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Evolution

Conservation of ribosomal protein binding sites in prokaryotic 16S RNAs

(ribosomal RNA/RNA–protein interaction/RNase digestion/molecular evolution)

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ABSTRACT **The *Escherichia coli* 30S ribosomal subunit proteins S4, S7, S8, S15, S17, and S20 that interact independently with 16S RNA from *E. coli* formed specific heterologous complexes with 16S RNAs extracted from 11 different prokaryotes covering a broad phylogenetic range. Complex formation was shown to be specific by saturation of binding in the presence of excess protein. Binding stoichiometries and the apparent affinities for a given protein varied depending on which 16S RNA was used, although the pattern of binding was not strictly correlated with phylogenetic relationships. The size-distribution of fragments resulting from limited hydrolysis of free prokaryotic 16S RNAs with T1 and pancreatic ribonucleases indicated that the structural organization of 16S RNA from *E. coli* is similar to that of 16S RNAs from closely related species, but differs, although to an unknown extent, from that of 16S RNAs from other prokaryotes tested. Digestions of RNA–protein complexes under similar conditions indicated that the proteins remain bound to specific RNA fragments. For those 16S RNAs isolated from species closely related to *E. coli*, the fragments were comparable to those generated by hydrolysis of the homologous complex.**

Both independent and cooperative interactions between ribosomal proteins and 16S RNA provide the basis for assembly of functional 30S ribosomal subunits in *Escherichia coli* (1–5). Binding sites for proteins S4, S7, S8, S15, S17, and S20, which associate independently with 16S RNA, have been localized on the 16S RNA molecule by partial RNase hydrolysis of protein–RNA complexes and by crosslinking with UV light (6–13). To further characterize the structural features of the 16S RNA molecule that are involved in these interactions, we have exploited the ability of ribosomal proteins from *E. coli* to form specific and functional complexes with 16S RNAs from other prokaryotes (14–16). Each of the six *E. coli* 30S subunit proteins that bind independently to homologous, phenol-extracted 16S RNA was tested for its capacity to form specific complexes with 16S RNAs from a number of other prokaryotes. The 16S RNAs were isolated from bacteria chosen to cover a wide phylogenetic range, including Gram-negative species closely related to *E. coli*, three species of *Bacillus*, a cyanobacterium, a Gram-positive anaerobe, and both purple and green sulfur bacteria (Fig. 1). Oligonucleotide catalogues available for several of the RNAs used here indicate that there are numerous differences in sequence among them (22–27) and, in two cases, it has been established that such differences occur within ribosomal protein binding sites (16, 28).

We report here that many specific heterologous complexes could be formed between *E. coli* ribosomal proteins and 11 different prokaryotic 16S RNAs. The ability of diverse prokaryotic RNAs to associate specifically with a unique set of *E. coli* ribosomal proteins reveals that ribosomal protein–RNA interactions have been extensively conserved throughout evolution and confirms the essential nature of such associations to proper ribosome assembly and function. The pattern of fragments obtained from RNase digests of the RNAs alone suggests that secondary, and possibly tertiary, structures of several of these 16S RNAs may be similar. Digestion of heterologous protein–RNA complexes implicates secondary and tertiary interactions in protein binding and demonstrates the feasibility of isolating protein-binding fragments. We expect that determination of the nucleotide sequences of the protein-binding regions from the different 16S RNAs will help to identify the structures within the RNA that are directly involved in protein–RNA interactions in the 30S subunit of prokaryotic ribosomes.

