RNA (2000), *6*:402–408. Cambridge University Press. Printed in the USA.

A hierarchy of RNA subdomains in assembly of the central domain of the 30 S ribosomal subunit

SULTAN C. AGALAROV[1,2] and JAMES R. WILLIAMSON[1]

[1]Department of Molecular Biology, The Scripps Research Institute, La Jolla, California 92037, USA
[2]Institute for Protein Research, Russian Academy of Sciences, Pushchino, Russia

ABSTRACT

Beginning with the framework that has been developed for the assembly of the 30 S ribosomal subunit, we have identified a series of RNAs that are minimal binding sites for proteins S15, S6, S18, and S11 in the central domain from *Thermus thermophilus*. The minimal binding RNA for proteins S15, S6, and S18 consists of helix 22 and three-way junctions at both ends composed of portions of helices 20, 21, and 23. Addition of the remaining portion of helix 23 to this construct results in the minimal site for S11. Surprisingly, almost half of the central domain (helices 24, 25, and 26) is dispensable for binding the central domain proteins. Thus, at least two classes of RNA elements can be identified in ribosomal RNA. A protein-binding core element (such as helices 20, 21, 22, and 23) is required for the association of ribosomal proteins, whereas secondary binding elements (such as helices 24, 25, and 26) associate only with the preformed core RNP complex. Apparently, there may be a hierarchy of ribosomal RNA elements similar to the hierarchy of primary, secondary, and tertiary binding ribosomal proteins.

Keywords: 30 S ribosome; ribosomal proteins; ribosome assembly; RNA–protein interactions; rRNA

INTRODUCTION

RNA–protein and protein–protein interactions play a key role in the structural organization and assembly of the ribosomal subunit. Despite numerous studies carried out over the past 30 years, the detailed structure and the mechanism of assembly of the subunit are still poorly understood. It is well known that the assembly process is sequential and highly cooperative. Only a few primary binding proteins interact directly and independently with 16 S RNA (Held et al., 1974). The secondary binding proteins require prior binding of a primary binding protein, and tertiary binding proteins require the presence of a primary binding protein and at least one secondary binding protein for incorporation into the assembling subunit. The primary binding proteins are a natural starting point for a comprehensive study of RNA–protein interactions in the 30 S subunit. Minimal RNA-binding sites for the S7, S8, and S15 proteins have been developed to study these interactions in greater detail (Dragon & Brakier-Gingras, 1993; Mougel et al., 1993; Wu et al., 1994; Batey & Williamson, 1996; Serganov et al., 1996). However, little progress has been made in understanding the incorporation of the secondary and tertiary binding proteins that are critical for the mechanism of ribosomal assembly.

Protein S15 binds independently to the central domain of 16 S RNA at the early stage of ribosome assembly, whereas proteins S6 and S18 bind only after S15 is bound, according to the assembly map of the 30 S subunit (Held et al., 1974). The subsequent binding of protein S11 depends on the presence of proteins S15, S6, and S18 (Held et al., 1974). Proteins S6/S18 apparently bind either cooperatively or as a heterodimer, and show very low binding to rRNA in the absence of S15 (Held et al., 1974; Gregory et al., 1984). The goal of the present study is to identify small RNA fragments that bind one or more secondary and tertiary binding proteins in addition to a primary binding protein. We have focused on preparing minimal RNP complexes derived from the central domain of the 30 S subunit that contain S15, S6, S18, and S11.

These four proteins have been shown by hydroxyl radical footprinting on 16 S RNA to interact exclusively within the central domain (Powers & Noller, 1995). In addition, proteins S15, S6, and S8 have been localized to the central domain in the 5.5 Å electron density maps for the 30 S ribosomal subunit (Clemons et al., 1999).

Reprint requests to: James R. Williamson, Department of Molecular Biology, MB-33, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA; e-mail: jrwill@scripps.edu.

The minimal S15 binding site is a fragment containing the three-way junction between helices 20, 21, and 22 (Batey & Williamson, 1996; Serganov et al., 1996). Recently, it was demonstrated that the central domain of the 16 S RNA (nt 547–895) from *Thermus thermophilus* can independently and specifically assemble with a defined subset of five ribosomal proteins, S6, S8, S11, S15, and S18 (Agalarov et al., 1998). Beginning with this central domain fragment, specific deletions were made in the RNA, and then the RNA fragments were incubated with a fractionated mixture of six ribosomal proteins (TP6). The reconstituted RNP complexes were purified from unbound proteins by sucrose gradient centrifugation, and the protein composition of the complexes was examined using SDS-PAGE. Our results demonstrate that a 127-nt RNA fragment composed of helices 22 and 23 and the three-way junction between helices 20, 21, and 22 is sufficient to bind ribosomal proteins S15, S6/S18, and S11. A slightly smaller 104-nt RNA fragment lacking the distal portion of helix 23 (nt 665–691) is sufficient to bind proteins S15 and S6/S18. The previously characterized S15–RNA complex, the new S15/S6/S18–RNA and S15/S6/S18/S11–RNA complexes, and the central domain complex provide model systems for a series of intermediates that recapitulate the 30 S subunit assembly pathway.

