The incubation of the 50 S ribosomal subunit of *Escherichia coli* with 1.5 M LiCl yields 1.5c core particles inactive in the peptidyl-tRNA hydrolysis activity of *in vitro* termination. The omission of L16 alone from reconstitutions of the proteins into the core results in inactive ribosomes. The single omission of a number of other proteins, in particular L7/L12, L10, L25, L27, and L15, gives ribosomes with intermediate activity. L16 alone is unable to restore significant activity to 1.5c cores, but together L16 and the above "stimulating" proteins produce particles as active as those reconstituted with the full complement of proteins.

The ribosomal proteins important for the expression of peptidyl-tRNA hydrolysis and peptidyl transferase activities are very similar. However, ribosomes lacking both L11 and L16, but not L16 alone, surprisingly can catalyze codon- and release factor 2-dependent peptidyl-tRNA hydrolysis. The addition of L16 dramatically increases the activity. L16 is, therefore, important but not essential for the expression of the release factor 2-dependent peptidyl-tRNA hydrolysis.

The peptidyl-tRNA hydrolysis step of peptide chain termination is believed to be catalyzed by peptidyltransferase, the enzymatic activity of the ribosome functioning in peptide bond formation. These two steps of protein biosynthesis have similar requirements (1) and similar antibiotic sensitivities (2). The environment of the peptidyltransferase may simply be altered by the interaction of the release factor with the ribosome thereby allowing the access of water to the catalytic center (3). Two 50 S proteins of the large ribosomal subunit of *Escherichia coli*, L11 and L16, were implicated in the peptidyltRNA hydrolysis step of termination using antibodies against each of the ribosomal proteins (4). L11 (5), L16 (6), and L2 and L16 (7) have all seemed the likely peptidyltransferase enzyme. The modification of a histidyl residue of L16 inactivates peptidyltransferase (8, 9) although so does similar modifications to L2 and L4 (7). The position of L11 has since been clarified. Particles lacking L11 were active in peptidyl-tRNA hydrolysis (10), and in peptidyl-tRNA hydrolysis (12), and it is now clear the importance of L11 in the restoration of peptidyltransferase activity is for the efficient binding of L16 to the core particles (13). L11 is important in termination for the function of the two release factors, RF-1 and RF-2 (14). A group of six proteins (including L2, L3, L15, L16, L18, possibly L4, and the 23 S rRNA) essential for the reconstitution of peptidyltransferase activity to 4.0c cores or rRNA has now been defined (13).

In this paper we have used the reconstitution of proteins into ribosomal core particles to establish the importance of L16 and other proteins for release factor-dependent peptidyl-tRNA hydrolysis.

### MATERIALS AND METHODS

70 S ribosomes and their subunits were prepared from *E. coli* K12 strain D10 (RNase 1, Met-, rel A -) (15) as previously described (16). The preparation of the LiCl core particles and split proteins was as described (17) with the later modifications (6). The isolation, fractionation, and purification of the ribosomal proteins have been described (18-20). The protein of the ribosomal core particles and ribosomal protein fractions was analyzed by two-dimensional gel electrophoresis according to Kaltschmidt and Wittmann (21) as modified by Roth and Nierhaus (22). Release factors were isolated and purified as described (23), and for most experiments fractions VI and VII (Ref. 23), RF-1 or RF-2, respectively, were used.

**Reconstitution and Activity of 1.5c Cores**—A typical reconstitution contained (in 100 μl) 2.5 A260 1.5c cores and ribosomal proteins as indicated at a 1.25 molar excess over the core. The reactions were incubated for 90 min at 50 °C in a buffer of 20 mM Tris-HCl, pH 7.5, 400 mM NH₄Cl, 0.2 mM EDTA, 20 mM Mg acetate, and 4 mM β-mercaptoethanol.