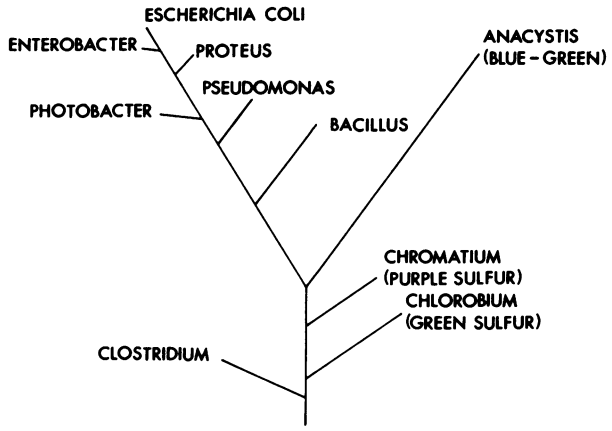

FIG. 1. Phylogenetic relationships among prokaryotes used in these experiments. The scheme is a tentative one based on sequence homologies among 5S RNA (17), cytochrome *c* (18), and ferredoxin (19) molecules, as well as on metabolic properties (20), of the organisms indicated and is not constructed to an absolute time scale. Strains used in the present study are: *Escherichia coli*, strain MRE600 (21), *Enterobacter aerogenes* (ATCC 10348), *Proteus vulgaris* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 10145), *Clostridium pasteurianum* (ATCC 6013), *Bacillus brevis* (ATCC 8185), *Bacillus stearotheromophilus* (ATCC 12980), *Bacillus subtilis*, strain 168 (provided by C. R. Thorne), *Photobacter* 8265 (provided by C. Woese), *Anacystis nidulans*, strain 28/10 (provided by R. J. Cedergren), *Chromatium vinosum*, strain D, and *Chlorobium limicola* (both provided by R. C. Fuller).

RESULTS

Some *E. coli* Ribosomal Proteins Bind to All Prokaryotic 16S RNAs Tested. Proteins S4, S7, S8, S15, S17, and S20 from *E. coli* were tested for their ability to interact with 16S RNAs from 11 other prokaryotes by measuring the cosedimentation of individual ^{3}H-labeled proteins with [^{14}C]RNA in sucrose

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Abbreviation: TMK, 0.05 M Tris·HCl, pH 7.6/0.02 M $MgCl_2$/0.35 M KCl.

gradients. Selected profiles for S4 and S7 incubated with 16S RNAs from four organisms are presented in Fig. 2. The results of such assays show that under our conditions each protein formed a specific complex with at least 5 different prokaryotic 16S RNAs and that two proteins, S8 and S15, associated with 10 of the 11 heterologous 16S RNAs (Table 1). Moreover, the proteins bound exclusively to 16S RNA when incubated with a mixture of 16S and 23S RNAs (unpublished data).

At least five of the six proteins tested interacted with 16S RNAs from species closely related to *E. coli*, such as *E. aerogenes* and *P. vulgaris*. However, 16S RNAs from more distantly related strains, such as *C. vinosum* and *A. nidulans*, bound an equivalent number of the *E. coli* proteins. It is thus evident that the ability of *E. coli* ribosomal proteins to associate with heterologous prokaryotic 16S RNAs is not solely dependent upon commonly accepted phylogenetic relationships (see Fig. 1).

Protein S4 interacted strongly with 5 of the 11 16S RNAs. The binding pattern for S17(S16) and, to a lesser extent, for S20 closely paralleled that for S4. These results are not surprising because the binding sites for all three proteins are located within the same region of the *E. coli* 16S RNA molecule (6, 7, 10, 11, 29).

Proteins S8 and S15, which have been shown to bind within a section of 150 bases near the middle of *E. coli* 16S RNA (7–10), associated with 10 of the 11 RNAs tested. Clearly, certain structural features of the S8/S15 binding region must be highly conserved. Because this part of *E. coli* 16S RNA is thought to consist of two adjacent hairpin loops (10, 11), it will be of particular interest to determine whether a similar secondary structure occurs in segments from other prokaryotic 16S RNAs that bind these two proteins.

Protein S7 from *E. coli* formed a specific complex with 8 of the 11 16S RNAs. Based on the concentration-dependence of binding, S7 appears to have a higher affinity for 16S RNAs from all three *Bacillus* spp. and from *C. vinosum* than for 16S RNA from *E. coli* (see *Discussion*).