RESULTS

Fractionation of the 30 S ribosomal proteins

Typically, reconstitution of 30 S subunits, or of individual domains, is performed with a complete mixture of proteins from the 30 S subunit (TP30). However, for shorter RNA fragments, using the same molar ratio between the proteins and RNA results in a large increase in the total protein-to-RNA mass ratio in the reconstitution mixture. A high protein-to-RNA mass ratio promotes nonspecific aggregation, resulting in a great decrease in the yield of the reconstituted RNPs. Fortunately, treatment of the *T. thermophilus* 30 S subunit with 5 M LiCl dissociates most of the proteins, leaving only six core proteins, S6, S8, S11, S15, S17, and S18, still bound to 16 S RNA (data not shown). This RNP can be collected by high-speed centrifugation, and subsequent dissociation of the bound proteins from 16 S RNA gives a mixture of the six core proteins (TP6). The components of the TP6 mixture can be readily separated using one-dimensional SDS gel electrophoresis. Conveniently, five of the six proteins in TP6, the exception being S17, are central-domain-binding proteins. In our reconstitution experiments with smaller RNA fragments, we used this TP6 mixture instead of TP30. The protein-to-RNA mass ratio is three times lower for the same protein-to-RNA molar ratio. The convenient preparation and analysis of the TP6 mixture greatly facilitates the monitoring of the reconstitution procedure with smaller RNAs.

Systematic deletions of the central domain RNA

Beginning with the previously characterized *Tth* central domain RNA (Agalarov et al., 1998), a series of deletions (Fig. 1) were constructed using the Quikchange mutagenesis protocol (Stratagene).

In the first deletion construct, helix 21 of 16 S RNA, which has been identified as the binding site of the primary binding protein S8, was removed (Mougel et al., 1993; Wu et al., 1994). In addition, most of helix 21 is known to be dispensable for the binding of the primary binding protein S15 (Batey & Williamson, 1996; Serganov et al., 1996). Reconstitution of the central domain of 16 S RNA lacking helix 21 (*Tth* T2 RNA; Fig. 1) results in a stable RNP complex that contains proteins S15, S6/S18, and S11, but not protein S8 (data not shown).

Hydroxyl radical footprinting experiments indicate that the loop of helix 26 is the region of 16 S RNA contacted by proteins S6/S18, and several nucleotides of helix 24 make contacts with protein S11 (Powers & Noller, 1995). To test if these regions are necessary for binding of proteins S6, S18, and S11, we prepared a series of 3′-terminal deletions of the *Tth* T2 RNA by runoff transcription from plasmid DNA truncated using four restriction sites (Fig. 1). Surprisingly, after reconstitution with TP6 and separation of the RNP complexes from unbound proteins, all of these fragments were still capable of binding proteins S15, S6, S18, and S11 (data not shown). Thus, helices 24, 25, and 26 do not contain important determinants for high-affinity binding of these four ribosomal proteins. A minimal construct was prepared lacking the residual nonpaired nucleotides from the 5′ and 3′ ends of the *Tth* T2 *Bsa*WI runoff transcript, which is the *Tth* T3 RNA (127 nt), composed of helix 22 and 23 and the three-way junction between helices 20, 21, and 22 (Fig. 1). As expected, reconstitution of the *Tth* T3 RNA with TP6 resulted in a stable RNP complex containing proteins S15, S6, S18, and S11 (Fig. 2).

There are 8 nt in the distal portion of helix 23 that are strongly protected from hydroxyl radicals by binding of protein S11 (Powers & Noller, 1995), and it seemed likely that this region was an important determinant for S11 binding. Deletion of the distal portion of helix 23 resulted in the *Tth* T4 RNA 104 nucleotide fragment. Reconstitution of this RNA with TP6 yielded a stable RNP complex containing proteins S15, S6, and S18, but lacking protein S11 (Fig. 2).

This fragment differs from the S15 minimal RNA binding site mainly by the presence of the proximal portion of helix 23. To demonstrate that this is the RNA region necessary for binding of S6 and S18, we reconstituted

