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# A Protein Involved in the Peptidyltransferase Activity of *Escherichia coli* Ribosomes*

**(LiCl 50S core/ribosomal split proteins/reconstitution/peptide-bond formation)**

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**ABSTRACT** **Cores were prepared from 50S ribosomal subunits by incubation with 0.4 M LiCl/$Mg^{++}$ (0.4c cores); 0.8c cores and corresponding $SP_{0.4-0.8}$ split proteins were obtained from 0.4c cores. In the fragment reaction 0.4c cores were active, but 0.8c cores were not. Activity of the 0.8c cores could be restored by reconstitution with the $SP_{0.4-0.8}$ fraction. The split proteins were separated by DEAE-cellulose chromatography and Sephadex gel filtration. The peptidyltransferase activity is correlated with the amount of protein L11 added to the 0.8c core under reconstitution conditions. Whether protein L11 displays the enzymatic activity itself or is part of the enzymatic center is discussed.**

Synthesis of peptide bonds, the primary function of ribosomes, does not require the entire complicated structure of the ribosome. The complexity is, however, required to ensure precision of translation during peptide-bond formation. The catalytic center of peptide-bond formation, referred to as peptidyltransferase, is located on the larger ribosomal subunit (1, 2). Peptide-bond formation can be tested in the "fragment reaction" (3), which uses 3′-fragments of a peptidyl-tRNA analogue [e.g., CACCA-(Ac[$^3$H]Leu)], puromycin, 50S subunits, and $Mg^{++}$ and $K^+$ ions. The last three bases (3′-end) of the tRNA in the donor site and the last two bases of the tRNA in the acceptor site seem to be crucial for peptidyltransferase activity (4). No factors and no GTP are involved in the reaction. Despite the abnormal requirement for 33% ethanol, this reaction is a model for peptide-bond formation in protein biosynthesis (4).

Staehelin *et al.* (5) described the preparation of a series of 50S derived cores ($\alpha,\beta,\gamma$) by isopycnic centrifugation in CsCl–$Mg^{++}$ solutions. The $\beta$-core showed only 20% activity in the poly(U) assay but it was fully active in the fragment assay; the $\gamma$-core was not active in either system (5, 6); the split proteins, $SP_{\beta-\gamma}$, could restore the activity in the fragment assay of the $\gamma$-core. We have prepared equivalent cores by LiCl treatment (7), which is more convenient for obtaining large amounts of cores and split proteins. In this paper, we show that the peptidyltransferase activity depends upon the presence of protein L11 in the core.

## MATERIALS AND METHODS

*Preparation and Analysis of LiCl Cores and Split Proteins.* 50S subunits were prepared as described (7). After heat activation (37° for 25 min), about 10,000 $A_{260}$ units of 50S subunits were suspended in about 500 ml of TM-buffer [10 mM Tris·HCl (pH 7.4)–10 mM Mg acetate] containing 0.4 M LiCl and stored for 5 hr at 0°. The cores (0.4c) were pelleted by centrifugation at 143,000 × $g$ for 5 hr and suspended in TM-buffer. The supernatant contained the split proteins ($SP_{0.4}$). The 0.4c fraction was dissolved in TM-buffer containing 0.8 M LiCl (1 ml/20 $A_{260}$ units) and, after 5 hr at 0°, was centrifuged for 5 hr at 176,000 × $g$. The pellet (0.8c cores) was suspended in core-buffer (about 400 $A_{260}$ units/ml) and dialyzed against core-buffer [20 mM Tris·HCl (pH 7.8)–20 mM Mg acetate–EDTA (1 M Mg acetate–EDTA contains 1 M Mg acetate and 50 mM EDTA)–200 mM $NH_4Cl$–2 mM 2-mercaptoethanol]. After clearing by low-speed centrifugation, $A_{260}$ was measured. The cores were kept frozen at −80° until use. About 20–25% of the $A_{260}$ units of the 0.4c cores was lost in preparation of the 0.8c cores.