Table 1. Binding of *E. coli* 30S subunit proteins to prokaryotic 16S RNAs

Source of 16S RNA	Molar protein:RNA binding ratio at saturation					
	S4	S7	S8	S15	S17 (S16)	S20
E. coli	1.0	0.8	0.8	1.0	0.5	0.6
E. aerogenes	1.0	+	+	0.3	0.6	0.5
P. vulgaris	1.0	0	+	0.9	0.7	+
P. aeruginosa	1.0	+	0.8	0.1	0.6	+
Photobacter 8265	1.0	0	0.9	0	0.5	0
C. vinosum	0.4	0.3	0.6	0.9	0.7	+
A. nidulans	+	+	0.4	1.8	0.3	0
B. subtilis	0	0.5	0.4	1.5	0	0
B. brevis	0	0.6	0	1.3	0	+
B. stearothermophilus	+	1.0	+	1.5	0	0
C. limicola	0	+	0.6	0.8	0	0
C. pasteurianum	0	0	0.7	1.5	0	0

30S ribosomal proteins S4, S7, S8, S15, S17(S16), and S20 from *E. coli* were individually tested for their ability to interact with 16S RNAs from 12 prokaryotes by the standard binding assay outlined in the legend to Fig. 2. The RNA-binding protein S17 was used in a mixture with S16, which does not bind independently to 16S RNA. Molar protein/RNA at saturation was computed for each complex as described by Muto *et al.* (9). The values for *E. coli* 16S RNA are from refs. 9 and 29. Symbols: +, binding with molar ratios <0.1 detected only for RNA concentrations >0.4 μM; 0, no binding detected.

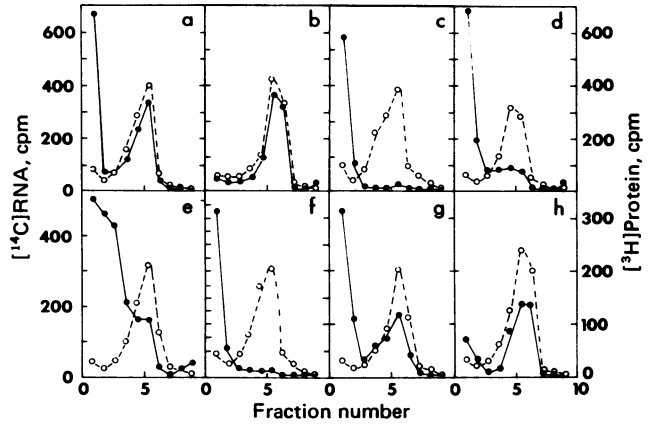

FIG. 2. Binding of *E. coli* 30S subunit proteins to prokaryotic 16S RNAs. *Preparation of components.* Exponentially growing bacteria were labeled with [^{14}C]uracil or ^{3}H-labeled amino acid mixture, harvested by centrifugation, and broken open by grinding with alumina. Following extraction in 10 mM Tris·HCl, pH 7.8/10 mM $Mg(OAc)_2$/60 mM $NH_4(OAc)$, the crude lysate was clarified and ribosomes were recovered by sedimentation for 90 min at 225,000 × *g*. 16S [^{14}C]RNA: After resuspension of the ribosomes in 10 mM Tris·HCl, pH 7.8/0.2 mM $MgCl_2$, RNA was isolated by phenol extraction and fractionated on 5–20% sucrose gradients in the same buffer. The 16S RNA was precipitated with 67% ethanol and resuspended in double-distilled water. ^{3}H-Labeled 30S subunit proteins: Ribosomes from *E. coli* MRE600 were resuspended in 20 mM Tris·HCl, pH 7.8/20 mM $Mg(OAc)_2$/500 mM NH_4Cl and washed by centrifugation through a cushion of 30% sucrose in this buffer. Ribosomal subunits were separated on 5–20% sucrose gradients in 10 mM Tris·HCl, pH 7.8/0.3 mM $Mg(OAc)_2$/30 mM $NH_4(OAc)$. The 30S subunit proteins were extracted with 67% HOAc and purified by chromatography on phosphocellulose (pH 6.5) and CM-cellulose (pH 5.6). These techniques have been described in detail (9). *Binding assay.* 16S [^{14}C]RNAs (5–30 μg) from *E. coli* (*a, e*), *P. vulgaris* (*b, f*), *B. subtilis* (*c, g*), and *C. vinosum* (*d, h*) were mixed with 1–2 molar equivalents of protein [^{3}H]S4 (*a, b, c, d*) or [^{3}H]S7 (*e, f, g, h*) in 100 μl of TMK buffer (0.05 M Tris·HCl, pH 7.6/0.02 M $MgCl_2$/0.35 M KCl) containing 5 mM 2-mercaptoethanol and 0.05% (wt/vol) bovine serum albumin. Mixtures were heated for 30 min at 40°, chilled for 15 min on ice, and centrifuged through 4-ml 3–15% sucrose gradients in TMK buffer for 3 hr at 50,000 rpm in a Spinco SW 60 rotor. After fractionation of the gradients, 200 μg of bovine serum albumin and 0.5 ml of 10% (vol/vol) Cl_3CCOOH were added to each tube and the precipitates were collected on glass-fiber filters. Radioactivity was measured in a scintillation counter with a cocktail containing 4 g of Omnifluor per liter of toluene. ^{3}H spillover in the ^{14}C channel was negligible; ^{14}C spillover in the ^{3}H channel varied (10–12%) and was substracted before the data were plotted. Because the specific activities of the components differed, radioactivity scales were varied accordingly. Marks on the left-hand ordinate of each frame correspond to 100 cpm of 16S [^{14}C]RNA, ○; marks on the right-hand ordinate correspond to 100 cpm of [^{3}H]protein, ●. Sedimentation is from left to right.