**In Vitro Termination**—The reconstituted 50 S particles were used to form 70 S substrates for *in vitro* termination assays. A typical reaction was incubated at 30 °C for 30 min in 140 μl and contained 0.4-0.8 A260 reconstituted 50 S subunits, 0.32 A260 30 S subunits, 0.03 A260 AUG, 6 pmol of [3H]Met-tRNA (4000 cpm/pmol) in a buffer of 20 mM Tris-HCl, pH 7.5, 200 mM NH₄Cl, 30 mM Mg acetate, 0.1 mM EDTA, and 2 mM β-mercaptoethanol. Following formation of the 70 S AUG-[3H]Met-tRNA complex either RF-1 or RF-2 (2-4 μg) was added and ethanol to a final concentration of 10% (v/v) if peptidyltRNA hydrolysis were to be measured, or 0.06 A260 UAA or UAG if the codon-dependent *in vitro* termination activity was determined. The final volume was 200 μl and the buffer concentration 25 mM Tris-HCl, pH 7.5, 140 mM NH₄Cl, 30 mM Mg acetate. The incubation was for 30 min at 0 °C (peptidyl-tRNA hydrolysis) or 20 °C (codon-dependent termination). The reaction was stopped by the addition of 250 μl of 0.1 M HCl, and the [3H]Met hydrolyzed was determined by extraction at pH 1 into 1.5 ml of ethyl acetate. The mixture was vortexed vigorously for a few seconds and after phase separation by centrifuging at 2000 × g for 5 min, 1.2 ml of the upper phase was assessed for the formation of [3H]Met.

**Peptidyltransferase**—50 μl of the reconstitution mixture containing 1.5c A260 of reconstituted core particles was mixed with 50 μl of buffer containing 2 pmol of N-acetyl-[3H]Leu-tRNA (50,000 cpm/pmol) and 5 μl of a 100 μM AUG-[3H]Met-tRNA complex and incubated for 30 min at 30 °C. The reaction was stopped by the addition of 50 μl of 0.1 M HCl, and the [3H]Met hydrolyzed was determined by extraction at pH 1 into 1.5 ml of ethyl acetate.
with 50 μl of ethanol containing 1 mg/ml of puromycin. The final concentrations were 50 mM Tris-HCl, pH 7.8, 135 mM NH₄Cl, 250 mM KCl, 14 mM Mg acetate, and 0.2 mM puromycin in 33% ethanol. After incubation for 3 h at 0 °C the reaction was stopped by the addition of 100 μl of 0.3 M Na acetate, pH 5.5, saturated with MgSO₄. Ethyl acetate (2.5 ml) was added, the mixture vortexed vigorously for 1 min, and after phase separation the upper 2 ml assessed for the formation of N-acetyl-[³H]Leu-puromycin.

RESULTS

Extraction of the 50 S ribosome subunit with 1.5 M LiCl yields a population of core particles (1.5c core) in which some of the proteins are completely removed while others are reduced significantly. One of the former group, L16, has been specifically implicated previously in the peptidyl-tRNA hydrolysis step of in vitro termination (3) but significant activity could not be restored to 1.5c cores by L16 alone, either in stoichiometric amounts or in excess. The 1.5c cores were reconstituted, therefore, with some or all of the missing proteins, and those reconstructed 50 S subunits were used to determine which proteins were important in peptidyl-tRNA hydrolysis of in vitro termination.

Most preparations of 1.5c cores lacked the proteins, L6, L10, L11, L7/L12, L16, L25, and L27 (the group 1 proteins) when compared with a typical electrophoretic map of the 50 S proteins. In general, most cores showed a significant but incomplete reduction in the amounts of L1, L2, L5, L9, L15, L18, and L30 (the group 2 proteins). Each of these proteins have been purified extensively, and the purity is greater than 95% when analyzed by two-dimensional gel electrophoresis (21). The protein complements of a typical preparation of 1.5c cores and artificial mixtures of group 1 and group 2 derived from fractionated individual components (see Fig. 2) are shown in Fig. 1, A, C, and D. For comparison, the gel electrophoretic pattern of the proteins of the 50 S subunit is shown in Fig. 1B. The 1.5c core lacked completely L7/L12, L6, L10, L11, and L16 although this particular preparation showed traces of L25 and L27 (group 1 proteins, positions shown by small arrows) while L1, L2, L5, L9, L15 and L30 were significantly reduced (group 2 proteins, large arrows). The artificial mixtures of the group 1 (Fig. 1C) and group 2 (Fig. 1D) proteins show the expected complements apart from minor proteins in the case of group 1 and L6 and L10 in group 2. These minor contaminants should not affect the qualitative conclusions drawn from the reconstitution studies described below since the proteins were added to the 1.5c cores at a slight molar excess only (1.25:1).

