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Cooperative interactions among protein and RNA components of the 50S ribosomal subunit of Escherichia coli

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ABSTRACT

Cooperative interactions among constituents of the 50S ribosomal subunit of *Escherichia coli* have been analyzed in order to elucidate its assembly and structural organization. Proteins L5 and L18 were shown to be necessary and sufficient to effect the association of the 5S and 23S RNAs into a quaternary complex that contains equimolar amounts of all four components. Measurement of diffusion constants by laser light scattering revealed that integration of the 5S RNA induced the 23S RNA to adopt a somewhat more open conformation. An investigation of relationships among proteins associated with the central and 3' portions of the 23S RNA demonstrated that attachment of L5, L10 + L11, and L28 depends upon the RNA-binding proteins L16, L2, and L1 + L3 + L6, respectively, and that L2 interacts with the central segment of the 23S RNA. These data, as well as the results of others, have been used to construct a scheme that depicts both direct and indirect associations of the 5S RNA, the 23S RNA, and over two-thirds of the subunit proteins. The 5' third of the 23S RNA apparently organizes the proteins required to nucleate essential reactions, whereas a region within 500 to 1500 bases of its 3' terminus is associated primarily with proteins involved in the major functional activities of the 50S ribosomal particle.

INTRODUCTION

The structural and functional properties of the 50S ribosomal subunit of *Escherichia coli* stem from specific interactions of its macromolecular constituents. Topographical relationships among the two RNAs and 32 different proteins of the subunit have been investigated by a variety of techniques (reviewed in ref. 1) and the discovery of conditions for the reconstitution of highly active 50S particles from their dissociated components represents a major asset in such studies (2-4). Assembly of the 50S subunit in vitro is thought to occur by a sequential and cooperative mechanism (5,6), much as in the case of the 30S subunit (7). Specifically, the ten or so large-subunit proteins that interact independently with the 23S RNA (8) stimulate the formation of binding sites for proteins unable to associate directly with the nucleic acid chain (5). This process guides the sequential addition of still

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other proteins as the growing ribonucleoprotein matures into a compact, functional subunit (3).

Valuable information on the organization of the 50S particle can be obtained from the study of subassemblies reconstituted from selected subunit constituents. Those containing the 5S RNA are of particular interest since they are believed to contribute to both the tRNA-binding and GTPase functions of the active ribosome (9,10). Other pathways lead to the association of proteins essential for the binding of elongation factors and the integrity of the peptidyl transferase center (1,5). In this report, we present further results on cooperative interactions among 50S subunit components with particular emphasis on the conditions that lead to the integration of the 5S and 23S RNAs. These data, in conjunction with the findings of others, permit the construction of an improved in vitro assembly map for the large ribosomal subunit which encompasses 23 of its 32 protein constituents and shows the relative positions of protein binding sites on both 5S and 23S RNAs.

MATERIALS AND METHODS

Ribosomal components. Ribosomes and ribosomal subunits were prepared from *E. coli* strain MRE600 as previously described (11). Unlabelled and [^{3}H]-aminoacid-labelled proteins were extracted from purified 50S subunits with 67% acetic acid and separated by ion-exchange chromatography (12). Unlabelled and [^{14}C]uracil-labelled 5S and 23S RNAs were extracted from 70S ribosomes with phenol and isolated by sucrose gradient centrifugation (11). In 5S RNA preparations, the ribosomes were first sedimented from the 30,000 x g supernatant through a cushion of 10 mM Tris·HCl, pH 7.6 - 1 mM $MgCl_2$ - 100 mM KCl in 30% sucrose (13). This procedure dissociates tRNA which would otherwise contaminate the 5S RNA fraction. Specific radioactivities of the labelled components were approximately 2500 cts/min per μg for [^{3}H]protein, 200 cts/min per μg for [^{14}C]5S RNA and 50 to 500 cts/min per μg for [^{14}C]23S RNA.

