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rRNA complementarity within mRNAs: A possible basis for mRNA-ribosome interactions and translational control

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ABSTRACT **Our recent demonstration that many eukaryotic mRNAs contain sequences complementary to rRNA led to the hypothesis that these sequences might mediate specific interactions between mRNAs and ribosomes and thereby affect translation. In the present experiments, the ability of complementary sequences to bind to rRNA was investigated by using photochemical cross-linking. RNA probes with perfect complementarity to 18S or 28S rRNA were shown to cross-link specifically to the corresponding rRNA within intact ribosomal subunits. Similar results were obtained by using probes based on natural mRNA sequences with varying degrees of complementarity to the 18S rRNA. RNase H cleavage localized four such probes to complementary regions of the 18S rRNA. The effects of complementarity on translation were assessed by using the mRNA encoding ribosomal protein S15. This mRNA contains a sequence within its coding region that is complementary to the 18S rRNA at 20 of 22 nucleotides. RNA from an S15-luciferase fusion construct was translated in a cell-free lysate and compared with the translation of four related constructs that were mutated to decrease complementarity to the 18S rRNA. These mutations did not alter the amino acid sequence or the codon bias. A correlation between complementarity and translation was observed; constructs with less complementarity increased the amount of translation up to 54%. These findings raised the possibility that direct base-pairing of particular mRNAs to rRNAs within ribosomes may function as a mechanism of translational control.**

Eukaryotic mRNAs contain cis-regulatory elements responsible for the posttranscriptional control of gene expression (reviewed in ref. 1). Although a number of studies have investigated the interactions of these cis-regulatory elements with trans-acting proteins (e.g., ref. 2), relatively few studies have focused on whether there are classes of cis-regulatory elements that function by interacting directly with ribosomes. Our interest in the interaction of mRNAs with ribosomes arose from the results of nucleic acid database searches and Northern analyses in which we identified sequences resembling those of 18S and 28S rRNAs within the untranslated and coding regions of a large number of eukaryotic mRNAs (3). These rRNA-like sequences appear both as similarities, or ''sense'' matches, and as complementarities, or ''antisense'' matches. These initial findings prompted us to suggest that mRNAs containing rRNA-like sequences might interact with ribosomes in two ways: complementary sequences could base-pair to rRNA, whereas those with similarities might mimic rRNA and bind ribosomal proteins (3). We further proposed that these interactions might directly affect translation.

The plausibility of these hypotheses depends on how accessible intact ribosomes are to mRNAs with complementary sequence matches. This accessibility might be limited by the extensive secondary structure of the rRNAs, or by the interactions among the four different rRNAs and 82 ribosomal proteins. In the present study, photochemical cross-linking was used to examine whether the 28S or 18S rRNAs within ribosomes were accessible to complementary sequences within mRNAs. To map the sites at which four of the probes were cross-linked to the 18S rRNA, complementary oligonucleotides were used to direct RNase H cleavage of the rRNA at specific flanking locations.

To investigate whether message complementarity to rRNA affects translation, we studied the mRNA encoding mouse ribosomal protein S15 (4) as a first example. This mRNA originally was identified as a highly expressed mRNA in rat insulinomas and was termed the rat insulinoma gene or *rig* (5). S15 mRNA contains a sequence within its coding region that is complementary to a stem-loop structure at the 3′ end of the 18S rRNA. This complementarity is conserved both among 18S rRNAs and S15 mRNAs in several species including chicken, toad, mouse, hamster, rat, and human (6, 7). To examine the possible effects of rRNA-complementarity on S15 mRNA translation, RNA molecules containing the complementary segment of the S15 gene, as well as selected variants of this sequence, were fused to the luciferase gene as a reporter and tested in a cell-free translation system.

Our findings suggest that particular mRNAs contain specific sequences that can interact with ribosomes by base-pairing with complementary sequences in the rRNA. Moreover, for the particular sequence examined within the S15 mRNA, we found that base-pairing with rRNA directly affects the translational efficiency of the S15 mRNA. We discuss the possibility that base-pairing between certain mRNAs and ribosomes may function as one mechanism of translational control in eukaryotic cells.

MATERIALS AND METHODS

Ribosomal Subunits. Crude ribosomes were prepared from the mouse P19 cell line as described (8). Ribosomes were dissociated into 40S and 60S subunits in the presence of puromycin, recovered by centrifugation, aliquoted, and stored at −80°C before use (9). Subunit dissociation was monitored by sucrose gradient profiles (10).

Synthesis of RNA Probes. Photochemical cross-linking was performed by using RNA probes transcribed from DNA templates that included fragments generated by PCR or by annealed oligonucleotides (see Fig. 1 and Table 1). In both cases the DNA templates contained the T7 promoter (11) at the 5′ end of the probe sequence. Radiolabeled RNA probes transcribed from these templates contained the cross-linking reagent 4-thiouridine (s^4U; ref. 12). The templates were incubated with 3 mM DTT, 500 μM each of GTP and ATP, a 350 mM:150 mM mixture of s^4U/UTP, 40 μM CTP (Ambion), 50

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Abbreviation: UTR, untranslated region.

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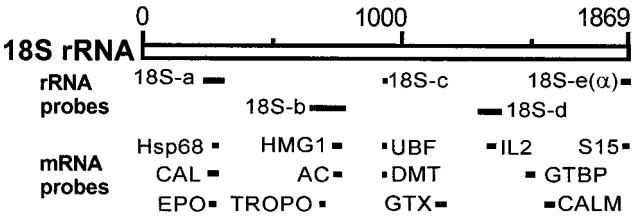

FIG. 1. Map of 18S rRNA indicating positions of rRNA and mRNA probes. The 18S rRNA is indicated as an open bar with nucleotide positions listed above the bar. The locations of the rRNA and mRNA probes are shown as lines below the regions of complementarity to the 18S rRNA. Details of the rRNA and mRNA probes are provided in Table 1.