The homogeneity of purified L11 and L16 is shown in Fig. 1, E and F, respectively. There is a high molecular weight spot within the fractionated L11 preparation which does not correspond to any of the known ribosomal proteins but it is believed to be a dimer of L11. Dialysis in urea of a similar preparation of L11 led to a significant decrease in the ratio between the component and L11 and was accompanied by an improved ability to act as an “L11” helper protein in peptidyltransferase (18).

Initially 1.5c cores were reconstituted with all but one of the missing proteins to assess the importance of that particular protein for the in vitro termination event. Such reconstituted 50 S subunits were then used in 70 S ribosomal substrates, with [³H]Met-tRNA bound at the P site as the model peptidyl-tRNA for the codon-independent hydrolysis step of in vitro termination. As shown in Fig. 2 the 1.5c cores are inactive in the partial reaction (bar A) but their activity can be restored by the unfragmented split proteins (bar B) or an artificial mixture of the fractionated group 1 and 2 proteins (bar E). Omission of the group 1 proteins in the reconstitution prevents the restoration of hydrolysis activity indicating the importance of these proteins (bar D) while omission of the group 2 proteins gives cores partially active in the event (bar C). The omission of a single protein not surprisingly results in a wide variation in the activity of the substrates for the in vitro termination partial reaction ranging from 10–100% of the control (bars F–S). Protein L16 is a critically important protein; it is the only protein whose omission gives almost inactive ribosomes (bar J). Of the other group 1 proteins, L7/L12, L10, L25, and L27 and the group 2 protein L15 contribute significantly to the activity of reconstituted cores; ribosomes lacking each of these proteins singly show intermediate activity only. The other proteins have little apparent effect on the reaction. Ribosomal cores reconstituted with artificial mixtures of the group 1 and 2 proteins in this study can form 70 S substrate with [³H]Met-tRNA as measured by binding to Millipore filters although ribosomes lacking L16 bind [³H]
Fig. 2. Single omission of a 50 S protein during reconstitution of 1.5c cores with mixtures of highly purified split proteins. Reconstitution of the 1.5c cores with artificial mixtures of purified ribosomal proteins was carried out in duplicate as described under "Materials and Methods" except that a single protein or a group of proteins were omitted as indicated. The formation of substrates for assay of the peptidyl-tRNA hydrolysis partial reaction (in the absence of codons) and the assay was as indicated. Individual backgrounds for each omission were determined from the cpm extracted in the absence of release factor and in most cases the background was about 4400 cpm.

### Table I

<table>
<thead>
<tr>
<th>Protein(s) omitted</th>
<th>Peptidyltransferase</th>
<th>Peptidyl-tRNA hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (+ Group 1 and 2)</td>
<td>100(18,200 cpm)</td>
<td>100(14,000 cpm)</td>
</tr>
<tr>
<td>None (+ SP1.5)</td>
<td>142</td>
<td>134</td>
</tr>
<tr>
<td>Group 1 and 2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 1</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td>Group 2</td>
<td>31</td>
<td>60</td>
</tr>
<tr>
<td>L6</td>
<td>69</td>
<td>82</td>
</tr>
<tr>
<td>L10</td>
<td>76</td>
<td>62</td>
</tr>
<tr>
<td>L1</td>
<td>71</td>
<td>88</td>
</tr>
<tr>
<td>L7/L12</td>
<td>87</td>
<td>56</td>
</tr>
<tr>
<td>L16</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>L25</td>
<td>55</td>
<td>61</td>
</tr>
<tr>
<td>L27</td>
<td>75</td>
<td>67</td>
</tr>
<tr>
<td>L1</td>
<td>101</td>
<td>91</td>
</tr>
<tr>
<td>L2</td>
<td>51</td>
<td>86</td>
</tr>
<tr>
<td>L5</td>
<td>96</td>
<td>107</td>
</tr>
<tr>
<td>L9</td>
<td>91</td>
<td>127</td>
</tr>
<tr>
<td>L15</td>
<td>50</td>
<td>56</td>
</tr>
<tr>
<td>L18</td>
<td>97</td>
<td>107</td>
</tr>
<tr>
<td>L30</td>
<td>90</td>
<td>100</td>
</tr>
</tbody>
</table>