The supernatant containing the $SP_{0.4-0.8}$ split proteins was dialyzed against core-buffer and lyophilized. By definition, the amount of equivalent units of split proteins was equal to the amount of $A_{260}$ units of 0.8c cores. The dry protein–salt mixture was suspended in 5 ml of glass-distilled water. For removal of most of the RNA, one volume of 8 M urea in 4 M LiCl solution was added; after 24 hr at 0°, the RNA was pelleted by centrifugation at 10,000 × $g$ for 20 min. The supernatant was dialyzed against TM-buffer (pH 8.6) containing 6 M urea and applied to a DEAE–cellulose column (1.5 × 20 cm) that had been equilibrated with the same buffer. The column was washed with about 30 ml of this buffer. The eluate containing the basic protein fraction of $SP_{0.4-0.8}$ was dialyzed against protein-buffer (same as core-buffer except 400 mM $NH_4Cl$ was used), and lyophilized. The acidic protein fraction of $SP_{0.4-0.8}$ was obtained by elution with TM-buffer containing 6 M urea and 1 M $NH_4Cl$.

The lyophilized basic fraction of $SP_{0.4-0.8}$ was dissolved in 5 ml of water, dialyzed against core-buffer with 6 M urea, and filtered through a Sephadex G-100 column (2 × 170 cm; core-buffer with 6 M urea; two drops per min, 120 drops per fraction). The proteins in each fraction were analyzed in the first dimension (pH 8.6) of a two-dimensional electrophoresis (10) to distinguish between acidic and basic proteins. Each fraction was concentrated by lyophilization to 0.1 of its volume and dialyzed against protein-buffer. For reconstitution, 20–70 $\mu$l was added to 5 $A_{260}$ units of 0.8c cores under reconstitution conditions (see next section).

*Partial Reconstitution of 50S Subunits.* Partial reconstitution was done according to Staehelin *et al.* (5): 5 $A_{260}$ units of core particles in 25 $\mu$l of core-buffer was added to 6 equivalent units of split proteins in 75 $\mu$l of protein-buffer. After incuba-

Abbreviations: CACCA-(Ac[$^3$H]Leu), CACCA-([$^3$H]leucyl-acetyl-N); SP, split proteins.

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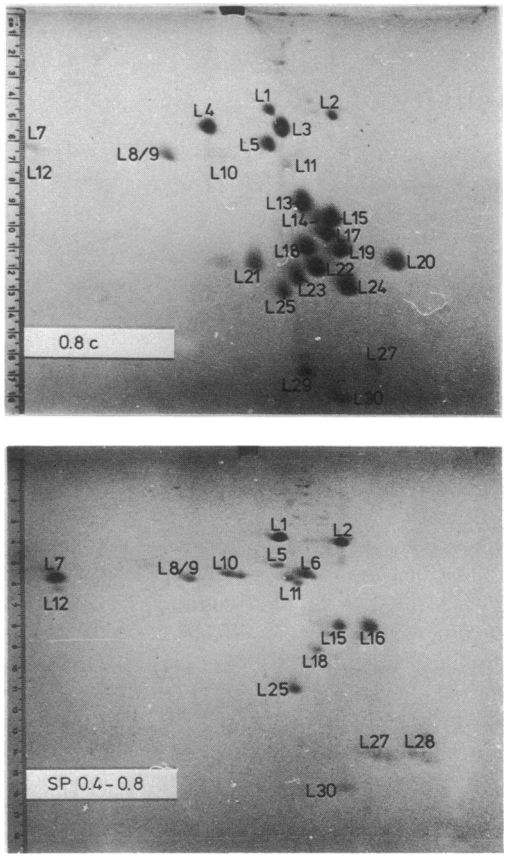


FIG. 1. Proteins in the 0.8c core and in the $SP_{0.4-0.8}$ split proteins demonstrated by two-dimensional electrophoresis.

tion (90 min at 50°), 50 $\mu$l of the reconstitution mixture was tested in the fragment assay for peptidyltransferase activity.

For large-scale reconstitution, 120 $A_{260}$ units of core particles in 600 $\mu$l of core-buffer was added to 144 equivalent units of split proteins in 1800 $\mu$l of protein-buffer. After incubation the reconstituted particles were pelleted by centrifugation at 106,000 $\times$ $g$ for 3 hr and suspended in 0.5 ml of core-buffer.