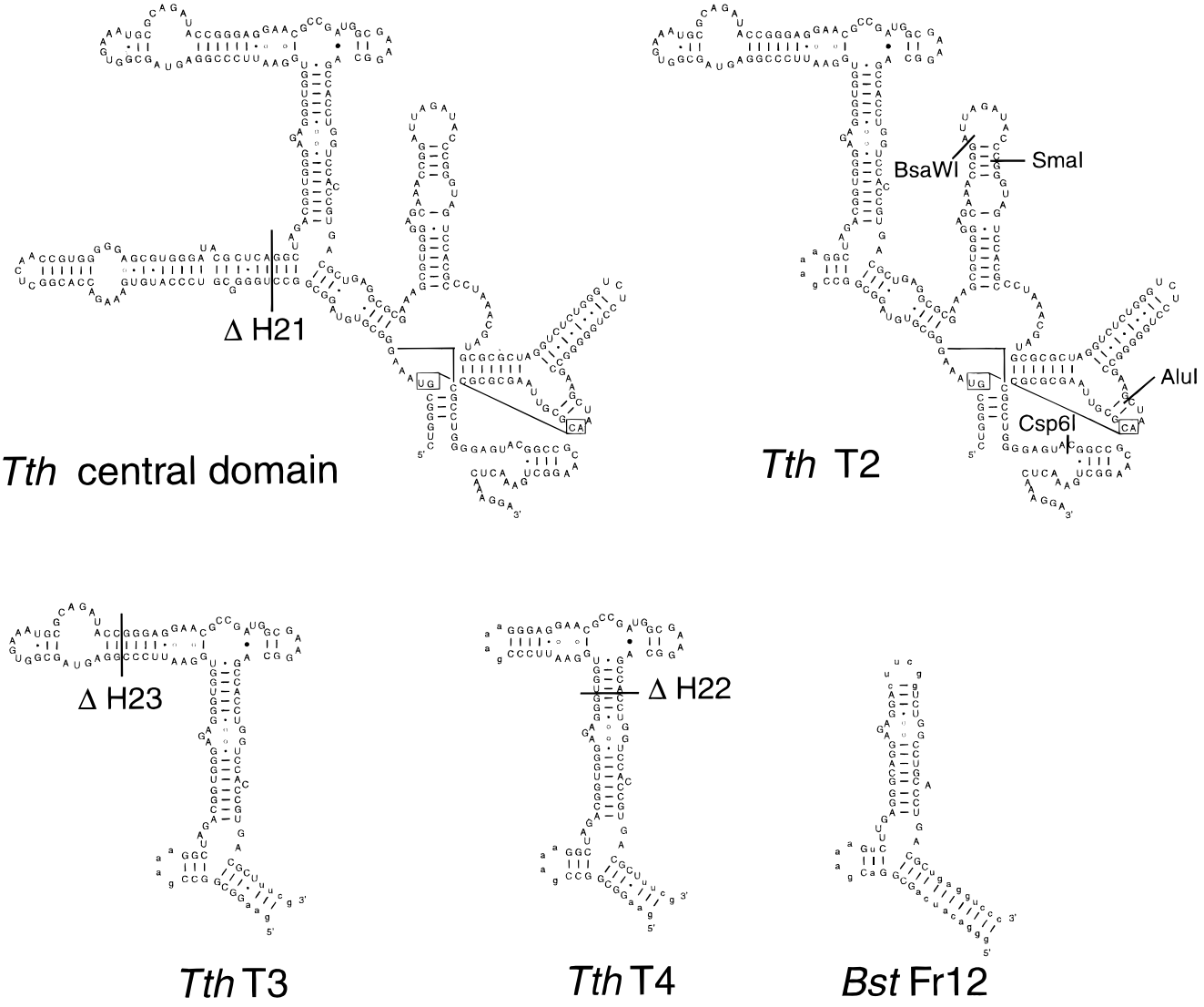

FIGURE 1. Secondary structures of central domain RNA constructs. The T2 RNA was constructed from the *T. thermophilus* central domain RNA (Agalarov et al., 1998) by deletion of helix 21 containing the S8 binding site, capping the short helix with a GAAA tetraloop. A series of 3′ terminal deletion fragments of the T2 RNA were prepared by runoff transcription at the four restriction sites shown. The T3 RNA was constructed by deletion of helices 24, 25, and 26 from the T2 RNA. The T4 RNA was constructed by deletion of the distal portion of helix 23, capping the helix with a GAAA tetraloop. The *B. stearothermophilus* Fr12 fragment contains the S15 binding site (Batey & Williamson, 1996). Nonribosomal nucleotides are indicated in lower case.

the *Bst* Fr12 RNA fragment (S15 minimal binding site from *Bacillus stearothermophilus*) with TP6 and only protein S15 was bound (Fig. 2). Hence, the proximal portion of helix 23 is the important site for specific interaction of S6 and S18 with 16 S rRNA.

To estimate the compactness and homogeneity of the RNP complexes, analysis of the sedimentation velocities in an analytical ultracentrifuge has been carried out. A representative experiment is shown in Figure 3, and the results for the series of RNPs are summarized in Table 1. All of the fragments and RNPs were homogeneous with the exception of the T3 RNP, which exhibits a tendency for dimerization that can also be seen in its sucrose gradient profile (Fig. 4). This heterogeneity in the T3 RNP makes it difficult to determine the true sedimentation coefficient of T3 RNP monomer.