Met-tRNA with somewhat lower efficiency than reconstituted cores containing L16. A comparison of the effects of omission of single proteins from reconstituted cores on peptidyltransferase and codon-independent peptidyl-tRNA hydrolysis is shown in Table I. Activity is restored to the inactive cores on reconstitution with the artificial mixtures of the group 1 and 2 proteins in each case. For many reconstituted cores the relative activities of the ribosomes are similar when compared with the controls for the two functions. For example, the absence of L16 results in almost total loss and that of L15 in partial loss of activity. There are exceptions, however. The loss of L7/L12 affects peptidyl-tRNA hydrolysis and poly(U) translation (data not shown) but not peptidyltransferase. The first two functions are mediated by protein synthesis factors, the elongation factors, and release factors, respectively, and a requirement for the proteins L7/L12 in these factor-mediated events is well established (3, 24, 25).

While L16 was critical for codon-independent peptidyl-tRNA hydrolysis activity clearly other proteins were also important, and it was not surprising that attempts to recover the in vitro termination activity of 1.5c cores with L16 alone even at several-fold excess proved relatively unsuccessful. Protein L16 was added, therefore, with these other proteins at a 1.25 molar excess. The results are illustrated in Table II. Very little activity is restored unless L16 is added together with the other important proteins of group 1. This results in ribosomes with significant activity which is further enhanced when L1, L2, and L15 of the group 2 proteins are included in the reconstitution. Therefore, a number of 50 S subunit proteins whose absence had reduced the activity of reconstituted cores (Fig. 2) were unable to restore peptidyl-tRNA hydrolysis function to the 1.5c cores by themselves but they were able to act as proteins supporting L16 in the restoration of this function. We term these proteins "stimulating proteins" (L1, L2, L6, L7/L12, L10, L11, L15, L25, and L27).

During reconstitution the proteins of the group 1 and 2 series are at a slight molar excess over cores (1.25:1) because this ratio gives optimum activity when the unfractionated SP1.5 proteins are used. The ratio may be lower if proteins have been partially inactivated during fractionation and purification. For this reason substrates prepared from the 50 S cores were reconstituted with L16 at various molar ratios along with the stimulating proteins. As shown in Fig. 3A the peptidyl-tRNA hydrolysis activity of the cores in 70 S substrate is restored on adding back L16 saturating at a molar ratio of about 2.5. Significant activity is recovered, however, when L16 is present at near molar equivalence with the cores. When all of the stimulating proteins are included (open circles) the activity of the cores is about 70% of that obtained with a mixture of all proteins present, and if only the most important of the stimulating proteins (L7/L12, L10, L11, L6, L15) are included the activity is somewhat lower saturating.
at just over 50% of the control (closed circles). Proteins L6, L10, L11 from group 1 were added since Hlamp et al. (13) have shown that at least two of these three are required for the binding of L16 to the ribosome core (“helper proteins” for the L16 binding). Neither L6 nor L11 had significant effect on the codon-independent termination reaction when omitted singly (Fig. 2).

In Fig. 3B the activity of the reconstituted cores in the codon-dependent termination reaction is shown. This reaction is also restored by addition of L16 and the stimulating proteins, but to a lesser degree (35–50% of control). The inclusion of the additional stimulating proteins together with the most important group similarly affects both the codon-mediated event and the hydrolysis event as shown in Fig. 3A (open circles).

