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Incorporation of 5S RNA into 16S•23S RNA complex

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5S RNA as such is not incorporated into 16S•23S RNA complex formed under reconstitution condition. However, the addition of 50S ribosomal proteins, L5, L18 and L25/L15 results in its incorporation in stoichiometric amount. None of the proteins added individually is capable of incorporating 5S RNA into the complex. Of the different combinations in pairs that are possible out of the four proteins, the pairs L5, L18 and L15, L18 stimulate the incorporation to some extent. Of the four possible triplets, L5, L18, L25 or L5, L15, L18 is the most efficient for maximum incorporation of 5S RNA. The presence of all the four proteins is no more effective than the combinations of the three.

It has been demonstrated for the first time in this laboratory that naked 16S and 23S RNAs are capable of forming a 1:1 stoichiometric complex under two well-defined conditions: reconstitution condition where high salt and high Mg^{++} are present and also in presence of 1 M alcohol with comparatively low salt and low Mg^{++} concentrations (1,2). Since 5S RNA is part of the structure of 50S ribosome and known to associate with 23S RNA through some 50S ribosomal proteins (3,4) it was of interest to find out whether 5S RNA can be incorporated into 16S•23S complex under the above mentioned conditions. The experiments described here, carried out under reconstitution condition, show that 5S RNA is not incorporated as such into the complex. Since the 50S ribosomal proteins L5, L18 and L25 are known to bind to 5S RNA (3,5-7) and also involved in the bind-

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ing of 5S RNA to 50S ribosome (6) these proteins were tested for their ability to incorporate 5S RNA into the complex. Further, in the assembly map of the 50S ribosome constructed by Nierhaus (8,9) L15 has been shown to play a prominent role in the binding of 5S RNA to 23S RNA. Therefore the effects of all the four proteins L5, L15, L8 and L25, in various combinations, on the incorporation of 5S RNA into the 16S·23S RNA complex were studied.

MATERIALS AND METHODS

Proteins L5, L15, L18 and L25 were gifts from Dr. A.T. Gudkov, of the Institute for Protein Research, Poustchino, Moscow Region, U.S.S.R.

Preparation of 5S, 16S and 23S RNAs - 16S and 23S RNAs were prepared from 30S and 50S subunits of E.coli MRE600 by phenolisation, combining the methods of Amils et al.(10) and Erdman et al. (11). 5S RNA was separated from 23S RNA by gel filtration through Sephadex G-100. ^{32}P-labeled 5S RNA was prepared from 70S ribosome isolated from ^{32}P-labeled cells; 16S and 23S RNAs were removed by precipitation with 1.5 M NaCl followed by gel filtration through Sephadex G-100.

Incorporation of 5S RNA into 16S·23S RNA complex - 6 µg of ^{32}P-5S RNA (1.2x10^{4}counts/min) was incubated at 37°C for 45 min with proteins (where indicated, 10-15 µg of each, representing a five fold molar excess over 5S RNA), in 20 mM Tris-HCl, pH 7.6, containing 400 mM KCl, 20 MM magnesium acetate and 6 mM β-mercaptoethanol. An equimolar amount (156 µg) of 23S RNA was then added. After 6 h at 4°C an equimolar amount (82 µg) of 16S RNA was added. The mixture was kept at 4°C for 12 hr and then subjected to sucrose density gradient (5-20%) centrifugation at 161,000xg for 9 hr. Fractions (0.25 ml) were collected and absorbancy at 260 nm as well as trichloroacetic acid-insoluble radioactivity in each fraction was measured in Rack Beta Counter of LKB Instrument Company, Sweden.

RESULTS AND DISCUSSION

That 5S RNA alone is not incorporated into 16S·23S RNA complex formed under reconstitution condition, even if excess of 5S RNA (in comparison to 16S and 23S RNAs) is added, is shown in Fig.1. This experiment confirms the specificity of complex formation between 16S and 23S RNAs, since another species of RNA (5S RNA) is not associated with the complex.