Protein-RNA complexes. Protein-RNA complexes were formed by incubating 23S RNA with 0.2 to 5 molar equivalents of [^{14}C]5S RNA and/or 0.2 to 5 molar equivalents of [^{3}H]protein and/or 2 molar equivalents of unlabelled protein in Tris/Mg/K buffer (50 mM Tris·HCl, pH 7.6 - 20 mM $MgCl_2$ - 300 mM KCl) as indicated in the figure legends. After heating, the reaction mixtures were placed on ice for 15 min and then sedimented through 4-ml 3 to 15% sucrose gradients in Tris/Mg/K buffer for 2.5 hr at 44,000 revs/min in a Spinco SW60 rotor. The gradients were fractionated and the radioactivity in each tube was analyzed (11). For the calculation of saturation data, molecular weights

for L5, L18, 5S RNA and 23S RNA were taken as 20,172 (14), 12,770 (15), 38,853 (16), and 1,100,000 (17), respectively. The 18S RNA fragment was prepared by limited pancreatic RNase hydrolysis of 50S ribosomal subunits (18). Ribosomal proteins were bound to the 18S RNA as described above and the complexes were digested with T_1 RNase at 0°C. The products were separated by centrifugation through 12-ml 3 to 15% sucrose gradients in Tris/Mg/K buffer for 16 hr at 32,000 revs/min in a Spinco SW41 rotor and each fraction was assayed for radioactivity.

Diffusion constants. Diffusion constants of the 23S RNA, the 5S RNA-L5-L18-23S RNA complex, and the 50S subunit were determined by laser light scattering. To prepare the complex, a 200-µl reaction mixture containing 4 µg of L5, 2.5 µg of L18, 4 µg of 5S RNA, and 100 µg of 23S RNA in Tris/Mg/K buffer was incubated for 15 min at 40°C, chilled, and dialyzed against 1000 ml of the incubation buffer. The complex was again heated to 40°C for 15 min and then dialyzed extensively against fresh Tris/Mg/K buffer containing 1 mM dithiothreitol. Portions of this solution were diluted as appropriate in the same buffer and filtered through a Millipore type GS membrane to remove unbound protein and particulate matter. Sucrose gradient centrifugation of the quaternary complex after filtration showed that the 5S RNA and the proteins remained associated with the 23S RNA and therefore confirmed the integrity of the reconstituted ribonucleoprotein. Aliquots of free 23S RNA and of intact 50S subunits in Tris/Mg/K buffer with dithiothreitol were incubated at 40°C for 15 min, dialyzed and filtered as described for the complex. Laser light-scattering measurements were performed according to previously published methods (19,20). For each sample, data were accumulated for at least 15 min at 20°C and the resulting values, generally obtained with a precision of ±1%, were corrected for solvent viscosity to yield values of $D_{20,w}$.

RESULTS

Minimum protein requirement for the association of 5S and 23S RNAs. 50S subunit proteins L5, L18 and L25, all of which bind individually to 5S RNA alone (9), were first implicated in the association of the 5S and 23S RNAs by Gray et al. (21), although L5 was incorrectly identified as L6 at that time (see ref. 22). In order to determine the minimum protein requirement for formation of the 5S RNA-23S RNA complex, unlabelled 23S RNA was incubated with ^{14}C-labelled 5S RNA in the presence of each of the three proteins and the mixtures were characterized on sucrose gradients. Neither L5, L18 nor L25 alone was able to promote the binding of the 5S RNA to the 23S

RNA, as judged from the absence of ^{14}C radioactivity at the position of 23S RNA in the gradients (Fig. 1a-c). Association of the two RNAs did occur, however, when both L5 and L18 were added to the incubation (Fig. 1d). Panels e and f of Figure 1 demonstrate that no other combination of two of the three proteins promoted formation of the 23S complex although the 5S and 23S RNAs interacted at least as well when all three proteins were present (Fig. 1g).