Heterologous Protein–RNA Interactions Are Specific. The specificity of all interactions with molar protein:RNA >0.1:1 has been confirmed by the saturation of binding in the presence of a 5-fold molar excess of protein. A binding ratio of approximately 1:1 is generally taken as evidence that RNA contains a single specific protein-binding site (3–5). Table 1 shows that, when complexes were formed, the 16S RNAs became saturated with individual proteins at a molar binding ratio of 1:1 or below, except in the case of S15. Representative saturation curves for S15, presented in Fig. 3, showed a range of plateau values from 0.1:1 to 1.8:1.

Prokaryotic Ribosomal RNAs Yield Characteristic RNase Digestion Patterns. Limited digestion of *E. coli* 16S RNA with

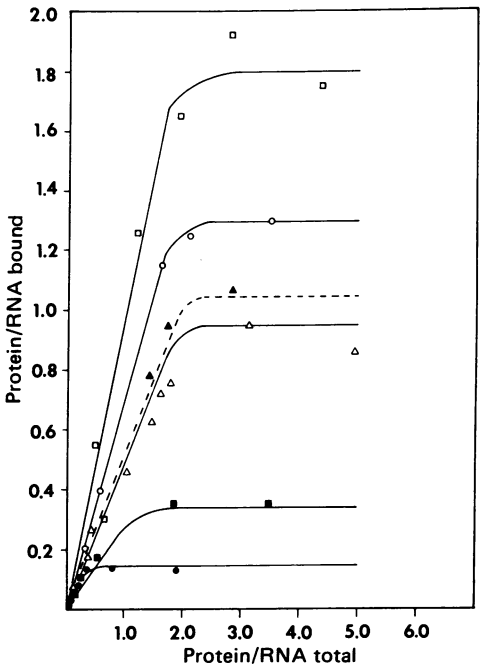

FIG. 3. Saturation of binding of *E. coli* S15 to prokaryotic 16S RNAs. 16S RNA (10 μg) was incubated with increasing amounts of [^{3}H]S15 and the complexes were analyzed as described in the legend of Fig. 2. The molar amounts of protein and RNA sedimenting in the 16S peak were determined by dividing the radioactivity of each component by its specific activity or, when unlabeled RNA was used, by absorbance measurements assuming $A_{260\,nm}^{1\,mg/ml} = 24$ for 16S RNA. The specific radioactivity of [^{3}H]S15 was 25 cpm/pmol and that of the various 16S RNAs ranged from 40 to 80 cpm/pmol. The molar ratio of protein and RNA in the complex was plotted vs. the molar ratio of total protein and RNA recovered in the gradient. The saturation curve for the binding of S15 to *E. coli* 16S RNA (9) is shown as a dashed line. Key to other 16S RNAs used: □, *A. nidulans*; ○, *B. brevis*; △, *C. vinosum*; ■, *E. aerogenes*; and ●, *P. aeruginosa*.