DISCUSSION

The sedimentation behavior for compact globular particles follows a well-defined relationship. A graph of the logarithm of the sedimentation coefficient S, corrected for the Archimedes factor, $1 - \nu\rho$, versus the logarithm of molecular mass M for different ribosomal RNA and RNP particles is shown in Figure 5. The data for the RNAs and RNPs fit well to the theoretical line with a slope of 2/3. The points for Fr12 RNA, the complex of Fr12 with S15, and the T4 RNP all lie on this straight

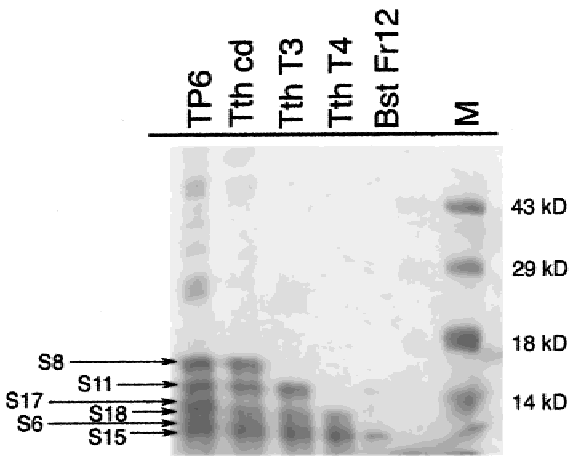

FIGURE 2. SDS-PAGE analysis of ribosomal RNA–protein complexes. Lane TP6 contains six proteins that were fractionated from *T. thermophilus* subunits. Proteins were identified based on the known mobilities of the *T. thermophilus* proteins (Eliseikina et al., 1995; Tsiboli et al., 1994). Lane Tth cd contains proteins isolated by reconstitution of Tth cd RNA with TP6, consistent with the previous results of Tth cd reconstitution with TP30 (Agalarov et al., 1998). Lane Tth T3 contains proteins S15, S6, S18, and S11 isolated by reconstitution of Tth T3 RNA with TP6. Lane Tth T4 contains proteins S15, S6, and S18 isolated by reconstitution of Tth T4 RNA with TP6. Lane Bst Fr12 contains protein S15 isolated by reconstitution of Bst Fr12 with TP6. Lane M contains standard molecular weight markers.

line and, therefore, they are highly compact. The points for free T3 and T4 RNA lie below the line (i.e., they are less compact). Apparently, proteins S15 and S6/S18 bind to the RNA fragment, inducing a conformational change in the RNA structure, resulting in a highly compact particle. We believe that the overall shape of the T4 RNP is consistent with their geometry within the 30 S subunit. In fact, the recent 5.5 Å crystal structure

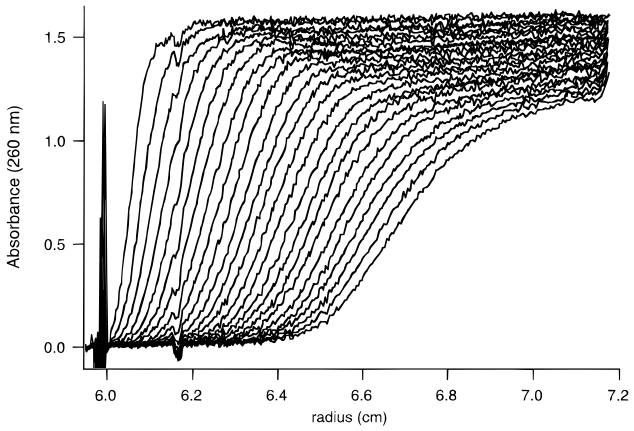

FIGURE 3. Sedimentation velocity profile for the T4 RNP. Absorbance scans were recorded every 2.2 min at 260 nm. Traces are shown for every other scan from 100 min to 206 min. Sedimentation coefficients were determined from the data by a global fit to multiple traces with well-resolved baselines using the program Svedberg.

TABLE 1. Sedimentation coefficients for central domain RNAs and RNPs.

	$S_{20,w}$	MW (kD)	$S/(1-\nu\rho)$
Fr12 RNA	3.9	25	8.4
T4 RNA	4.3	35.5	9.3
T3 RNA	4.9	43.5	10.6
Fr12•S15	4.3	35.5	10.5
T4 RNP	5.8	70	15.7
T3 RNP	6.1	93	16.9

of the *Tth* 30 S subunit revealed the positions of S15 and S6 near helix 22 in the central domain, and this geometry is consistent with our observations (Clemons et al., 1999). The T3 particle may also be compact, but it is difficult to determine the S value for the T3 RNP because of its tendency for dimerization or aggregation.

The minimal sites for the primary binding proteins S8 and S15 have been previously identified, and the hydroxy radical footprints (Powers & Noller, 1995) for all of the central domain proteins are shown in Figure 6. There are a variety of other chemical protection data for the central domain; however, we present only the hydroxy radical footprints, which generally give the most localized protections. In the present study, we have determined RNA regions that are the most important determinants for interaction of the secondary binding proteins S6 and S18 and the tertiary binding protein S11, which are the proximal and distal portions of helix 23, respectively. These regions of the RNA coincide with a part of the hydroxy radical footprints for these proteins on 16 S rRNA. In addition, portions of helix 23 were identified in RNase-resistant RNPs containing proteins S15, S8, S6, and S18 (Gregory et al., 1984). However, in addition to their footprints on helix 23, proteins S6 and S18 also footprint the loop of helix 26, and protein S11 footprints with helix 24 (Fig. 6; Powers &