That L5 and L18 are required to stimulate 5S RNA-23S RNA association is in accord with the results of Gray et al. (21) but, in contrast to their findings, we could not reconstitute the complex with L5 and L25. The relatively small amounts of protein and RNA used in the experiments of Figure 1 are insufficient for the quantitative binding of L5 or L25 to the 5S RNA alone (13). However, even at component concentrations as high as 5×10^{-6} M, where the 5S RNA becomes saturated with both L5 and L25 (13,23), no 23S complex was formed in the presence of either of the combinations L5 + L25 or L18 + L25 (data not shown).

Stoichiometry of the components in the 5S RNA-L5-L18-23S RNA complex. Increasing amounts of ^{14}C-labelled 5S RNA were added to a fixed amount of unlabelled 23S RNA and a twofold molar excess of ^{3}H-labelled L18 and unlabelled L5. After sucrose gradient centrifugation, the molar ratio of 5S RNA to 23S RNA in each complex was calculated from the quantities of 23S RNA added to the incubation mixture and of $[^{14}C]$5S RNA sedimenting at 23S. The results, illustrated in Figure 2, show that the 5S RNA:23S RNA ratio at

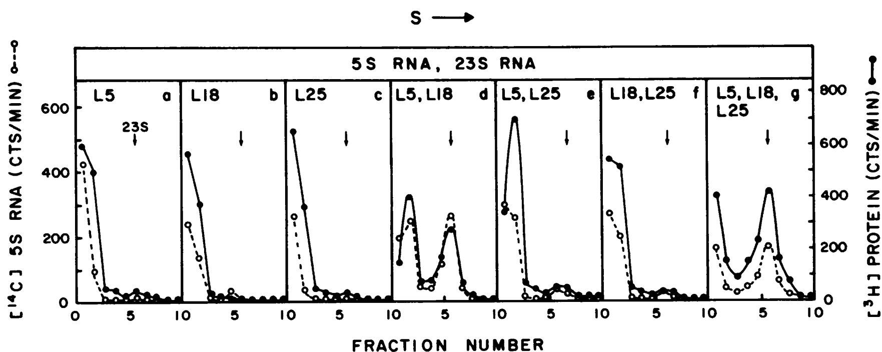


Figure 1. Protein requirement for the association of 5S and 23S RNAs. Mixtures containing 0.2 μg each of the indicated ^{3}H-labelled protein(s), 0.5 μg of ^{14}C-labelled 5S RNA and 15 μg of unlabelled 23S RNA were incubated in 100 μl Tris/Mg/K buffer for 15 min at 30°C and analyzed by sucrose gradient centrifugation as described in Materials and Methods. (●—●), $[^{3}H]$protein; (O---O), $[^{14}C]$5S RNA.

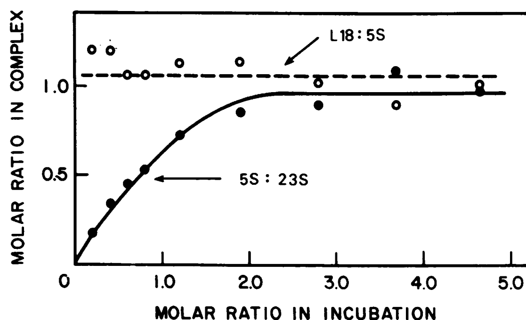


Figure 2. Saturation curve for the binding of 5S RNA to 23S RNA in the presence of L5 and L18; molar ratio of L18 to 5S RNA in the 23S complex. Increasing amounts of [^{14}C]5S RNA were added to mixtures containing 15 μg of unlabelled 23S RNA and a twofold molar excess of unlabelled L5 and [^{3}H]L18 relative to 23S RNA in 100 μl Tris/Mg/K buffer. Following incubation and sucrose gradient analysis, molar 5S RNA:23S RNA and L18:5S RNA ratios were calculated from the quantities of [^{14}C]5S RNA and [^{3}H]L18 coincident with the 23S peak, and of 23S in the reaction mixture, according to the specific radioactivities and molecular weights given in Materials and Methods. (●——●), molar 5S RNA:23S RNA ratio; (O---O), molar L18:5S RNA ratio.

saturation is 1:1 and are thus consistent with the measurements of Gray and co-workers (21) in which a membrane filter assay was employed. The use of ^{3}H-labelled L18 in this series of experiments also allowed us to compute the relative stoichiometries of L18 and 5S RNA in the 23S complex. Figure 2 demonstrates that the molar L18:5S RNA ratio at each point is approximately 1:1, even before the 23S RNA is saturated by 5S RNA. We conclude that the association of L18 with the 23S complex is proportional to the amount of 5S RNA present and that the stoichiometry of L18, 5S RNA and 23S RNA in the complex is 1:1:1.