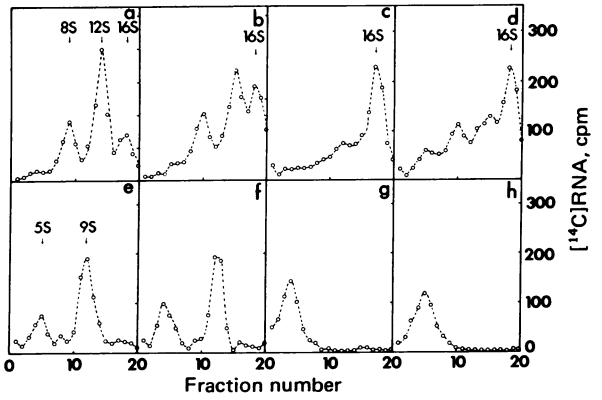

FIG. 4. Limited hydrolysis of prokaryotic 16S RNAs by T1 and pancreatic RNases. 16S [^{14}C]RNAs (25 μg) from *E. coli* (*a*, *e*), *Photobacter* (*b*, *f*), *B. brevis* (*c*, *g*), and *C. pasteurianum* (*d*, *h*) were incubated for 30 min at 40° in 100 μl of TMK buffer, chilled on ice, and treated for 30 min at 0° with 0.25 μg (1 unit) of T1 RNase (T1:RNA = 1:100) (*a*, *b*, *c*, *d*) or 5 μg of pancreatic RNase A (A:RNA = 1:5) (*e*, *f*, *g*, *h*). The mixtures were layered onto 12-ml 3–15% sucrose gradients in TMK buffer and centrifuged for 16 hr at 32,000 rpm in a Spinco SW 41 rotor; gradient fractions were analyzed and plotted as described in the legend of Fig. 2. Sedimentation is from left to right.

RNase T1 and pancreatic RNase A yielded characteristic fragment patterns as shown by the sucrose gradient profiles in Fig. 4 *a* and *e*. At RNase T1:RNA = 1:100 three main peaks were evident: undigested 16S RNA, a 12S fragment which has been shown to consist of about 900 nucleotides from the 5′ terminus of the 16S RNA, and an 8S fragment of 500–600 nucleotides extending from the 3′ terminus of the 12S fragment to within 50 bases of the 3′ terminus of the 16S RNA molecule (9).

Of the prokaryotic RNAs under investigation, the four isolated from *E. aerogenes*, *P. vulgaris*, *P. aeruginosa*, and *Photobacter* each yielded a fragment profile essentially identical to that of *E. coli* 16S RNA when digested with RNase T1 under the same conditions (see Fig. 4*b*). Digestion of the RNAs from the three *Bacillus* spp. and *C. limicola* resulted in a pattern in which a large portion of the 16S RNA seemed to remain intact and no discrete 8S or 12S fragments were evident, although some material in the 8S–12S size range did appear to be present (see Fig. 4*c*). The 16S RNAs from *C. pasteurianum*, *C. vinosum*, and *A. nidulans* gave intermediate patterns in which most of the 16S RNA appeared intact but some fragments sedimenting at approximately 8S and 12S were detectable (see Fig. 4*d*).