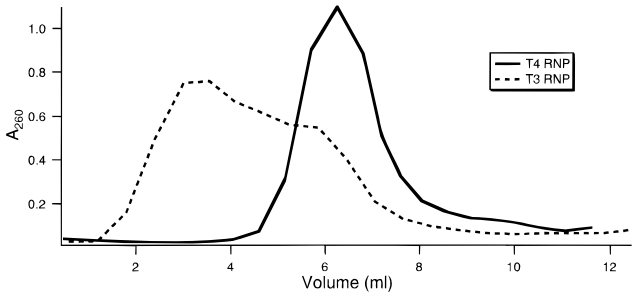

FIGURE 4. Sucrose gradient profiles from purification of central domain RNPs. Traces for the T3 and T4 RNPs are shown from the sucrose gradient purification after reconstitution. The top of the gradient is at the right of the graph. The T4 RNP is very homogeneous; however the T3 RNP displays a tendency for aggregation, as can be seen from the high molecular weight peak observed.

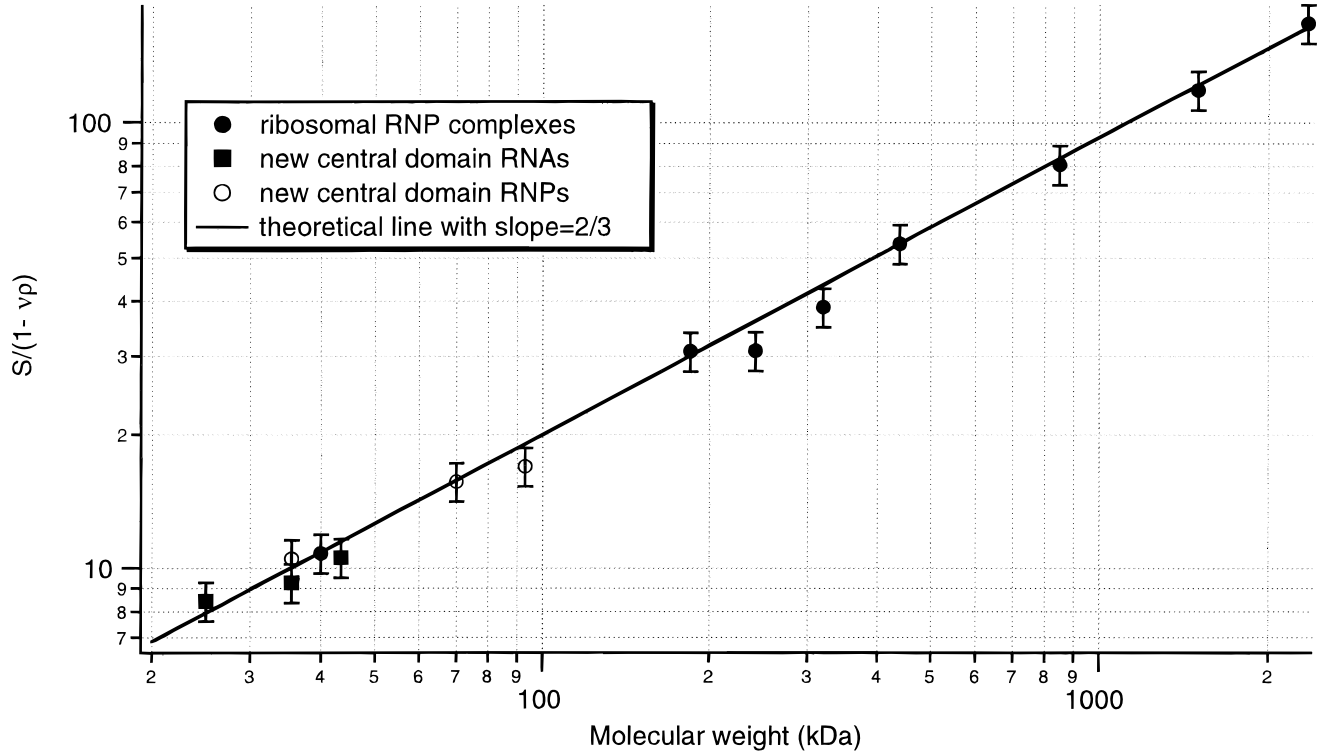

FIGURE 5. Comparison of the sedimentation coefficients for a series of ribosomal RNA protein complexes. ●: Previously characterized RNAs and RNPs: 5 S RNA (40 kD); central domain RNP (185 kD) (Agalarov et al., 1998); 5′-domain RNP (242 kD) and 3′-domain RNP (320 kD) (Agalarov et al., 1999); 22 S 5′+ central domain RNP (Ulitin et al., 1997); 30 S subunit; 50 S subunit; and 70 S ribosome. ■: Central domain RNAs from this study: *Bst* Fr12 (25 kD); *Tth* T4 (35.5 kD); *Tth* T3 (43.5 kD). ○: Central domain RNPs from this study: *Bst* Fr12-S15 complex (35.5 kD); T4 RNP (70 kD); T3 RNP (93 kD). The line is a least-squares fit to the theoretical and empirically observed $M^{2/3}$ dependence of the sedimentation coefficient for the 5 S RNA, central domain, 22 S RNP, 30 S and 50 S subunits, and the 70 S ribosome. The error bars were arbitrarily set to ±10% of the reported S values as a gross overestimation of the experimental errors, which are typically ≪5%. Compact near-spherical particles give values on the theoretical line, whereas significant deviations observed for the T3 RNP, as well as the 5′- and 3′-domain RNPs, suggest these RNPs are not compact.