In a second set of analyses, increasing quantities of ^{3}H-labelled L5 were mixed with a standard amount of unlabelled 23S RNA in the presence of a twofold molar excess of ^{14}C-labelled 5S RNA and unlabelled L18. The molar ratios of L5 to both 5S and 23S RNAs were estimated from the amount of [^{3}H]L5 and [^{14}C]5S RNA in the 23S peak of the sucrose gradients and from the quantity of 23S RNA in the incubation mix. As seen in Figure 3, protein L5 saturates the 23S complex at a stoichiometric ratio of about 1:1, whereas L5 and 5S RNA are present in equimolar amounts throughout the range of concentrations tested. In addition, we infer from the amount of 5S RNA in the complex that association of the two RNAs is proportional to bound L5 when L18 is in excess. Taking the results of Figures 2 and 3 together, it is clear that the molar

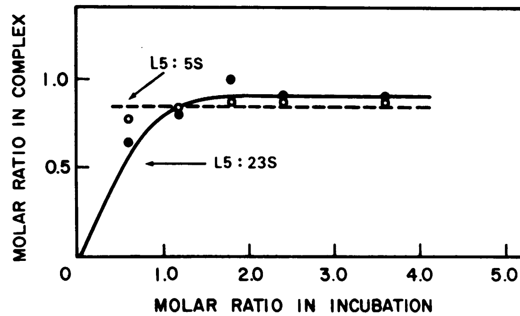


Figure 3. Saturation curve for the binding of L5 to 23S RNA in the presence of L18 and 5S RNA; molar ratio of L5 to 5S RNA in the 23S complex. Increasing amounts of [^{3}H]L5 were incubated with 15 μg of unlabelled 23S RNA in the presence of a twofold molar excess of unlabelled L18 and [^{14}C]5S RNA relative to 23S RNA. Molar ratios of components in the 23S complex were determined as in Fig. 2 (●——●), molar L5:23S RNA ratio; (O---O), molar L5:5S RNA ratio.

ratio of the components of the 5S RNA-L5-L18-23S RNA quaternary complex is 1:1:1:1.

Conformation of the 23S RNA in the quaternary complex. The influence of 5S RNA attachment on the conformation of the 23S RNA was assessed by comparing the translational diffusion constants of the 5S RNA-L5-L18-23S RNA complex and the 23S RNA. Values of $D_{20,w}$ were determined by laser light scattering and are presented as a function of concentration in Figure 4 along with the corresponding least-squares plots. The $D^o_{20,w}$ of the quaternary complex, obtained by extrapolation, was about 6% less than that of the free RNA. Since L5, L18 and 5S RNA contribute little to the scattering of the ribonucleoprotein particle, the results suggest that the 23S RNA becomes somewhat less compact--or more asymmetric--when associated with the protein-5S RNA subassembly. Although the negative slopes of the plots indicate that both samples aggregated somewhat with increasing concentration, the data do not deviate materially from linearity between 0.05 and 0.5 mg/ml.