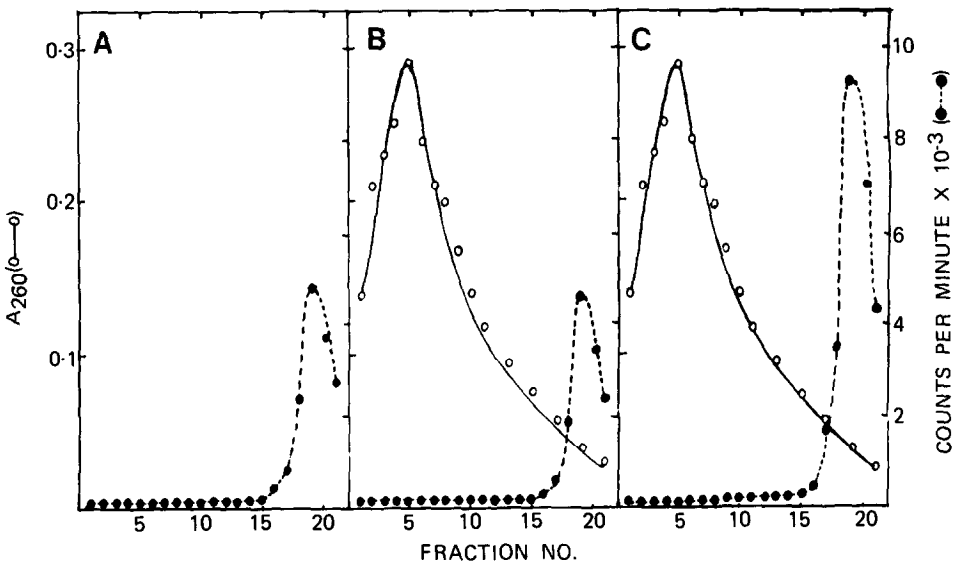

Fig.1. Sucrose density gradient centrifugation of 16S.23S RNA complex along with 5S RNA. A mixture of 23S RNA (156 µg) and 16S RNA (82 µg) was incubated with 6 µg (B) and 12 µg (C) of ^{32}P-5S RNA ($2x10^3$ counts/min/µg) under reconstitution condition and subjected to density gradient centrifugation as described under 'Materials and Methods'. In the control experiment (A) 5S RNA alone (6 µg) was subjected to centrifugation.

As mentioned in the introduction, 50S ribosomal proteins L5, L18 and L25 bind to 5S RNA and these proteins have been implicated in the incorporation of 5S RNA into 50S ribosome through binding to 23S RNA. Recently L15 has also been thought to be involved in such process. When 5S RNA was incubated individually with each of these four proteins no incorporation of 5S RNA into the complex was observed (results not presented). When pairs of these proteins were tested, it was observed that all the combinations promoted small but significant amounts of incorporation of 5S RNA (Fig.2). The pair L5 and L18 and L15 and L18 produced somewhat more incorporation than the others although the incorporation was much less than stoichiometric.

Maximum incorporation was obtained when 5S RNA was incubated with a mixture of two other specific proteins (Fig.3).