Noller, 1995). Protein S8 similarly footprints a region of helix 25, but its primary binding site is localized within helix 21. Our experiments confirm that helix 25 is not a strong binding site for S8, as the RNA fragment lacking helix 21 in which other structural elements including helix 25 are present does not bind protein S8 under our reconstitution conditions. For S8, S6, S18, and S11, it is possible to distinguish the primary binding site on the RNA from the secondary site of interaction.

The most significant result of our studies is that only helices 21, 22, and 23 and the three-way junction between helices 20, 21, and 22 are required for binding of all of the central domain proteins, with the exception of S8, as a stable RNP. Surprisingly, almost half of the central domain RNA is dispensable for protein recognition. This remarkable fact will facilitate the study of RNA–protein interaction at a structural level. Toward this end, we have succeeded in obtaining crystals of the T4 RNP that diffract to 2.6 Å, and we are proceeding with determination of the structure of this RNP (S. Agalarov, S. Prasad, P. Funke, D. Stout, and J. Williamson, unpubl. results).

The fact that half of the central domain RNA is dispensable for binding the central domain proteins may reflect an important principle of ribosome assembly. Our results demonstrate that a core RNA consisting of helices 20, 21, 22, and 23 is capable of nucleating a core RNP containing proteins S15, S8, S6, S18, and S11. The helix 24, 25, and 26 region corresponds to a secondary element that can only dock to the core element. Thus, there is a hierarchy of RNA elements, where core elements bind the proteins and secondary elements bind to the core RNP.

The hierarchical assembly of the central domain shows a very strong parallel to the folding of the *Tetrahymena* ribozyme, which consists of two phylogenetically conserved domains. The first domain, composed of the P4–P6 regions, forms a very stable, independently folding structure that folds before the second domain, both kinetically and thermodynamically (Laggerbauer et al., 1994; Zarrinkar & Williamson, 1994). The second domain, composed of the P3–P7 regions, does not form a stable structure, but assembles onto the preformed P4–P6 domain (Doudna & Cech, 1995). In