Despite its substantially greater mass, the 50S subunit exhibits a value of $D^o_{20,w}$ approximately 40-45% higher than that of either the 23S RNA or the quaternary complex (Fig. 4). It follows directly that the RNA chain must be much more tightly packed in the mature ribosomal particle than in the free state. The light scattering measurements show that attachment of the L5-L18-5S RNA unit does not stimulate the nucleic acid molecule to fold into the compact structure it possesses in the intact subunit and suggest that integration

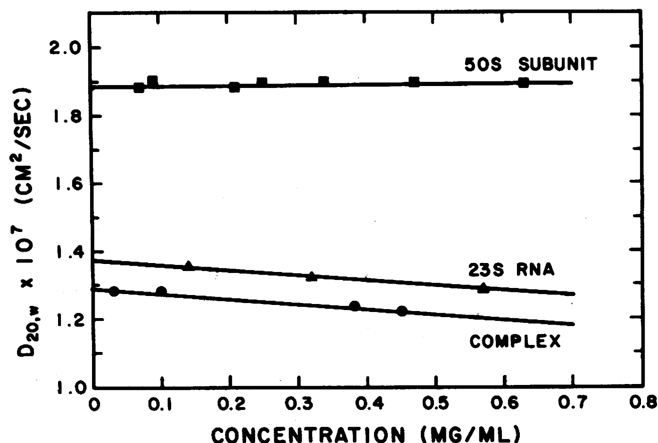


Figure 4. Diffusion constants of the 23S RNA, the 5S RNA-L5-L18-23S RNA complex and the 50S subunit. $D_{20,w}$ was obtained at a variety of concentrations as described in Materials and Methods. Lines were fitted to the experimental data by the method of least squares. Values of $D^{o}_{20,w}$ for the 23S RNA, the complex and the 50S subunit were 1.38, 1.29 and 1.89 x 10^{-7} cm^2/sec, respectively. (▲—▲), 23S RNA; (●—●), 5S RNA-L5-L18-23S RNA complex; (■—■), 50S subunit.

of the 5S RNA does not in itself draw together two or more segments of the 23S RNA which are widely separated from one another in the primary structure.

Binding of L5, L10, L11 and L28 to protein-23S complexes. We previously reported that six proteins unable to interact directly with the 23S RNA bind specifically to complexes containing one or more RNA-binding proteins from the 50S subunit (5). In particular, L5, L10 and L11 each bound to the 23S RNA in the presence of both L2 and L16. Further dissection of these cooperative interactions showed that L16 promotes association of L5 (Fig. 5a) and that L2 directs the attachment of L10 and L11 (Fig. 5b,c). Moreover, L5 did not bind in the presence of L2, nor did either L10 or L11 in the presence of L16 (data not shown). A systematic screening for additional cooperativity of this kind revealed that L28 was able to interact with a 23S complex containing L1, L3 and L6 (Fig. 5d), all of which bind to sites in the 3' third of the RNA molecule (24).

Location of protein binding sites in the 23S RNA. The location of binding sites for ten 50S subunit proteins that associate independently with the 23S RNA has been accomplished by a number of different methods (18,24-27). Our approach entails the analysis of interactions between individual proteins

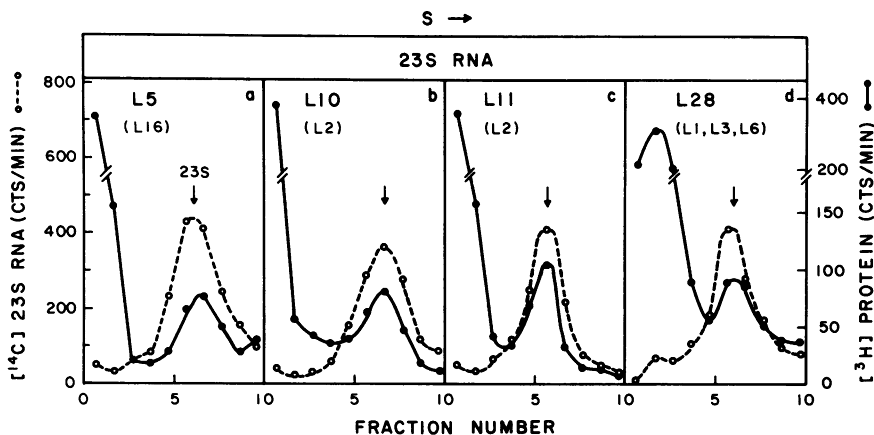


Figure 5. Association of L5, L10, L11 and L28 with 23S RNA in the presence of RNA-binding proteins. From 1 to 2 μg of ^{3}H-labelled proteins L5, L10, L11 and L28 were mixed with 50 μg of ^{14}C-labelled 23S RNA and two molar equivalents of unlabelled RNA-binding proteins in 100 μl Tris/Mg/K buffer and incubated for 15 min at 40°C. The complexes were separated from unbound protein by sucrose gradient centrifugation. ^{3}H-labelled proteins are indicated in boldface; unlabelled proteins in each mixture are shown in parentheses. (●——●), [^{3}H]protein; (O---O) [^{14}C]23S RNA.