Hydrolysis of *E. coli* 16S RNA with pancreatic RNase A at enzyme:substrate = 1:5 generated two main products: a 9S fragment of about 500 nucleotides originating some 20 bases from the 5′ terminus of the 16S RNA molecule and a 5S fragment containing about 150 bases that arose from near the middle of the 16S RNA (9). Under these conditions, RNA from *E. aerogenes*, *P. vulgaris*, *P. aeruginosa*, and *Photobacter* each again gave a digestion profile nearly identical to that for *E. coli* 16S RNA (see Fig. 4*f*). Digestion of 16S RNA from *C. vinosum* with pancreatic RNase also produced fragments which sedimented at about 5S and 9S, although the yield of 9S fragments was much lower. The remaining six RNAs gave no detectable 9S fragments (see Fig. 4 *g* and *h*). Thus, in terms of the structural characteristics which determine susceptibility to nuclease attack, there are four 16S RNAs which were very similar to 16S RNA from *E. coli* and seven which differed, although to an unknown extent.

E. coli Ribosomal Proteins Are Retained by Specific Fragments of Prokaryotic 16S RNAs. When *E. coli* 16S RNA–protein complexes were digested under the conditions described above, the proteins remained bound to specific RNA fragments (6–11). Protein S4, for example, was retained by the *E. coli* 12S fragment derived from RNase T1 hydrolysis (Fig. 5*a*) and to the 9S fragment after digestion with RNase A (Fig. 5*e*). Analogous profiles were obtained for the 16S RNAs isolated from *E. aerogenes*, *P. vulgaris*, *P. aeruginosa*, and *Photobacter* (see Fig. 5 *b* and *f*). In a like manner, S15 remained associated with the *E. coli* 12S fragment from RNase T1 digestion (Fig. 5*c*) and with the 5S fragments produced by treatment with RNase A (Fig. 5*g*). When complexes of protein S15 and 16S RNAs from the same four species were treated with T1 and pancreatic RNases, comparable patterns of protein binding were obtained (see Fig. 5 *d* and *h*).

Ribosomal proteins also remained attached to specific RNA fragments during hydrolysis of other heterologous RNA–protein complexes with RNase T1 or pancreatic RNase A, although these fragments were not necessarily equivalent to those from *E. coli* 16S RNA. Protein S7, for example, cosedimented with discrete segments of RNA from all three *Bacillus* spp. (Fig. 6*a*) as well as from *C. vinosum*. Fragments of RNA retaining S8 were observed in digestion profiles of 16S RNA from *P. aeruginosa*, *C. vinosum*, *Photobacter*, *C. pasteurianum*, and *C. limicola* (Fig. 6*b*). Finally, fragments specific for S15 have been generated in all cases in which S15–16S RNA complexes could be formed (Fig. 6*c*).