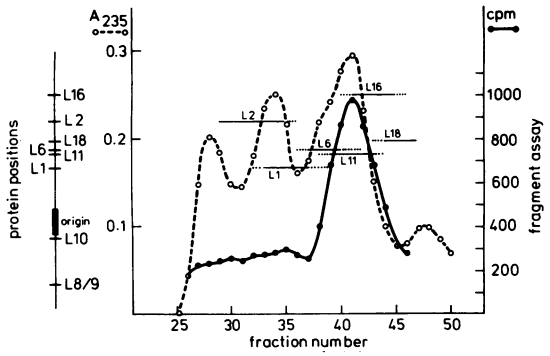


FIG. 2. Sephadex G-100 gel filtration. The elution profile (O- - -O) of the basic protein fraction of RNA-free $SP_{0.4-0.8}$ proteins is presented. The *lines* show the presence of proteins in the corresponding fractions as determined by the first dimension of the two-dimensional acrylamide gel electrophoresis. Acidic and basic proteins can be distinguished by this electrophoresis technique. L5 and L30 were not seen in the gels. L25, L27, and L28 gave weak bands in the gels from fractions 47–50. Each fraction was tested for peptidyltransferase activity (●——●).

2.5 $A_{260}$ units were used for peptidyltransferase assay. The proteins from 100 $A_{260}$ units were extracted by the acetic acid method and subjected to two-dimensional electrophoresis. For this purpose the particle suspension ($Mg^{++}$ concentration raised to 0.1 M) was stirred for 45 min at 0° in 66% acetic acid. After centrifugation at 30,000 $\times$ $g$ for 30 min, the protein supernatant was dialyzed against 1% acetic acid, lyophilized, and resolved in sample gel (10).

*tRNA Fragments Were Prepared* as described by Monro (11) with some modifications. The tRNA was charged with [$^3$H]leucine as described (12). The mixture was extracted with water-saturated phenol, and the [$^3$H]leucyl-tRNA was

TABLE 1. *Protein composition of different cores derived from the 50S subunit*

| Protein | β-core | γ-core | 0.4c core | 0.8c core | (0.8c + peak III) |
|---|---|---|---|---|---|
| L 1 | - | - | + | (+) | (+) |
| 2 | + | + | + | (+) | (+) |
| 3 | + | + | + | + | + |
| 4 | + | + | + | + | + |
| 5 | + | (+) | + | (+) | (+) |
| 6 | (±) | - | (+) | - | + |
| 7 | - | - | (+) | (±) | (±) |
| 8, 9 | + | (±) | + | (+) | + |
| 10 | + | - | + | (±) | (±) |
| 11 | + | (±) | + | (±) | + |
| 12 | - | - | (+) | (±) | (±) |
| 13 | + | + | + | + | + |
| 14 | + | + | (+) | + | + |
| 15 | + | - | + | + | + |
| 16 | (±) | - | (+) | - | + |
| 17 | + | + | + | + | + |
| 18 | + | (±) | + | + | + |
| 19 | + | + | + | + | + |
| 20 | + | (+) | + | + | + |
| 21 | + | + | + | + | + |
| 22 | + | + | + | + | + |
| 23 | + | + | + | + | + |
| 24 | + | + | + | + | + |
| 25 | - | - | (+) | (+) | (+) |
| 27 | + | (±) | + | (±) | (±) |
| 28 | + | (±) | (+) | - | - |
| 29 | + | + | + | + | + |
| 30 | + | (±) | + | + | + |
| 31 | (+) | - | - | - | - |
| 32 | + | + | + | + | + |
| 33 | - | - | - | - | - |
| 34 | n.d. | n.d. | n.d. | n.d. | n.d. |

The proteins of the different particles were separated by two-dimensional electrophoresis. In the fragment assay, the (0.8c + peak III) particle (see Table 2, Exp. 2) showed an activity of 43% compared to that of the 50S subunit. +, present in normal amount; +, present in reduced amount; ±, present in traces; −, not detectable; n.d., not determined.

precipitated with ethanol. T1 ribonuclease digestion of leucyl-tRNA was as described (11). For paper electrophoresis of the leucyl-tRNA fragments on Whatman 3 MM, the buffer system of Ghosh *et al.* (13) was used [pyridine–glacial acetic acid–$H_2O$ 1:10:189 (pH 3.5)]. After electrophoresis for 3 hr at 3000 V and 5°, the CACCA-Leu fragment migrated about 3–4 cm toward the cathode. The fragment was eluted, acetylated (14), and again subjected to paper electrophoresis under the same conditions. The CACCA-(AcLeu) fragment, which migrated 5–6 cm towards the anode, was eluted, lyophilized, and dissolved in water.