While investigating the importance of L16 in the termination event we discovered a very significant differential effect of L11 on the codon-dependent function of the two release factors, RF-1 and RF-2, and this is reported in detail in the accompanying paper. This finding, however, led to the observation that ribosome cores lacking both L11 and L16 are active in the codon-dependent RF-2-mediated termination reaction. These observations are summarized in Table III. Addition of L16 and of the stimulating proteins in the reconstitution of 1.5c cores led to particles as active as those reconstituted with unfractionated SP1.5 in RF-2-mediated codon-dependent termination. Peptidyltransferase activity of the reconstituted cores was 80% of those where SP1.5 had been added. When L16 was omitted there was, as expected, virtually no termination activity (1%) or peptidyltransferase (12%). Surprisingly, when both L11 and L16 are omitted the termination activity is very significant, namely 54% of that of the control, while the peptidyltransferase activity remains low (10%). When L16 is present but L11 is omitted the activity of the reconstituted cores is now 2.6-fold higher than that of the control where all proteins are present. This results because the efficiency of the release factor 2 is several-fold better on ribosomes lacking L11 in the codon-dependent reaction (14). No such stimulation of peptidyl-tRNA hydrolysis, the codon-independent partial reaction of in vitro termination, was seen when L11 was omitted from the reconstitution mixture as shown in Fig. 2.

To investigate these paradoxical results further, the activity of the reconstituted cores in codon-dependent termination was compared with their ability to form potential substrate in each case. The 50 S subunits, reconstituted with stimulating proteins alone or with L11 and/or L16 together with stimulating proteins, are able to form 70 S complexes containing f^3HMet-tRNA. The amount of f^3HMet-tRNA bound into the complex was assessed on Millipore filters. The UAA-dependent hydrolysis of the f^3HMet-tRNA mediated by release factor 2 was measured for each complex. The conditions for hydrolysis were set at 20°C for 30 min using 1 μg of RF-2. For f^3HMet-tRNA binding 1.5c cores alone (with 30 S subunit) bound 2,260 cpm.

### Table III

**Hydrolysis of peptidyl-tRNA dependent upon codon and RF-2 on ribosomes lacking L11 and/or L16**

<table>
<thead>
<tr>
<th>Peptidyl-tRNA hydrolysis N-acetyl-[3H]Leu-puromycin</th>
<th>% of bound, released as f^3HMet</th>
<th>% of bound, released as f^3HMet-tRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>f^3HMet released</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L16, L11 stimulators</td>
<td>36</td>
<td>4</td>
</tr>
<tr>
<td>L11 stimulators</td>
<td>34</td>
<td>7</td>
</tr>
<tr>
<td>L16 stimulators</td>
<td>50</td>
<td>10</td>
</tr>
</tbody>
</table>

### Table IV

**A comparison of the formation of 70 S substrates for in vitro termination and their hydrolysis using reconstituted 50 S subunits lacking L11 and/or L16**

<table>
<thead>
<tr>
<th>Source of 50 S</th>
<th>Protein</th>
<th>Bound f^3HMet-tRNA</th>
<th>% of added f^3HMet</th>
<th>% of bound f^3HMet-tRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>L11, L16, stimulators</td>
<td>16,000</td>
<td>94</td>
<td>64</td>
</tr>
<tr>
<td>1.5c cores</td>
<td>L11, L16, stimulators</td>
<td>10,500</td>
<td>62</td>
<td>44</td>
</tr>
<tr>
<td>1.5c cores</td>
<td>L11, L16, stimulators</td>
<td>9,000</td>
<td>56</td>
<td>114</td>
</tr>
<tr>
<td>1.5c cores</td>
<td>L11, L16, stimulators</td>
<td>6,500</td>
<td>38</td>
<td>1</td>
</tr>
<tr>
<td>1.5c cores</td>
<td>L11, L16, stimulators</td>
<td>6,470</td>
<td>38</td>
<td>38</td>
</tr>
</tbody>
</table>

Downloaded from http://www.jbc.org/ by guest on September 23, 2017
of L16 but not L11. This is in complete contrast to the activity of those substrates in the in vitro termination assay. Under the conditions chosen almost half of the bound fMet-tRNA was hydrolyzed from substrates containing L11 and L16 (44%), somewhat less (38%) from substrates lacking both these proteins, none from substrates containing L11 lacking L16, and more than that potentially available from substrates lacking only L11 (114%). This latter result may reflect either some recycling of unbound fMet-tRNA on the active ribosomes or an underestimation by Millipore analysis of active substrates for in vitro termination.