and several large fragments derived from the 23S RNA by limited RNase digestion. By this procedure, L4, L20 and L24 were assigned to the 5'-terminal 13S RNA whereas L1, L2, L3, L6, L13, L16 and L23 were found to bind to the 3'-terminal 18S RNA (18). The latter fragment was further hydrolyzed to subfragments of 8S and 12S which encompass the central and 3' portions of the 23S RNA, respectively. Of the proteins that bound to the 18S fragment, all except L2 and L16 were shown to interact with one or the other subfragment (24). We here report that L2 associates specifically with the 8S RNA (Fig. 6). L16, however, does not remain attached to either subfragment when the L16-18S RNA complex is partially digested with RNase, suggesting that the binding site for this protein is destroyed by the scission(s) which produce the 8S and 12S RNAs. A similar observation was made when the 13S fragment was cleaved into roughly equal halves by hydrolysis with T_1 RNase. While the interactions of L20 and L24 were not seriously impaired by this treatment, L4 was unable to associate with either of the two resulting subfragments (data not shown). Thus, although these negative results must be interpreted with caution, it is possible that the binding sites for L4 and L16 span or adjoin the major T_1 RNase cleavage points in both 13S and 18S RNAs.

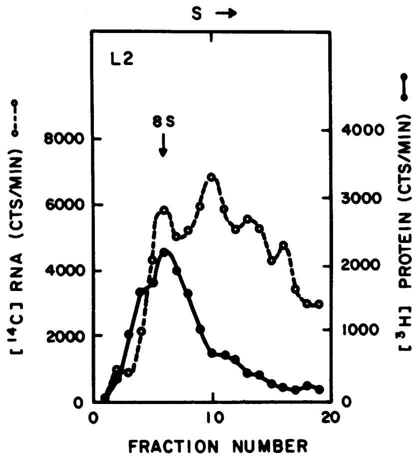


Figure 6. RNase T_1 digest of L2-18S RNA fragment complex. 8 μg of ^{3}H-labelled L2 was incubated with 100 μg of ^{14}C-labelled 18S RNA fragment for 20 min at 40°C in 200 μl of Tris/Mg/K buffer and chilled. The mixture was then digested with 2 μg of RNase T_1 for 10 min at 0°C and fractionated by centrifugation through a 12-ml sucrose gradient. (●——●), [^{3}H]L2; (O---O), [^{14}C]RNA fragments.

DISCUSSION

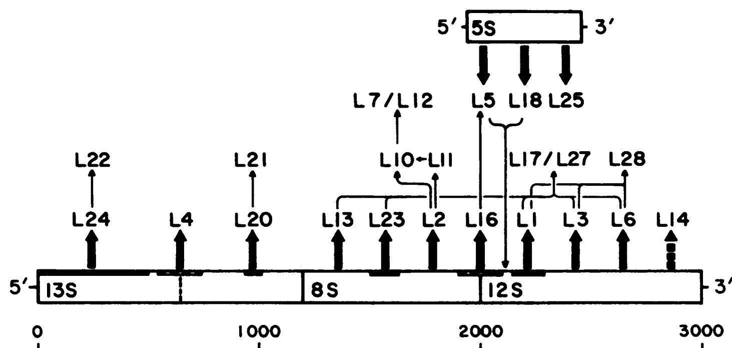
Integration of L5, L18 and 5S RNA with the 23S RNA. We have shown that 50S ribosomal subunit proteins L5 and L18 mediate the association of 5S RNA with 23S RNA in the presence of 20 mM Mg^{2+} and 300 mM KCl. This result agrees with earlier findings of Gray et al. (21), although we failed to confirm their observation that L18 and L25 also stimulate 5S RNA-23S RNA interaction under similar conditions. The quaternary complex was judged to be specific from saturation binding studies and all four components were found to associate in equimolar amounts when excess RNA and/or protein was added to the incubation mixture. In particular, our experiments unambiguously demonstrate that the molar ratio of L18 to both RNAs in the complex is 1:1 at saturation. Our conclusions are thus consistent with those of Newton and Brimacombe (28), who isolated a specific ribonucleoprotein fragment from the 50S subunit that consisted of 5S RNA, a portion of the 23S RNA, and equimolar quantities of proteins L5, L18 and L25.