*The Fragment Was Assayed* according to Monro (11) with the following modifications: 50 $\mu$l of the reconstitution mixture was added to 50 $\mu$l of a mixture containing Tris·HCl (pH 7.8), KCl, $MgCl_2$, and about 5000 cpm of the CACCA-(Ac[$^3$H]Leu) fragment. The reaction was started by addition of 50 $\mu$l of cooled ethanol containing 4 mM puromycin. The final concentrations of buffer and salts in 150 $\mu$l were: 30 mM Tris·HCl (pH 7.8)–115 mM $NH_4Cl$–250 mM KCl–20 mM Mg acetate. After incubation for 10 min at 0°, the reaction was stopped by addition of 100 $\mu$l of 0.3 M Na acetate (pH 5.5) saturated with $MgSO_4$. 1.5 ml of ethylacetate was added; the mixture was agitated for 30 sec and centrifuged briefly at low speed. 1 ml of the upper layer was mixed with 0.5 ml of Soluene, and after addition of 4 ml of scintillation fluid, radioactivity was determined.

*Other Methods.* $\beta$- and $\gamma$-cores and the $SP_\beta$ and $SP_{\beta-\gamma}$ split proteins were prepared according to Staehelin *et al.* (5). RNA was extracted as described (9). For analysis, 0.5 $A_{260}$ units were subjected to electrophoresis on 3.1% polyacrylamide gels for 90 min [buffer: 90 mM Tris·HCl 90 mM boric acid (pH 8.3)–2.5 mM $Na_2EDTA$] at 2 mA per gel.

TABLE 2. *Activity of 50S subunit and derivates in the fragment assay*

| Experiment No. 1 | | Experiment No. 2 | |
|---|---|---|---|
| Particle | Peptidyltransferase (% activity) | Particle | Peptidyltransferase (% activity) |
| 50S | 100 (3565 cpm) | 50S | 100(2156 cpm) |
| 0.4c core | 67 | 0.8c | 6.5 |
| $\beta$-core | 64 | (0.8c + $SP_{0.4-0.8}$) | 51 |
| 0.8c core | 5 | (0.8c + ac–$SP_{0.4-0.8}$) | 55 |
| $\gamma$-core | 3 | (0.8c + DEAE–$SP_{0.4-0.8}$) | 48 |
| (0.8c + $SP_{0.4-0.8}$) | 75 | (0.8 + peak III) | 43(7) |
| ($\gamma$-core + $SP_{\beta-\gamma}$) | 78 | | |
| (0.8c + $SP_{\beta-\gamma}$) | 46 | | |
| ($\gamma$-core + $SP_{0.4-0.8}$) | 82 | | |
| (0.8c + $SP_{0.4}$) | 3 | | |
| (0.8c + $SP_{0.4}$ + $Sp_{0.4-0.8}$) | 87(92) | | |
| $SP_{0.4-0.8}$ | 1.3 | | |

When particles or split proteins were tested alone, they were subjected to reconstitution conditions before the test. ac–$SP_{0.4-0.8}$ are proteins extracted from $SP_{0.4-0.8}$ by the acetic acid method; DEAE–$SP_{0.4-0.8}$ is the basic protein fraction obtained after LiCl–urea treatment and DEAE–cellulose chromatography of $SP_{0.4-0.8}$; peak III contains the proteins of the third peak obtained on Sephadex G-100 gel filtration (see Fig.2). The values in parentheses represent the activity in the poly(U) translation system.

