# INTERDEPENDENCE OF *E. COLI* RIBOSOMAL PROTEINS AT THE PEPTIDYLTRANSFERASE CENTRE

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## 1. Introduction

A model reaction for peptide bond formation is the fragment assay [1]. This assay uses the 50 S subunit, an analogue of the 3′-end fragment of a peptidyl–tRNA (C–A–C–C–A–N–acetyl–leucin) and a related analogue of an aminoacyl–tRNA (puromycin). All 50S structural elements directly involved in binding of these fragments and in the peptidyltransferase activity itself belong by definition to the peptidyltransferase centre.

Reconstitution experiments have shown that protein L11 is involved in peptidyltransferase activity [2]. Chloramphenicol interferes with the binding of the aminoacyl terminus of the aminoacyl–tRNA [3,4], i.e., the attachment of the last (3′-end) two or three nucleotides [5]. The drug binds to protein L16 on the 50S subunit as shown by reconstitution [5] and affinity labelling experiments with iodoamphenicol [6]. Thus, L16 is located at the A site moiety of the peptidyltransferase center.

The P site region of the transferase centre has been analysed by affinity labelling using *N*-substituted phenylalanyl–tRNA [7,8] and methionyl–$tRNA_F^{Met}$ analogues [9]. These experiments indicated that proteins L2 and L27 are located at or near this region.

The role of L11 is not yet clear. Either, (a), this protein is the peptidyltransferase itself or, (b), it is necessary to maintain the active conformation of the enzymatic structure. If hypothesis (a) is valid proteins L11 and L16 must be neighbours. Here we describe experiments which test the neighbourhood hypothesis. We show that in addition to L16, protein L11 is part of the single chloramphenicol binding site on the 50 S subunit. Furthermore, L6 is identified as a protein at or near the A site moiety of the peptidyltransferase centre.

## 2. Materials and methods

### 2.1. *Materials*

Preparation of cores and isolation of C–A–C–C–A–Leu were described elsewhere [2]. Proteins L6, L11 and L16 were isolated by DEAE column chromatography and Sephadex G-100 gel filtration as described [2], and in addition a second G-100 gel filtration step was included for purification of L11 and L16.

### 2.2. *Methods*

Reconstitution experiments were performed as described [2]. The peptidyltransferase activity of each reconstituted particle was tested by a kinetic fragment assay. For the kinetic measurements 12.5 $A_{260}$ units of 50 S subunits or particles were incubated at 0°C in a final volume of 810 μl containing 30 mM Tris–HCl, pH 7.8, 250 mM KCl, 115 mM $NH_4Cl$, 20 mM Mg acetate, 1.3 mM puromycindihydrochloride in 33% ethanol. Each assay contained the fragment C–A–C–C–A–(Ac [$^3$H] Leu) (about 130 000 cpm). At the times indicated in fig. 2, 150 μl was removed and mixed with 100 μl of 0.3 M Na acetate (pH 5.5) saturated with $MgSO_4$. After addition of ethylacetate (1.5 ml) 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 4 ml of scintillation fluid and counted.