A small subpopulation of particles still containing L16, on which new substrate could be reformed during the reaction, could explain the observation that ribosomes apparently lacking L11 and L16 can hydrolyze peptidyl-tRNA. As shown in Fig. 4 the kinetics of the release of fMet from substrates formed with ribosomes lacking only L11 (closed circles) or both L11 and L16 (open circles) were compared. The rate of release from the ribosomes without L11 and L16 was lower and seemed to reflect a homogeneous population catalyzing the reaction. Nonradioactive fMet-tRNA was added at a 4-fold excess during the reaction to assess whether recycling of substrate was occurring. In both ribosomal substrates (without L11 or without both L11 and L16) there was a similar decrease in the rate of release. No cessation in the rate of release from the substrate lacking both L11 and L16 occurred to indicate extensive recycling of fMet-tRNA on a small population of active ribosomes. It should also be noted that the cores contain no trace of L16 even when their protein complements are analyzed by heavily overlapping two-dimensional gels (Fig. 1A) although up to 5% contamination may not have been detected.

As an alternative approach active subpopulations of substrate have been artificially constructed where 5, 10, and 25% of the ribosomes were active. Such populations were detected in approximately the expected amounts, suggesting no significant recycling of the active ribosomes. The release of the f[3H]fMet reached a plateau when the expected proportion had been released in contrast to the result shown in Fig. 4 where a relatively low but continuous rate of hydrolysis occurred with the substrate lacking both L11 and L16. Moreover, when a small proportion of active ribosomes (rather than active substrate) were added to inactive substrate a modest equilibration of f[3H]fMet-tRNA to the active ribosomes was observed, consistent with that seen with both substrates in Fig. 4 when excess nonradioactive fMet-tRNA was added.

**Fig. 4. Evidence that recycling of a small subpopulation of ribosomes is not responsible for the activity of reconstituted ribosomes lacking L16.** 1.5c cores were reconstituted with mixtures of purified proteins omitting only L11 (■—■) or L11 and L16 (○—○) as described. Such 50 S cores were formed into 70 S complexes and the UAA-dependent release of fMet mediated by RF-2 measured with time. Nonradioactive fMet-tRNA was added at a 4-fold excess during the time course as indicated by the arrows on the diagram (A—A and A—A, respectively). Background values of 525 and 440 cpm at zero time were subtracted in each case.

**DISCUSSION**

The assembly of the ribosomal protein L16 into reconstituted 50 S subunits is required for the recovery of the codon-independent peptidyl-tRNA hydrolysis step of the termination of protein synthesis. From the protein fraction split off the 50 S subunit by 1.5 M LiCl it is the only protein which, when omitted by itself, results in particles inactive in this function. A major effect of L16 may be on the conformation of the peptidyltransferase center since it induces a conformational change with a high activation energy (123 kJ/mol) when reconstituted into cores (26). The inhibition of the peptidyl-tRNA hydrolysis step of termination by anti-L16 and the reversible modification of a histidine on L16 correlated with reversible inhibition of peptidyltransferase activity (9) suggest that the position of and/or possible contribution of L16 to the catalytic center may also be important.

Peptidyl-tRNA hydrolysis is factor mediated in contrast to the peptidyltransferase function and, therefore, it is not surprising that additional proteins are important in restoring the former function. Several studies have indicated the importance of L7/L12 for the release factor interaction with the ribosome (24, 25, 27). The partial dependence of both activities on some proteins may reflect the involvement of these proteins in critical assembly processes during reconstitution of the ribosome subunit. For example, L10, L16, L18, and L25 are strongly dependent upon L15 for their assembly (28). The 1.5c cores (Fig. 1A) show a significant reduction in L15 and correspondingly a partial dependence on this protein for the restoration of the functional activities.