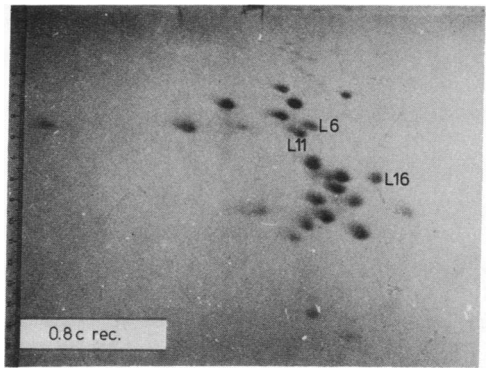


FIG. 3. Two-dimensional electrophoresis pattern of the proteins in the reconstituted (0.8c + peak III) particle active in the fragment reaction. Peak III means the third peak of Sephadex G-100 eluate. Three new proteins appear: L6, L11, and L16.

## RESULTS

*Characterization and Comparison of Cores.* Two methods were used to prepare cores and split proteins from 50S subunits: isopycnic centrifugation in CsCl–$Mg^{++}$ solutions [$\beta$ and $\gamma$-core (5)] and incubation with different LiCl concentrations [0.4c and 0.8c cores (7)]. The protein composition of the various cores is listed in Table 1. The $\beta$-core contains seven proteins less (either present in traces or not detectable) than the 50S subunit (L1, L6, L7, L12, L16, L25, and L33); nine or 10 additional proteins (L8/L9, L10, L11, L15, L18, L27, L28, L30, and L31) are removed from the $\beta$-core upon conversion to the $\gamma$-core. The 0.4c core has only two proteins (L31 and L33) less than the 50S subunit, and it largely loses eight additional proteins (L6, L7, L10, L11, L12, L16, L27, and L28) upon conversion to the 0.8c core. The 0.8c core contains intact 23S RNA and the same amount of 5S RNA as the 50S subunits (determined by RNA gel electrophoresis). The same RNA pattern was found for the $\gamma$-core (5).

Table 2, Exp. 1, demonstrates the functional equivalence of the 0.4c core to the $\beta$-core and the 0.8c core to the $\gamma$-core. Both 0.4c core and $\beta$-core have about 65% activity in the fragment assay compared to the 50S subunit; whereas the 0.8c core and

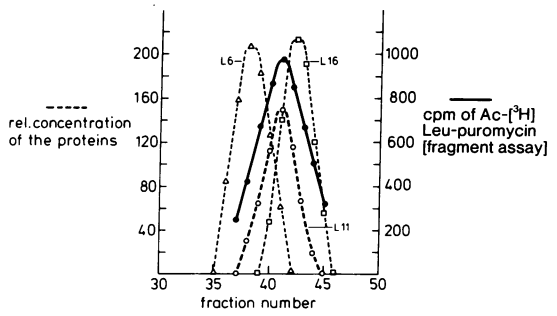


FIG. 4. The proteins of each Sephadex G-100 fraction (Fig. 2) were separated in the first dimension of the two-dimensional electrophoresis. The gels were scanned, and the relative concentration of specific protein in each fraction was calculated. – – –, concentration of L6, L11, and L16; ●——●, peptidyltransferase activity.

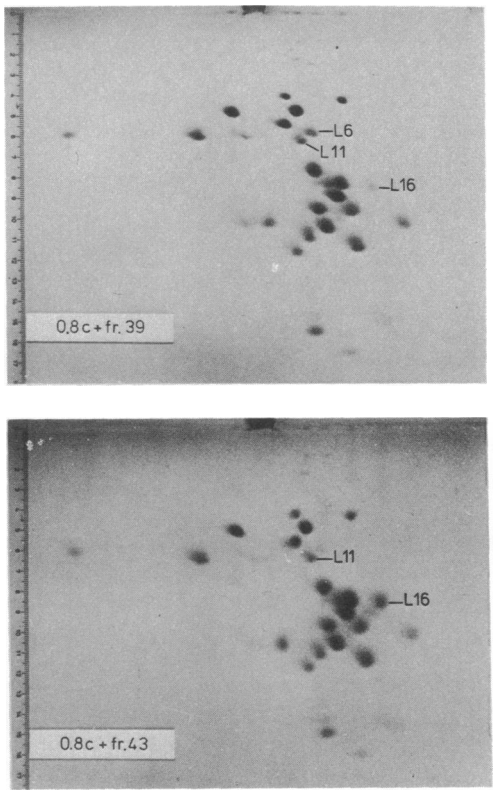


FIG. 5. Protein patterns of the reconstituted (0.8 + fraction 39) and (0.8c + fraction 43) particles that had about the same activity in the fragment reaction (compare Fig. 4).

the $\gamma$-core show very little activity (5 and 3%, respectively). The activity of the 0.8c core and the $\gamma$-core increased, after homologous and heterologous reconstitution with the split proteins, to the same extent, about 80%, except for the combination (0.8c + $SP_{\beta-\gamma}$), which had an activity of less than 50%.