The interactions that lead to formation of the 5S RNA-L5-L18-23S RNA complex in vitro are evidently sufficient to promote the integration of the 5S RNA into nascent 50S particles during subunit assembly, although the precise stage at which this occurs is not yet certain (10,29). Moreover, the arrangement of components in the reconstituted complex may well represent their actual disposition in the ribosome since a ribonucleoprotein of similar composition and stoichiometry can be generated by nuclease digestion of the 50S subunit (28). A remarkable aspect of quaternary complex formation de-

rives from the fact that, while L5 and L18 each associate specifically with 5S RNA (13), none of the three molecules can bind independently to the 23S RNA (8,21). This problem would be resolved if interactions among L5, L18 and the 5S RNA were to result in cooperative structural changes which, in turn, increase the affinity of the components for the large ribosomal RNA. Indeed, the ability of L18 to alter both the conformation of the 5S RNA and its strength of interaction with L5 provides support for this suggestion (13, 23,30).

The site of 5S RNA integration is believed to lie some 500 to 1000 residues from the 3' terminus of the 23S RNA on the basis of nucleotide sequences present in a ribonucleoprotein fragment containing L5, L18, L25 and segments of both small and large RNA molecules (27). It is possible that a second integration site exists as well, since a sequence near the 5' terminus of the 23S RNA possesses extensive complementarity to a highly conserved region of the 5S RNA (31,32). These two loci are separated by at least 2000 bases, however, and if binding of the L5-L18-5S RNA subassembly were to bring them into close proximity, the 23S RNA should assume a more compact configuration and its diffusion constant should rise accordingly. In fact, the $D^{o}_{20,w}$ of the quaternary complex is somewhat lower than that of the free nucleic acid molecule and it is therefore unlikely that 5S RNA effects the juxtaposition of distant portions of the 23S RNA chain.

Cooperative interactions among 50S subunit components. Our past and present observations on the association of 50S subunit proteins with the 5S and 23S RNAs are summarized, together with the results of others, in Figure 7. The sites of attachment of ten RNA-binding proteins along the 23S RNA have been located with varying degrees of precision by three methods: investigation of nucleotide sequences protected from RNase digestion by individual proteins (25); interaction of single proteins or groups of proteins with specific fragments of the 23S RNA (18,24,26); and analysis of ribonucleoprotein fragments isolated by nuclease treatment of 50S subunits (26,27). The results obtained by the different approaches are in quite good agreement. Proteins L24 and L20 associate with the 5' and 3' ends of the 13S RNA, respectively (25), and circumstantial evidence suggests that L4 may interact with sequences at the middle of this fragment. The binding site for L23 has been placed near the center of the 8S RNA (25), although the relative positions of L2 and L13 are unknown. L1 has been shown to occupy the 5' end of the 12S fragment (25); the placement of L3 and L6 is once again arbitrary, however. Protein L14, which is unable to interact independently with the 23S RNA, binds to the 18S