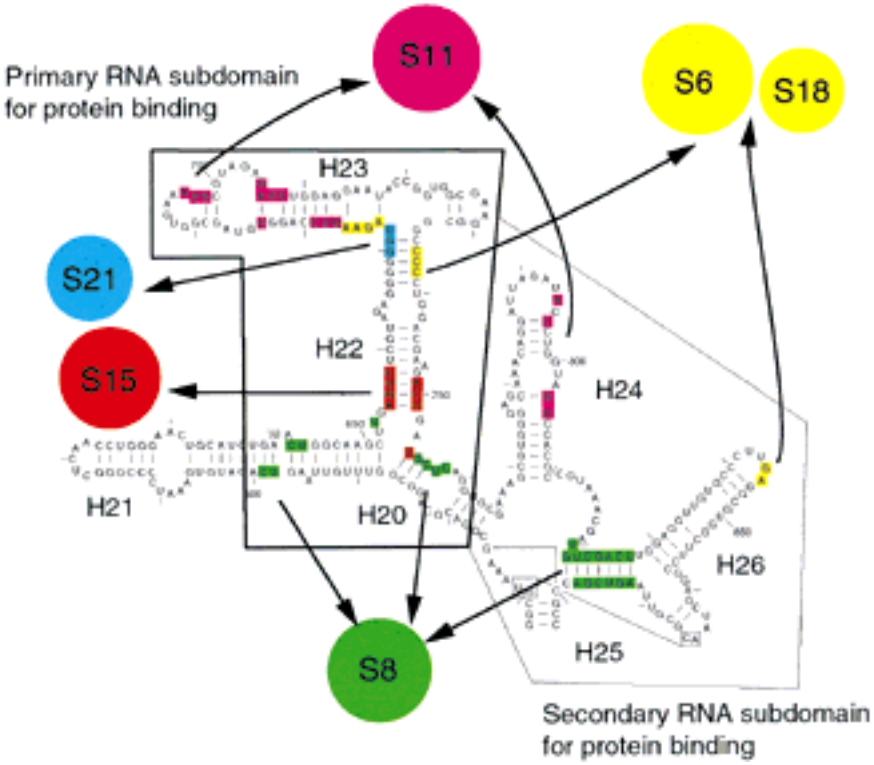

FIGURE 6. Proposed hierarchy of protein-binding RNA subdomains in the central domain. The hydroxy radical footprints for each of the central domain proteins is superimposed on the secondary structure of the *E. coli* central domain (Powers & Noller, 1995). The footprints are color coded as S15 (red), S6/S18 (yellow), S11 (magenta), S8 (green), S21 (blue). Note that there is no counterpart to S21 in *T. thermophilus*. The core central subdomain RNP is composed of helix 20 and the proximal portions of helices 21, 22, and 23, which are sufficient to assemble an RNP-containing proteins S8, S15, S6, S18, and S11. This RNA corresponds to the region primarily responsible for protein binding. The secondary binding subdomain, composed of helices 24, 25, and 26, is not necessary to form the core RNP, but nevertheless can assemble onto this preformed complex to form a compact RNP. Note that the footprints for proteins S8, S6, S18, and S11 are bipartite, with one portion of each footprint residing in each of the two subdomains.

the *Tetrahymena* ribozyme, the functionally important residues for substrate binding are in the P3–P7 domain, and the P4–P6 domain appears to be largely responsible for providing a scaffold for stabilization of the P3–P7 domain. This may be an additional parallel to the central domain, where the core RNP serves as a scaffold for the functionally important regions, such as the universally conserved 790 loop, which is important for P-site tRNA association.

By analogy to the *Tetrahymena* ribozyme, we propose that the central domain RNA can be divided into two subdomains. The region corresponding to the T3 RNA constitutes a primary subdomain for protein binding, and the helix 24, 25, and 26 region corresponds to a secondary subdomain for protein binding (Fig. 6). Thus, in general, it may be that primary protein-binding subdomains of rRNA bind a certain set of ribosomal proteins forming a compact RNA–protein framework, and only this preformed RNP is recognized by other secondary subdomains of rRNA. In principle, this hierarchy of RNA subdomains could apply to the whole ribosome, as well as other large RNPs. Further investigations with both the 30 S and 50 S subunits will reveal if this concept is general, as the complex process of ribosome assembly continues to be elucidated.

MATERIALS AND METHODS

Ribosomal 30 S subunits were isolated from ribosomes of *T. thermophilus* HB8 according to Gogia et al. (1986).

RNP particles containing six ribosomal proteins were obtained by treating 30 S subunits with a solution of 5 M LiCl, 100 mM $MgCl_2$, in 20 mM Tris-Cl, pH 7.6, at 25 °C for 1 h. The particles were collected by centrifugation at 50,000 rpm for 6 h at 20 °C in a 50Ti rotor (Beckman). To obtain TP6, the RNP particles were incubated with 6 M LiCl in 20 mM Tris-Cl, pH 7.6, at 4 °C for 1 h. The precipitated 16 S rRNA was removed by low-speed centrifugation, and the supernatant containing TP6 was dialyzed against the reconstitution buffer: 20 mM Tris-HCl, pH 7.6, 20 mM $MgCl_2$, 330 mM KCl.

DNA fragment corresponding to the central domain (nt 547–895 in the 16 S rRNA sequence of *T. thermophilus*) was inserted in the plasmid pGEM-9Zf(−) under control of the SP6 promoter (Agalarov et al., 1998). Deletions were made from this construct protocol using the Quikchange Mutagenesis Kit (Stratagene), and mutants were verified by sequencing. Plasmids were purified from 500 mL culture using a Mega Prep DNA purification kit (Qiagen).

Restriction enzymes for plasmid linearization were obtained from New England Biolabs. Linearized plasmids were extracted using phenol/chloroform/isoamyl alcohol (25:24:1). Transcription in vitro was carried out as described previously (Pokrovskaya & Gurevich, 1994). After transcription, the mixture was treated with pancreatic DNase I (Boehringer Mannheim) at a final concentration of 40 U/mL for 30 min at 37 °C and then dialyzed against reconstitution buffer.

Prior to reconstitution, the RNA was heated for 10 min at 50 °C. Reconstitution was performed at a final RNA concentration of 0.1–0.2 mg/mL using a 1.5 molar excess of TP6 in the reconstitution buffer for 30 min at 48 °C. The reconstituted RNPs were purified from unbound proteins using sucrose gradient centrifugation. The reconstitution mixture was concentrated using a Centricon concentrator (Amicon) to a volume of

0.5–0.6 mL, loaded on a 5–20% linear sucrose gradient in the reconstitution buffer, and centrifuged for 18 h at 40,000 rpm at 4 °C in a Beckman SW40 rotor. The protein composition of the RNPs was analyzed by loading the RNPs suspended in 1% SDS onto precast 16.5% polyacrylamide minigels (Biorad), using 0.025 M Tris, 0.192 M glycine, pH 8.3, as the electrophoresis buffer.

Velocity sedimentation experiments were carried out on a Beckman analytical ultracentrifuge equipped with ultraviolet optics, at 20 °C. The speed in all the experiments was 50,000 rpm. The samples were dissolved in reconstitution buffer at a concentration of approximately 1 A_{260} U/mL.