*Active Proteins in the $SP_{0.4-0.8}$ Fraction.* All experiments were performed with the 0.8c core and the $SP_{0.4-0.8}$ split proteins shown in Fig. 1. 16 (or 17) Proteins are seen in the $SP_{0.4-0.8}$ preparations: L1, L2, L5, L6, L7, L8/L9, L10, L11, L12, L15, L16, L18, L25, L27, L28, and L30. In some preparations L15 was not found in the split proteins but was found in normal amounts in the corresponding 0.8c core. This was the case with the 0.8c core used in the experiments of this section. As 0.8c core has only background activity in the fragment assay and as it displays an intense L15 spot (see Fig. 1), L15 can be excluded from the proteins that play a role in restoring activity.

The yield of 0.8c cores is 20% lower than the input of 0.4c cores. The missing particles must dissociate rather completely for, as mentioned above, we found intact 5S and 23S RNA in the supernatant. The ratio $A_{260/280}$ of the supernatant was similar to that of RNA. In contrast to this result, Siddiqui and Hosokawa (16) found no release of 5S RNA from 50S subunits on incubation with 1 M LiCl for 30 min at 4°. The supernatant fraction has no activity by itself in the fragment assay (Table 2, Exp. 1). One may ask whether the RNA in the supernatant is important for reconstitution of peptidyltransferase activity. In order to answer this question we extracted the proteins in the $SP_{0.4-0.8}$ supernatant by the acetic acid method (the ratio $A_{260}/A_{280}$ = 0.9 of the extracted proteins was as expected for proteins) and tested their activity after reconstitution in the fragment assay. The results are shown in Table 2, Exp. 2. The $SP_{0.4-0.8}$ proteins extracted with acetic acid were as active as the $SP_{0.4-0.8}$ fraction containing RNA. Therefore, the RNA in this fraction is not relevant for the activity.

In large-scale experiments with $SP_{0.4-0.8}$, we precipitated most of the RNA and removed the rest by DEAE–cellulose chromatography. The buffer of the DEAE–cellulose column was adjusted to pH 8.6 because this is between the reported isoelectric points (17) of L8/L9 (pI = 6.4) and L11 (pI = 9.7) or L6 (pI = 10.0). The acidic proteins L7, L12, and L8/L9 were quantitatively bound to the column; even L10 was almost completely removed The DEAE eluate, containing the basic proteins, was as active as the initial sample whereas the acidic proteins showed no activity. Thus, it is clear that proteins L7, L8/L9, L10, and L12 do not restore activity of the 0.8c core.

The basic fraction, containing proteins L1, L2, L5, L6, L11, L15, L16, L18, L25, L27, L28, and L30, was subjected to Sephadex G-100 gel filtration (Fig. 2). Only the third peak, containing L6, L11, L15, and L16, restored peptidyltransferase activity after reconstitution (Table 2, Exp. 2). The protein pattern of the reconstituted particles revealed three new proteins (L6, L11, and L16) compared to the 0.8c core (Fig. 3). In contrast to the (0.8c core + $SP_{0.4}$ + $SP_{0.4-0.8}$) particle (Table 2), the reconstituted particle was not active in poly(phenylalanine) synthesis (7% compared to 50S).

For further study, a large-scale reconstitution was done with each G-100 fraction, and an aliquot (2.5 $A_{260}$ units) of the reconstituted particles was tested in the fragment assay (Fig. 2). The remainder was used for identification of the proteins present by two-dimensional electrophoresis. This procedure identified the split proteins that became attached to the 0.8c core particles under reconstitution conditions.