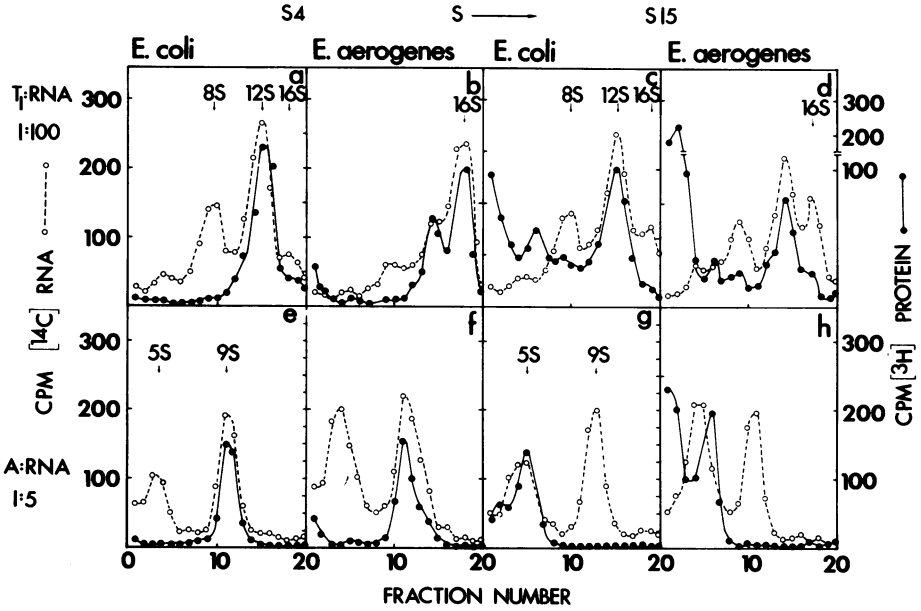

FIG. 5. Limited digestion of 16S RNA–protein complexes with T1 and pancreatic RNases. Complexes of 16S [^{14}C]RNA from *E. coli* and *E. aerogenes* with [^{3}H]S4 (*a, b, e, f*) and [^{3}H]S15 (*c, d, g, h*) from *E. coli* were formed by incubation in TMK buffer for 30 min at 40°; they were chilled, and then digested for 30 min at 0° with T1 RNase (T1:RNA = 1:100) (*a, b, c, d*) or pancreatic RNase A (A:RNA = 1:5) (*e, f, g, h*). Reaction mixtures were centrifuged through 3–15% sucrose gradients for 16 hr at 32,000 rpm in a Spinco SW 41 rotor. Analysis of the gradient fractions was as in the legend of Fig. 2. ○, [^{14}C]RNA; ●, [^{3}H]protein. Sedimentation is from left to right.

DISCUSSION

The ability of six RNA-binding proteins from the 30S ribosomal subunit of *E. coli* to interact with 16S RNAs from a wide variety of prokaryotes, as shown by the present results and by those of others (14–16), indicates that such associations have been highly conserved throughout evolution. We infer that once functional protein–RNA interactions evolved, the essential structural basis for complex formation persisted in relatively unchanged form throughout the development of these organisms. Although we cannot yet say which properties of the primary, secondary, or tertiary structures of the 16S RNA must be maintained, sequence analysis of the heterologous binding fragments should provide detailed information toward this end.

All of the heterologous interactions observed appear to be site-specific as shown by the saturation of binding in the presence of excess protein. Nonetheless, binding stoichiometries for each protein differ with the particular 16S RNA used, as can be seen from the variation in saturation plateau values for S15 in Fig. 3. Molar protein:RNA <1:1 may simply mean that some of the attachment sites in the RNA are inaccessible for protein interaction. However, the highly reproducible association of more than one molecule of S15 per molecule of 16S RNA from three species of *Bacillus*, from *C. pasteurianum*, and from *A. nidulans* remains puzzling.

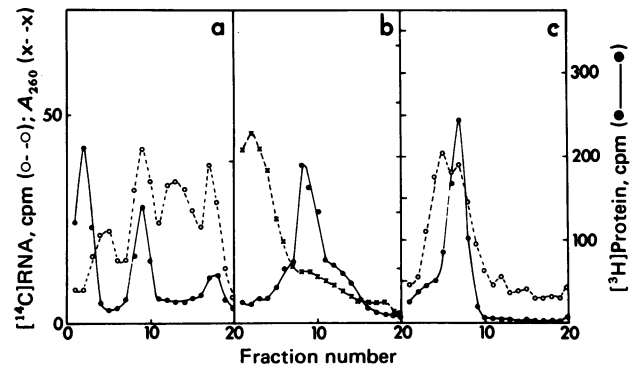

FIG. 6. Isolation of prokaryotic RNA fragments bound to *E. coli* ribosomal proteins. ^{3}H-Labeled proteins S7, S8, and S15 from *E. coli* were incubated with [^{14}C]RNA from *B. stearothermophilus* (*a*), *C. limicola* (*b*), and *A. nidulans* (*c*), respectively, and treated with T1 or pancreatic RNase as described in Fig. 5 except that T1:RNA = 1:20 was used for *a* and *b* and A:RNA = 1:5 was used for *c*. Incubation mixtures were fractionated and analyzed in the standard way except that the RNA profile in *b* was monitored by absorbance at 260 nm (100 is equivalent to $A_{260\ nm}$ = 0.5). Sedimentation is from left to right.