The partial specific volume of the RNPs were calculated from the weight fraction of the RNA and the proteins in the complex, using partial specific volume of the RNA and the proteins as 0.537 cm^3/g and 0.73 cm^3/g, respectively, and assuming their additivity.

ACKNOWLEDGMENTS

We would like to thank Dr. Hilal Rashuel for assistance with ultracentrifugation experiments, and Dr. Jeffrey W. Orr for critical comments on the manuscript. We thank Dr. N. Matvienko (Pushchino, Russia) for kindly providing the SP6 RNA polymerase. This work was supported by a grant from the National Institutes of Health (GM 53757) to JRW.

Received August 27, 1999; returned for revision September 23, 1999; revised manuscript received November 24, 1999

REFERENCES

Agalarov SC, Selivanova OM, Zheleznyakova EN, Zheleznaya LA, Matvienko NI, Spirin AS. 1999. Independent in vitro assembly of all three major morphological parts of the 30 S ribosomal subunit of *Thermus thermophilus*. *Eur J Biochem 266*:533–537.

Agalarov SC, Zheleznyakova EN, Selivanova OM, Zheleznaya LA, Matvienko NI, Vasiliev VD, Spirin AS. 1998. In vitro assembly of a ribonucleoprotein particle corresponding to the platform domain of the 30S ribosomal subunit. *Proc Natl Acad Sci USA 95*:999–1003.

Batey RT, Williamson JR. 1996. Interaction of the *Bacillus stearothermophilus* ribosomal protein S15 with 16 S rRNA: I. Defining the minimal RNA site. *J Mol Biol 261*:536–549.

Clemons WM Jr, May JLC, Wimberly BT, McCutcheon JP, Capel MS, Ramakrishnan V. 1999. Structure of a bacterial 30S ribosomal subunit at 5.5Å resolution. *Nature 400*:833–840.

Doudna JA, Cech TR. 1995. Self-assembly of a group I intron active site from its component tertiary structural domains. *RNA 1*:36–45.

Dragon F, Brakier-Gingras L. 1993. Interaction of *Escherichia coli* ribosomal protein S7 with 16S rRNA. *Nucleic Acids Res 21*:1199–1203.

Eliseikina I, Agalarov SC, Muranova T, Markova L, Kashparov I, Aviliyakulov N, Garber M. 1995. Preparative isolation of proteins from *Thermus thermophilus* ribosomal 30 S subunits in non-denaturing conditions. *Biochimia (Russia) 60*:1720–1730.

Gogia Z, Yusupov M, Spirina T. 1986. Structure of *T. thermophilus* ribosomes. I. Method for isolation and purification. *Mol Biol (Russia) 20*:519–526.

Gregory RJ, Zeller ML, Thurlow DL, Gourse RL, Stark MJR, Dahlberg AE, Zimmerman RA. 1984. Interaction of ribosomal proteins S6, S8, S15, and S18 with the central domain of 16 S ribosomal RNA from *Escherichia coli*. *J Mol Biol 178*:287–302.

Held WA, Ballou B, Mizushima S, Nomura M. 1974. Assembly mapping of 30 S ribosomal proteins from *Escherichia coli*. Further studies. *J Biol Chem 249*:3103–3111.

Laggerbauer B, Murphy FL, Cech TR. 1994. Two major tertiary folding transitions of the *Tetrahymena* catalytic RNA. *EMBO J 13*:2669–2676.

Mougel M, Allmang C, Eyermann F, Cachia C, Ehresmann B, Ehresmann C. 1993. Minimal 16S rRNA binding site and role of conserved nucleotides in *Escherichia coli* ribosomal protein S8 recognition. *Eur J Biochem 215*:787–792.

Pokrovskaya I, Gurevich V. 1994. In vitro transcription: Preparative RNA yields in analytical scale reactions. *Anal Biochem 220*:420–423.

Powers T, Noller HF. 1995. Hydroxyl radical footprinting of ribosomal proteins on 16S rRNA. *RNA 1*:194–209.

Serganov AA, Masquida B, Westhof E, Cachia C, Portier C, Garber M, Ehresmann B, Ehresmann C. 1996. The 16S rRNA binding site of *Thermus thermophilus* ribosomal protein S15: Comparison with *Escherichia coli* S15, minimum site and structure. *RNA 2*:1124–1138.

Tsiboli P, Herfurth E, Choli T. 1994. Purification and characterization of the 30 S ribosomal proteins from the bacterium *Thermus thermophilus*. *Eur J Biochem 226*:169–177.

Ulitin AB, Agalarov SC, Serdyuk IN. 1997. Preparation of a “beheaded” derivative of the 30 S ribosomal subunit. *Biochimie 79*:523–526.

Wu H, Jiang L, Zimmerman RA. 1994. The binding site for ribosomal protein S8 in 16S rRNA and spc mRNA from *Escherichia coli*: Minimum structural requirements and the effects of single bulged bases on S8-RNA interaction. *Nucleic Acids Res 22*:1687–1695.

Zarrinkar PP, Williamson JR. 1994. Kinetic intermediates in RNA folding. *Science 265*:918–924.