In another set of measurements, an aliquot of each G-100 fraction was analyzed in the first dimension of the two-dimensional electrophoresis. This procedure separates all the proteins present in peak III (Fig. 2). Each gel was scanned with a Joyce–Loebl scanner, and the areas of the various proteins were computed. Since we applied a constant amount of each fraction to the electrophoresis gels, the area was a measure of the relative concentration of each protein in a given fraction. A curve can thus be drawn of the relative concentration of each protein in a fraction. This was done for L6, L11, and L16 (Fig. 4). These results show that the activity curve fits only the L11-concentration curve. The above results are confirmed by the results of the two-dimensional electrophoresis. Comparison of the protein patterns of the particles reconstituted with fraction 39 and with fraction 43 (Fig. 4) should be conclusive. From Fig. 4, the (0.8c core + fraction 38) particle would be expected to contain L6 and L11 and traces of L16, whereas the proteins of the (0.8c core + fraction 43) particle would be expected to contain L16 and L11 but no L6.

As seen in Fig. 5, this was in fact the case. L11 displays a spot of about the same density in both cases, whereas L6 shows a clear spot for the (0.8c + fraction 39) particle, but is not seen in (0.8c + fraction 43). L16 is present in traces in (0.8c + fraction 39) and gives an intense spot in (0.8c + fraction 43). As both particles have the same activity in the fragment assay, about 65% of the activity of particle (0.8c + fraction 41), L11 must be the protein responsible for the ac-

tivity. The spot intensities of L6, L11, and L16 in the reconstituted particles (Fig. 5) are correlated with their concentrations in the corresponding fractions. This result indicates that the three proteins are attached independently and not cooperatively to the 0.8c core.

## DISCUSSION

In order to start with a defined population of 50S subunits, we heat-activated the 50S ribosomes before splitting. Heat activation seems to induce a more stable structure, for the 0.8c cores derived from 50S subunits that were not heat-activated (7) have six proteins (L1, L2, L8, L14, L15, and L20) less than the 0.8c cores described in this paper.

Peptidyltransferase activity is lost after the transition 0.4c core → 0.8c core or β-core → γ-core. If we assume that a single protein can restore the activity, this protein must be among those removed both from the 0.4c core *and* the β-core by LiCl and CsCl, respectively. These proteins are L10, L11, L27, and L28. We tested particles reconstituted with the individual proteins [isolated by carboxymethylcellulose-column chromatography (18)] in all possible combinations but did not find any appreciable activity. Reconstitution with each of the single proteins present in the $SP_{0.4-0.8}$ fraction gave activity with one L6 preparation, but we could not reproduce this result with other preparations of this protein. The most likely explanation of these results is that the isolation procedure leads to a loss of the capacity of these proteins to reconstitute a particle with peptidyltransferase activity. Since the total $SP_{0.4-0.8}$ proteins were able to reconstitute an active particle (Table 2, Exp. 1), we decided to separate these proteins by a different method, and our results showed that activity of the 0.8c core can be restored by protein L11 alone. Does this finding mean that protein L11 itself is the peptidyltransferase?

Binding of the aminoacyl- and peptidyl-residues and transfer of the peptidyl unit seem to be three distinct ribosomal functions occurring on nonidentical sites. This conclusion follows from studies by Celma *et al.* (19): In concentrations sufficient to give almost complete inhibition of the fragment reaction, celesticetin affects neither the UACCA-Leu binding to the a-site moiety of the active center nor the CACCA-(AcLeu) binding to the p-site moiety. On the other hand chloramphenicol, which completely blocks the UACCA-Leu binding, does not alter the CACCA-(AcLeu) binding. This makes it unlikely that L11 is the aminoacyl (or puromycin) binding site. Aminoacyl binding is blocked by chloramphenicol (15, 20) and puromycin inhibits chloramphenicol binding (20, 21). Moreover, it has been found by two different techniques (Nierhaus & Nierhaus, in preparation; Pongs, Bald & Erdmann, in preparation) that L16 is the chloramphenicol-binding, and therefore possibly the aminoacylbinding, protein. If L11 is the peptidyltransferase, L11 and L16 must be close neighbors on the 50S ribosome. Both proteins exist in one copy per 50S subunit (22), L16 binds directly to 23S RNA, and L11 is a "nonbinding" protein (23).