The catalytic function of the peptidyltransferase, namely ester aminolysis resulting in peptide bond formation or ester hydrolysis resulting in release of the polypeptide, are not unique to the ribosome. Of all the ribosomal functions they have seemed the most likely to be catalyzed by one or two components rather than a domain involving many components. However, the search for such a component(s) has proven an enigma; several reports have identified different possible catalytic components (L11, L2, or L16) in apparent conflict (5–7). A recent systematic study involving the reconstitution of a functional core particle (minimal particle) starting with 23 S rRNA and 16 purified proteins demonstrated that any defined component(s) responsible for catalytic function must come from five proteins, L2, L3, L4, L15, L16 and the 23 S rRNA (18). Of these proteins L2 and L15 have central positions in the ordered assembly of the particle (28).

L16 has remained a prime candidate throughout this saga for providing at least part the catalytic function of peptidyltransferase. In no case previously has there been a clear demonstration of active ribosomes lacking the protein, nor have E. coli mutants been isolated lacking L16 in their ribosomes as has been the case with L1 and L11 (29, 30). In this study we have obtained an apparent paradox; L16 seems essential for the maintenance and restoration of the codon-independent RF-2-induced termination event (Table I), and yet ribosomes lacking this protein show significant activity in the codon-dependent RF-2-induced peptidyl tRNA hydrolysis (Table III) but only when protein L11 is also missing. As shown in the accompanying paper, protein L11 allows RF-2 to function much more efficiently at lower concentrations of factor and codon. Therefore, the amplification of the facet of the reaction involving the release factor has apparently in-
increased the sensitivity of the assay and enables the measurement of another facet, namely the peptidyl-tRNA hydrolysis.

There are at least two explanations for our L16 findings. The first makes use of a hybrid of two possible models for the molecular mechanism of peptide bond formation on ribosomes (4). One postulates the participation of histidine and a carboxyl function from a ribosomal component analogous to the active center of a serine protease. In the alternative model the ribosome is seen solely as a template to align precisely the reactants and allow a spontaneous reaction to occur. A hybrid model would postulate that if the alignment of the reactants were not precise or rigid then some rate enhancement could be gained by the involvement of a functional group from a ribosomal protein. The requirement for L16 in peptidyl-tRNA hydrolysis adds to the evidence for its involvement in the catalysis. The activity observed with ribosomes lacking L11 and L16 might reflect less dependence on L16 for rate enhancement because the enhanced interaction of the ribosome with the release factor provides better access of water to the peptidyltransferase center.

A second alternative explanation is that L16 is important for an optimal conformation of the peptidyltransferase center and is essential for the positioning of the substrate at the A site moiety of the peptidyltransferase center (A′ site) but not the substrate at the P′-site or in the catalysis. L16 in fact induces conformational changes when reconstituted back in L16 lacking cores (26). This explains the importance of L16 for both the peptidyltransferase and peptidyl-tRNA hydrolase activity. The postulated L16 effect on the A′ site would result in its absolute requirement for the peptidyltransferase activity but not for the peptidyl-tRNA hydrolysis reaction during termination, since H₂O as an acceptor molecule has obviously less defined binding constraints than an aminoacyl residue with its tRNA 3′-end. Further, the access of water to the peptidyltransferase center induced by release factor might be blocked by L11 if L16 is missing. Either adding back L16 or removing L11 would allow water access to the center again.

Acknowledgment—We thank Dr. H. G. Wittmann for constant advice and encouragement.

REFERENCES
The importance of the Escherichia coli ribosomal protein L16 for the reconstitution of the peptidyl-tRNA hydrolysis activity of peptide chain termination.

W P Tate, H Schulze and K H Nierhaus


Access the most updated version of this article at [http://www.jbc.org/content/258/21/12810](http://www.jbc.org/content/258/21/12810)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/258/21/12810.full.html#ref-list-1](http://www.jbc.org/content/258/21/12810.full.html#ref-list-1)