TP 50 the total proteins from the 50S subunit. For details of the two-step reconstitution see *Materials and Methods*.

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1. Traub, P. & Nomura, M. (1968) *Proc. Nat. Acad. Sci. USA* **59**, 777–784.
2. Nomura, M. (1973) *Science* **179**, 864–873.
3. Maruta, H., Tsuchiya, T. & Mizuno, D. (1971) *J. Mol. Biol.* **61**, 123–134.
4. Chu, F. K. & Maeba, P. Y. (1973) *Can. J. Biochem.* **51**, 129–139.
5. Staehelin, T. & Meselson, M. (1966) *J. Mol. Biol.* **16**, 245–249.
6. Hosokawa, K., Fujimura, R. K & Nomura, M. (1966) *Proc. Nat. Acad. Sci. USA* **55**, 198–204.
7. Staehelin, T., Maglott, D. & Monro, R. E. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34**, 39–48.
8. Kischa, K., Möller, W. & Stöffler, G. (1971) *Nature New Biol.* **233**, 62–63.
9. Hamel, E., Koka, M. & Nakamoto, T. (1972) *J. Biol. Chem.* **247**, 805–814.
10. Nierhaus, K. H. & Montejo, V. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1931–1935.
11. Nierhaus, D. & Nierhaus, K. H. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2224–2228.
12. Nomura, M. & Erdmann, V. A. (1970) *Nature* **228**, 744–748.
13. Homann, H. E. & Nierhaus, K. H. (1971) *Eur. J. Biochem.* **20**, 249–257.
14. Hardy, S. J. S., Kurland, C. G., Voynow, P. & Mora, G. (1969) *Biochemistry* **8**, 2897–2905.
15. Nierhaus, K. H., Bordasch, K. & Homann, H. E. (1973) *J. Mol. Biol.* **74**, 587–597.
16. Funatsu, G., Nierhaus, K. H. & Wittmann-Liebold, B. (1972) *J. Mol. Biol.* **64**, 201–209.
17. Kaltschmidt, E. & Wittmann, H. G. (1970) *Anal. Biochem.* **36**, 401–412.
18. Ceri, M. & Maeba, P. Y. (1973) *Biochim. Biophys. Acta* **312**, 337–348.
19. Marcot-Queiroz, J. & Monier, R. (1967) *Bull. Soc. Chim. Biol.* **49**, 477–494.
20. Yu, R. S. T. & Wittmann, H. G. (1973) *Biochim. Biophys. Acta* **319**, 388–400.
21. Voigt, J. & Parmeggiani, A. (1973) *Biochem. Biophys. Res. Commun.* **52**, 811–818.
22. Hosokawa, K., Kiho, Y. & Migata, L. K. (1973) *J. Biol. Chem.* **248**, 4135–4143.
23. McEwen, C. R. (1967) *Anal. Biochem.* **20**, 114–149.