Quantitative variation in the heterologous protein–RNA interactions can also be considered in terms of thermodynamic stabilities. Direct measurement of association constants was not feasible since the complexes were analyzed in sucrose gradients under nonequilibrium conditions. It is possible to derive minimum estimates of the affinities, however, from the fact that all incubations were carried out at protein and RNA concentrations of 0.1–1 μM. Assuming in addition that the complexes were diluted by an order of magnitude during centrifugation, we conclude that the binding constants for the interactions described here are at least 10^{7}–10^{8} M^{-1}. These estimates are likely to be accurate for associations that could be detected only when RNA concentrations were raised from 0.1 to 0.4 μM and are consistent with values reported for other ribosomal protein–RNA interactions (ref. 30; unpublished data). In several cases, we failed to detect binding even when RNA and protein were present at 0.4 μM or greater. Although we cannot exclude the possibility that complex formation might occur at still higher component concentrations, the corresponding association constants must be less than 10^{7} M^{-1}. From this argument, it is evident that certain RNAs differ substantially in their affinities for a given protein. As a specific example, *E. coli* S7 associated with 16S RNAs from all three *Bacillus* species and from *C. vinosum* at RNA concentrations approximately 1/5 of those required for interactions with *E. coli* 16S RNA. The heterologous complexes therefore were more stable than the homologous complex.

The pattern of fragments produced by limited RNase digestion of free prokaryotic 16S RNAs suggests that many of them possess a similar structural organization. Our reasoning is based on the observation that the 12S and 8S fragments generated by RNase T1 hydrolysis of *E. coli* 16S RNA contain a number of hidden breaks (31). Hence, their hydrodynamic stability can be accounted for only if the discontinuous sequences are linked together by secondary and tertiary interactions within the RNA molecule. Such interactions apparently exist in 16S RNAs from *E. aerogenes*, *P. vulgaris*, *P. aeruginosa*, and *Photobacter* as well, since their RNase T1 digestion profiles closely resembled the profile of *E. coli* 16S RNA. Moreover, the

16S RNAs of *C. pasteurianum*, *C. vinosum*, and *A. nidulans* may also have similar structural features although the digestion data are less convincing in these instances. The greater resistance of the four other prokaryotic 16S RNAs under these conditions could mean either that the sequence joining the 12S and 8S domains is less suceptible to nuclease attack or that the two segments are spanned by RNA·RNA interactions not present in 16S RNA from *E. coli*.

Our findings also suggest that the secondary and tertiary folding of the 16S RNA is important in its association with ribosomal proteins as well as in the maintenance of its structure *per se*. Partial hydrolysis of *E. coli* 16S RNA with pancreatic RNase A produced a 9S fragment consisting of several noncontinuous sequences from the 5′-terminal 550 residues of the molecule that together comprise the binding region for S4 (7, 29, 32, 33). In this report we have shown that, of 12 different prokaryotic 16S RNAs, only those that associated strongly with S4 also yielded stable 9S fragments when digested with pancreatic RNase A. The ability of the ribosomal RNAs to bind S4 may therefore be governed by the same intramolecular interactions that preserve the integrity of the 9S fragments. We cannot yet say whether the particular configuration adopted by the nucleic acid chain is necessary to position short contact sites correctly in the primary sequence or whether specific features of the secondary and tertiary structures are themselves recognized by complementary portions of the interacting proteins.

The S8/S15 binding region, which consists of about 150 nucleotides near the middle of *E. coli* 16S RNA, may prove to be more useful in elucidating structural homologies because it is relatively small and thought to be mainly double-stranded (7–11). In addition, both S8 and S15 associated with most of the prokaryotic 16S RNAs tested here and, after RNase hydrolysis, remained attached to small, specific RNA fragments that can be readily isolated for further structural analysis. The importance of RNA secondary structure in this region has recently been affirmed through analysis of a segment of *B. stearothermophilus* 16S RNA protected from nuclease digestion by S8 (28). This region of RNA can be arranged in two adjacent hairpin loops comparable to those proposed for the corresponding segment from *E. coli* 16S RNA, whereas the primary sequences are only partially homologous. It is our hope that the isolation and sequencing of analogous fragments from several different 16S RNAs will lead to a better understanding of the structural basis for RNA–protein interaction in the prokaryotic ribosome.

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