After the reconstitution the protein patterns of

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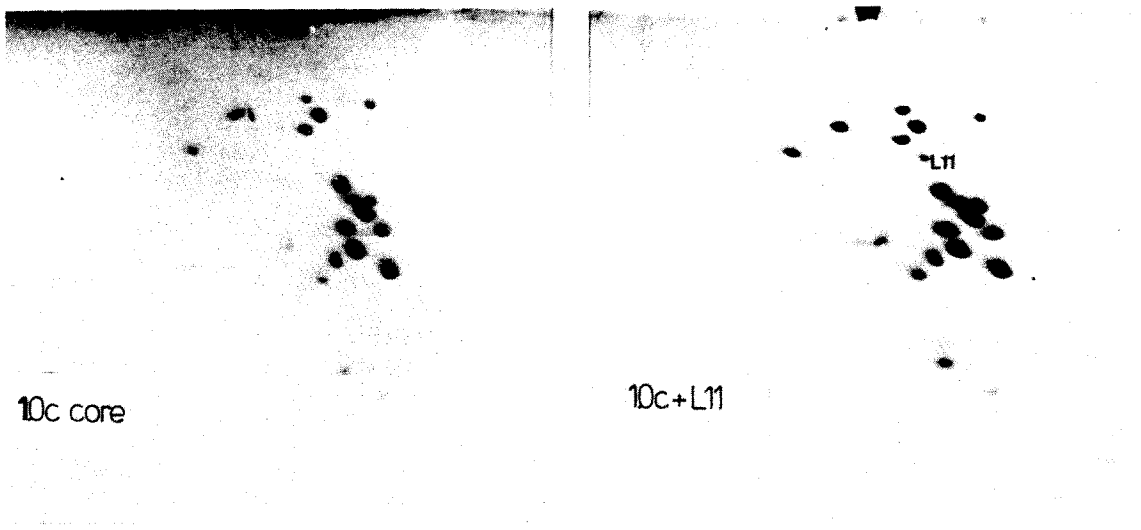


Fig. 1. Two-dimensional electrophoresis pattern of the proteins in the 1.0c core and the reconstituted particle (1.0c + L11).

the particles were analyzed in the two-dimensional gel electrophoresis. The proteins found on the particle and not on the 1.0 c core are listed in table 1.

For C–A–C–C–A–[$^3$H]Leu binding, 8 $A_{260}$ units of 50 S subunits, core particles or 23 S RNA were incubated with the fragment C–A–C–C–A–Leu (30 000 cpm) in a volume of 100 μl containing 50% ethanol, 30 mM Tris–HCl, pH 7.4; 25 mM Mg acetate, 20 mM $NH_4Cl$, 200 mM KCl and, where indicated, 0.15 mM chloramphenicol. After incubation for 15 min at 0°C the particles of RNA were pelleted. 60 μl of the supernatant was mixed with 0.5 ml soluene and 4 ml scintillation liquid and counted. The difference in counts (compared to a control sample containing no particle or RNA) is a measure of the amount of the tRNA fragment bound.

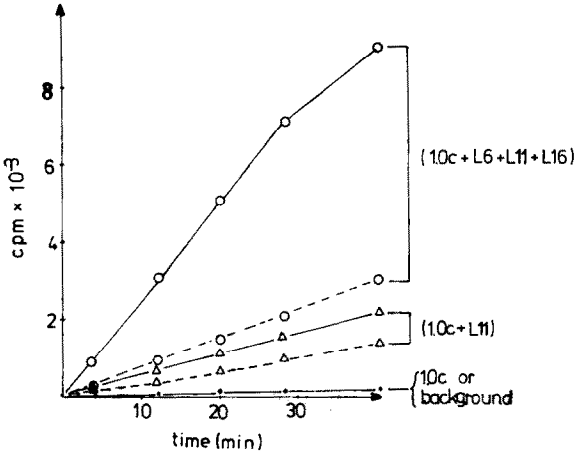


Fig. 2. Kinetics of the fragment assay of the 1.0c core and the reconstituted particles (1.0c + L11) and (1.0c + L6 + L11 + L16). (——) without chloramphenicol, (– – – –) with chloramphenicol (final concentration: 0.04 mM).

## 3. Results and discussion

The proteins L6, L7, L10, L11, L12, L16, L27, L28, L31 and L33 were split off the 50 S subunit by incubation with 1M LiCl. The remaining core (1.0c) was not active in either the fragment assay or chloramphenicol binding. Proteins L6, L11 and L16 were purified by DEAE-cellulose column chromatography followed by one (L6) or two (L11, L16) gel filtration steps as described [2]. Reconstitution experiments were performed with the 1.0c core and the three proteins in all possible combinations (see table 1). The reconstituted particles were tested for peptidyltransferase activity, chloramphenicol binding and binding of the aminoacyl–tRNA fragment C–A–C–C–A–Leu, which binds to the A site part of the peptidyltransferase centre [11].