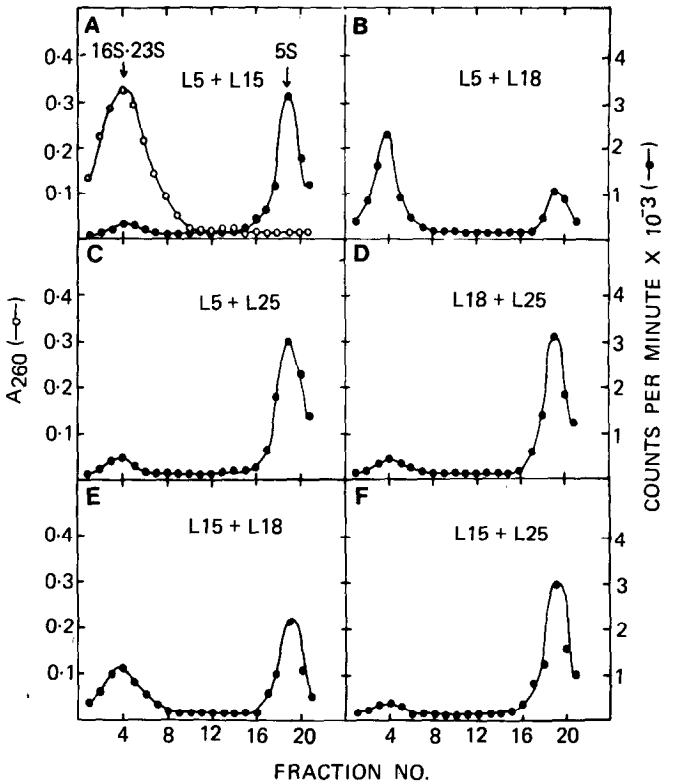

Fig.2. Effect of pairs of the proteins L5, L15, L18 and L25 on the incorporation of 5S RNA into 16S·23S RNA complex. 6 µg of ^{32}P-5S RNA was preincubated with the pairs of proteins as indicated in the figure and then incubated with the 16S·23S RNA complex containing 82 µg of 16S RNA and 156 µg of 23S RNA according to the procedure described under 'Materials and Methods'. The remaining procedure was as described in the legend to Fig.1.

The most effective combinations were L5, L15, L18 and L5, L18, L25. The other combinations (L5, L15, L25 and L15, L18, L25) were not more effective than the protein pairs. It appears that L5 and L18 are essential and that either L15 or L25 must also be included for maximum incorporation. The addition of all the four proteins does not produce any better effect, on the other hand, leads to somewhat less incorporation (data not shown).

From the results presented in Fig.3 it may be calculated that approximately 1 mole of 5S RNA binds to 1 mole of the 16S·23S binary complex. To confirm this two fold and fourfold molar excesses of 5S RNA were added to the complex (Fig.4). It

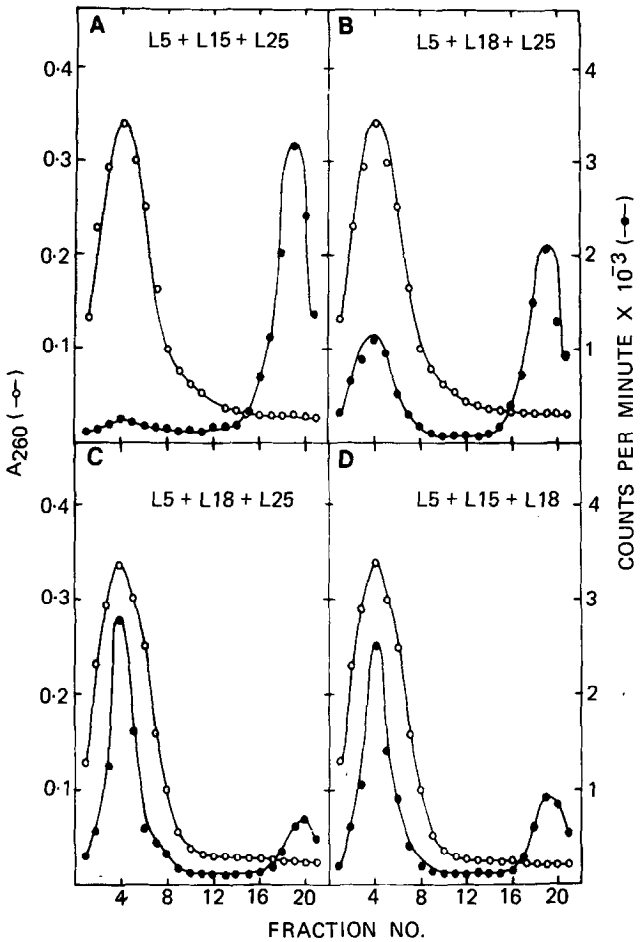

Fig.3. Effect of proteins L5, L15, L18 and L25, in combination of three, on the incorporation of 5S RNA into complex. The incubation and other procedures were as described in the legend to Fig.2 except that three proteins (in stead of two) were added in each combination, as indicated in the figure.

was found that only stoichiometric amount of 5S RNA was incorporated.

5S RNA is part of the 50S ribosome structure but its mode of binding to the subunit is not definitely known. As shown here, the combination of three proteins, either L5, L18 and L25 or L5, L18 and L15, leads to almost stoichiometric amount of incorporation of 5S RNA. It has also been observed that the same proteins lead to the binding of 5S RNA to 23S RNA (for example, references 3, 5-7 and also unpublished results of this laboratory).