Unassigned Proteins: L9, L15, L19, L29, L30, L31, L32, L33, L34

Figure 7. Partial reactions in 50S ribosomal subunit assembly. Heavy arrows represent direct and specific interaction of proteins with the 5S and 23S RNAs. Assignment of proteins to the 13S, 8S and 12S fragments of the 23S RNA was carried out as previously described (18,24; see text). The approximate positions of binding sites for L1, L20, L23 and L24 are indicated by solid bars (25). Interrupted bars marks possible locations of binding sites for L4 and L16 at junctions between the major subfragments. Dashed line in 13S RNA indicates the T_1 RNase cleavage site described in the text. L14 associates with the 18S RNA but not with intact 23S RNA (24); it has not yet been assigned to either the 8S or 12S subfragments. The ordering of protein binding sites on the 5S RNA has recently been reported (38). Light arrows denote cooperative interactions characterized by us in this and earlier communications (5), with the exception of the L11→L10→L7/L12 pathway which was described by Highland and Howard (34).

fragment (5), but has not been located in either of the subfragments that arise from it. Although L13 has been assigned to the central fragment in the assembly map, its association with the 13S fragment has also been reported (26). It is possible that this protein is capable of association with two distinct portions of the 23S RNA since it was found to be crosslinked to both 13S and 18S fragments prepared from formaldehyde-treated subunits (33).

The attachment of one or more RNA-binding proteins to the 23S RNA paves the way for a variety of cooperative interactions (Fig. 7). Thus, L21 and L22 associate with the 23S RNA in the presence of L20 and L24, respectively, L2 stimulates the binding of L10 and L11, L16 promotes the attachment of L5, L28 binds in the presence of L1, L3 and L6, and the L17/L27 mixture interacts with a complex containing L1, L3, L6, L13 and L23 (5; this report). The map has been extended to show assembly relationships among L10, L11 and L7/L12 that were inferred from experiments on the reconstitution of protein-deficient core particles (34). Interactions of the 5S RNA are also included in

the scheme. Although L5, L18 and L25 can all bind to the 5S RNA individually, L18 stimulates the association of L5 approximately tenfold (13) and the latter proteins together are responsible for the integration of the 5S and 23S RNAs (21; this report). In addition, L5 is linked to the 23S RNA via its cooperative interaction with L16. Figure 7 therefore relates most of the proteins present in the main 50S subunit assembly intermediate described by Nierhaus and Dohme (6) either directly or indirectly to the 23S RNA and accounts for 23 of the 32 proteins that must associate with the growing ribonucleoprotein particle. It is assumed that the map portrays only the strongest interactions, however, since the mature subunit is undoubtedly stabilized by a much more intricate array of protein-protein and protein-RNA contacts.

From the evidence presently available, two regions of the 23S RNA can be tentatively distinguished from one another on the basis of their structural and functional roles. The 5' end of the molecule, represented by the 13S RNA fragment, appears to be part of an *assembly nucleus* whose formation ensures the efficient accomplishment of further assembly reactions. Five proteins associated with this fragment, L4, L13, L20, L22 and L24, have been shown to stimulate a temperature-dependent conformational change in the RNA that is essential for the reconstitution of active 50S particles in vitro (6). Moreover, L4, L13, L20, L21, L22 and L24 are among the very few proteins that remain bound to ribonucleoprotein core particles derived from intact 50S subunits by treatment with LiCl or trypsin under relatively harsh conditions (35,36). In contrast, most of the protein groups believed to participate directly in protein biosynthesis are linked to the 3' half of the 23S RNA. These include L2, L11, L16, L18 and L27, which have been consistently identified with tRNA binding and peptidyl transferase activity, L2, L5, L7/L12, L10, L11, L18, L23, L25 and L28, which have all been implicated in factor binding and factor-dependent GTPase activity, and a number of proteins thought to mediate subunit-subunit interaction (1). In addition, many of the proteins in this *functional cluster* have been crosslinked to one another or to other proteins that associate with the 3' portion of the 23S RNA. Prominent among them are L2, L3, L5, L7/L12, L10, L11, L14, L17, L23 and L25 (37). Thus, despite the tightly folded configuration of the 23S RNA in the 50S subunit, the nucleic acid molecule can be resolved into two principal domains which differ markedly from each other in their biological activity.

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