Peptidyltransferase activity is only seen when L11

Table 1

| 1.0 c cores reconstituted with | | | Fragment assay | | CAM-binding equilibrium dial. | C–A–C–C–A[$^3$H]Leu-binding | |
|---|---|---|---|---|---|---|---|
| L6 | L11 | L16 | (cpm) | (% CAM-inhibition) | (pM/nM particle) | (cpm) | (% CAM-inhibition) |
| – | – | – | 0 | | 4 | 6222 | 0 |
| + | – | – | 7 | | 3 | 5864 | 10 |
| – | + | – | 756 | 43 | 40 | 5977 | 7 |
| – | – | + | 12 | | 78 | 6083 | 6 |
| + | + | – | 749 | 47 | 43 | 6142 | 21 |
| + | – | + | 53 | | 101 | 5923 | 15 |
| – | + | + | 1892 | 51 | 98 | 6185 | 10 |
| + | + | + | 2934 | 66 | 150 | 5931 | 27 |
| 1.0 c + all split proteins | | | 2621 | 62 | 156 | 6016 | 26 |
| 50 S | | | 3133 | 74 | 300 | 6920 | 52 |
| 23 S RNA | | | | | 13 | 5190 | 4 |

Peptidyltransferase activity of the reconstituted particles and their chloramphenicol (CAM)- and C–A–C–C–A–[$^3$H]Leu-binding. Where indicated we added chloramphenicol to the fragment assay (final concentration: 0.04 mM). The 12 min values of the fragment assay kinetics are listed under 'fragment assay' (first column).

is present (see the first column of table 1). The protein patterns of the 1.0c core and the reconstituted particle (1.0c + L11) is demonstrated in figure 1, and their peptidyltransferase activity in fig. 2. The L11 dependent activity is stimulated by L16, whereas L6 shows a stimulating effect only when protein L16 is present in addition to L11 (see table 1).

The simplest interpretation is that L11 and L16 are neighbours and that L6 is functionally linked to L16. If we assume that L16 alone is responsible for chloramphenicol binding, then the particle containing L11 but neither L6 nor L16 should not bind chloramphenicol and, therefore, be resistant to chloramphenicol in the fragment assay. Surprisingly, the peptidyltransferase activity of this particle could be inhibited to a remarkable extent by chloramphenicol (first column). Therefore, another protein in addition to L16 must be involved in chloramphenicol binding. The binding of the drug was tested directly by equilibrium dialysis and the results are summarized in the second column of the table. As expected, the greatest binding was detected in the presence of L16, indicating the dominant role of L16 for this ribosomal function. However, the particle reconstituted with L11 alone also shows significant binding. As different groups have reported only one binding site for chloramphenicol per 50 S subunit [12,13,5], our findings strongly indicate that proteins L11 and L16 are neighbours.

In a last series of experiments the binding of the fragment C–A–C–C–A–Leu was tested (third column). Unfortunately, 23 S RNA binds large amounts of this fragment under the conditions used. This binding is clearly unspecific as we find similar binding to 16 S RNA and a mixture of tRNAs (data not shown). We therefore need a tool to reveal the portion of C–A–C–C–A–Leu fragment which is specifically bound to the particles. This tool is chloramphenicol: As pointed out above, the drug interferes with the binding of the tRNA 3′-terminal

fragment. Accordingly, the chloramphenicol induced inhibition of this fragment binding gives a measure of the specific as opposed to non-specific reaction.

The particles reconstitued with one of the three proteins show insignificant or low inhibition. However, when L6 is present in addition to L11 and/or L16 chloramphenicol inhibits the fragment binding significantly. Thus, L6 is important for the inhibitory effect of chloramphenicol, although it is not involved directly in chloramphenicol binding.

In summary, L11 is involved in the peptidyltransferase activity and is a neighbour of L16. The chloramphenicol binding site consists of both L11 and (more pronouncedly) of L16. L6 is important for the inhibitory effect of chloramphenicol. It influences directly the L16 dependent function (chloramphenicol binding) and via L16 the L11 dependent function (peptidyltransferase activity).

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