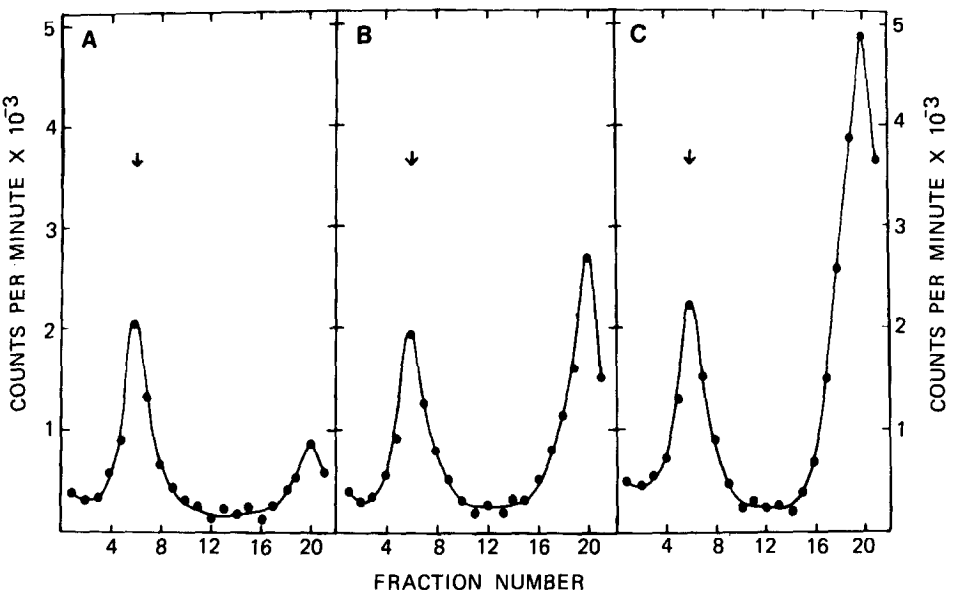

Fig.4. Stoichiometry of the incorporation of 5S RNA into 16S•23S RNA complex. ^{32}P-5S RNA was incubated with proteins L5, L18 and L25 as described in the legend to Fig.2 and then varying amounts of this preparation were incubated with a mixture of nonradioactive 16S and 23S RNAs under reconstitution condition as described under 'Materials and Methods'. The subsequent procedure was as described. 16S, 23S and 5S RNAs were present in the following molar ratios : (A) 1:1:1 (B) 1:1:2 (C) 1:1:4 . The arrow indicates the position of 16S•23S RNA complex.

Therefore it is not surprising that 5S RNA becomes part of 16S•23S RNA complex in presence of these proteins. It should be mentioned here that 5S RNA does not bind to 16S RNA under these conditions (results not presented). The same results were obtained whether 5S RNA was incubated with these proteins first and then the mixture of 23S and 16S RNAs (or 16S•23S RNA complex) was added or *vice versa* (results not presented).

Extensive studies led Nierhaus to present the assembly map of 50S ribosome (8,9). In this L15 instead of L25 has been implicated in the binding of 5S RNA to ribosome. It is interesting to observe from the present experiments that L5 and L18 in combination with either L15 or L25 lead to maximum incorporation of 5S RNA; thus, L15 and L25 can be exchanged for the purpose.

That 16S and 23S RNAs associate in stoichiometric amounts and 5S RNA is incorporated into the complex, also in stoichiometric amount, may be of considerable physiological significance. Preliminary studies (unpublished results) carried out in this laboratory on the effect of kethoxal treatment of ribosomes, ribosomal subunits and rRNAs on the capacity of 16S and 23S RNAs to form the binary complex show that the regions of 16S and 23S RNAs that are assumed to be involved in the association of 30S and 50S subunits (12-15) appear to be involved in the association of naked 16S and 23S RNAs as well. The present studies with 5S RNA indicate that these sites are apparently not involved in the incorporation of 5S RNA into the complex.

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