The other possibility, that L11 belongs to the binding site of the peptidyl residue, can be excluded by binding experiments with CACCA(AcLeu) (Nierhaus & Nierhaus, in preparation). The 0.8c core has about the same capacity for binding the CACCA-(AcLeu) fragment as does the 50S subunit. Because this fragment binds to the p-site moiety of the peptidyltransferase center (15) and because L11 is not on the 0.8c core, L11 cannot be a part of the binding site for the peptidyl residue.

Three alternatives remain: (*a*) the peptidyltransferase is composed of two or more proteins, and L11 is one of them. (*b*) The peptidyltransferase is still on the 0.8c core but L11 is required for active conformation. (*c*) L11 is the peptidyltransferase, which must be integrated onto the core for steric reason, e.g., binding of the substrates in the correct configuration. If $SP_{\beta-\gamma}$ contains, in addition to L11, another component that participates in the peptidyltransfer reaction to about the same extent as L11, we should expect significant activity with a (0.8c + all fractionated proteins minus L11) particle. This particle, however, is not active at all (unpublished observation). Therefore, we do not favor alternative (*a*). A conformational change—if there is any—must be small because the 0.8c core has the correct conformation to bind the CACCA-(AcLeu) fragment. Also the S-values of the 0.8c core and the active (0.8c + fraction 41) particle do not differ significantly. Therefore, we conclude that probably L11 itself has enzymatic activity when present in its native environment in the reconstituted 50S-derived cores.

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1. Monro, R. E. (1967) *J. Mol. Biol.* **26**, 147–151.
2. Maden, B. E. H., Traut, R. R. & Monro, R. E. (1968) *J. Mol. Biol.* **35**, 333–345.
3. Monro, R. E., Cerná, J. & Marcker, K. A. (1968) *Proc. Nat. Acad. Sci. USA* **61**, 1042–1049.
4. Monro, R. E., Staehelin, T., Celma, M. L. & Vazquez, D. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34**, 357–368.
5. Staehelin, T., Maglott, D. & Monro, R. E. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34**, 39–48.
6. Maglott, D. & Staehelin, T. (1971) *Methods Enzymol.* **XX**, 408–417.
7. Homann, H. E. & Nierhaus, K. H. (1971) *Eur. J. Biochem.* **20**, 249–257.
8. Gesteland, R. F. (1966) *J. Mol. Biol.* **16**, 67–84.
9. Nierhaus, K. H., Bordasch, K. & Homann, H. E. (1973) *J. Mol. Biol.* **74**, 587–597.
10. Kaltschmidt, E. & Wittmann, H. G. (1970) *Anal. Biochem.* **36**, 401–412.
11. Monro, R. E. (1971) *Methods Enzymol.* **XX**, 472–481.
12. Nishizuka, Y., Lipman, F. & Lucas-Lenard, J. (1968) *Methods Enzymol.* **XII**, 708–721.
13. Ghosh, H. P., Söll, D. & Khorana, H. G. (1967) *J. Mol. Biol.* **25**, 275–298.
14. Haenni, A. L. & Chapeville, F. (1966) *Biochim. Biophys. Acta* **114**, 135–148.
15. Celma, M. L., Monro, R. E. & Vazquez, D. (1970) *FEBS Lett.* **6**, 273–277.
16. Siddiqui, M. A. Q. & Hosokawa, K. (1968) *Biochem. Biophys. Res. Commun.* **32**, 1–8.
17. Kaltschmidt, E. (1971) *Anal. Biochem.* **43**, 25–31.
18. Hindennach, I., Kaltschmidt, E. & Wittmann, H. G. (1971) *Eur. J. Biochem.* **23**, 12–16.
19. Celma, M. L., Monro, R. E. & Vazquez, D. (1971) *FEBS Lett.* **13**, 247–251.
20. Pestka, S. (1969) *Biochem. Biophys. Res. Commun.* **36**, 589–595.
21. Fernandez-Muñoz, R., Monro, R. E., Torres-Pinedo, R. & Vazquez, D. (1971) *Eur. J. Biochem.* **23**, 185–193.
22. Weber, H. J. (1972) *Mol. Gen. Genet.* **119**, 233–248.
23. Stöffler, G., Daya, L., Rak, K. H. & Garrett, R. A. (1971) *Mol. Gen. Genet.* **114**, 125–133.