μCi of α-^{32}P-CTP (3,000 Ci/mmol, NEN) and 25 units of T7 RNA polymerase (Stratagene). Nonradioactive RNA competitors were transcribed by using 500 μM each of GTP, CTP, ATP, and UTP. Transcription proceeded at 37°C for 3 h and was terminated by DNaseI (Ambion) digestion of the template. RNA was extracted with phenol/chloroform (1:1) and further purified by passage through Microspin G25 columns (Pharmacia). In addition to the specific competitor RNAs, the nonspecific competitor poly(C) RNA, a 50-nt long string of C residues, was similarly generated by *in vitro* transcription.

Cross-Linking. Cross-linking reactions contained 20 pmol of probe and 2 pmol of ribosomal subunits in binding buffer (13) in the presence of 150 pmol of tRNA. Samples were incubated at 37°C for 10 min, cooled on ice, and cross-linked by exposure to 365 nm UV light for 10 min. To visualize cross-linking of the probe to the rRNA, the ribosomal proteins were digested with proteinase K and extracted with phenol/chloroform (1:1), and the RNA was precipitated and electrophoresed on 1.2% agarose/formaldehyde gels (3). The RNA in the gel was visualized with ethidium bromide, transferred to HybondN$^+$ nylon membrane (Amersham), and exposed to film.

RNase H Localization of Cross-Linked Probes. To localize the position within the rRNA at which the probe was cross-linked, the cross-linked RNA was purified away from the ribosomal proteins. Short complementary DNA oligonucleotides were annealed to the rRNA at sequences flanking the region of complementarity, in RNase H buffer (14). The mixtures were heated to 50°C for 3 min, then incubated at 30°C for 30 min. RNase H (0.5 μg) then was added, and incubation continued at 30°C for 30 min. The RNA was purified and electrophoresed as described above.

Cell-Free Translation of S15-Luciferase mRNA. Reporter constructs containing nucleotides 31–93 of the S15 cDNA sequence were fused to the luciferase gene in the pGL3-control reporter vector (Promega), resulting in a fusion protein containing the first 21 amino acids of the S15 protein fused immediately upstream of the initiation codon of the luciferase protein. The wild-type sequence and various mutations of that sequence were synthesized as oligonucleotides, annealed, and cloned into the reporter vector by using the *Hin*dIII and *Nco*I restriction sites. The T7 promoter sequence (11) then was cloned upstream of the S15-luciferase gene by using the *Bln*I and *Xho*I restriction endonuclease sites. S15-luciferase constructs were linearized with the restriction endonuclease *Sal*I, and RNA was transcribed by using T7 RNA polymerase.

Lysates for cell-free translation were prepared from P19 embryonal carcinoma cell lines, frozen as aliquots in liquid nitrogen, and stored at −70°C (15, 16). Cell-free protein synthesis was performed as described (17) with the following modifications: the reaction mixture contained 20 mM Hepes, pH 7.4, 100 mM potassium acetate, 1.1 mM magnesium acetate, 0.6 mg creatine kinase, 2 mM DTT, 10 mM creatine phosphate, 25 mM amino acid mixture (Promega), 1 mM ATP, 50 mM GTP, 75 mg/ml tRNA, 240 mM spermidine, 2.5 mM cAMP, and 2.5 mM fructose-1,6-bisphosphate. The translation reaction volumes were 25 μl, including 10 μl of extract and 0.5 μg of RNA, and were incubated at 30°C for 1.5 h. Luciferase activity was assayed as described (18).

RESULTS

Complementary rRNA Sequences Cross-Link Specifically to Ribosomes. The accessibility of the ribosome to various RNA probes was determined by using photochemical cross-linking. For these initial experiments, cross-linking was performed by using sense and antisense probes based on sequences from four regions of the 18S rRNA and one from the 28S rRNA (see Table 1 and Fig. 1). These RNA probes were synthesized with the modified nucleotide s^{4}U, radiolabeled with ^{32}P, and cross-linked by exposure to UV light to dissociated 60S and 40S subunits. UV-induced cross-linking of the s^{4}U nucleotide has been shown to occur between base-paired nucleotides, or at the borders of stable helical structures (12). The rRNA and probe were purified away from ribosomal proteins and separated on an agarose gel. The cross-linked probe was localized to the 28S or 18S rRNA by autoradiography. As shown in Fig. 2*A*, the four probes that were complementary to the 18S rRNA cross-linked to the 18S rRNA, but not to the 28S rRNA. In addition, the probe that was complementary to the 28S rRNA cross-linked to the 28S rRNA, but not to the 18S rRNA. The bands observed below the major 18S rRNA band with probes 18S-c(α) and 18S-d(α) are minor degradation bands of the 18S rRNA. In contrast, probes that contained sense sequences corresponding to the same regions of the 18S or 28S rRNAs as the complementary probes showed no evidence of cross-linking to ribosomes under the same conditions. One exception was the probe 18S-a(s), which contains a sequence identical to nucleotides 227–312 of the 18S rRNA, yet it cross-linked weakly to the 28S rRNA. This finding was not completely unexpected because the 18S-a(s) probe has a region complementary to the 28S rRNA (74% in 34 nt). These results demonstrate that the rRNAs in ribosomes have regions accessible to probes that are complementary in sequence and that these regions can be cross-linked to them.

To explore whether the cross-linking to rRNA might require interactions with some of the ribosomal proteins, the 18Sb(α) probe was cross-linked to rRNA that had been purified away from ribosomal proteins (Fig. 2*B*). These results indicated that cross-linking of this probe to rRNA could occur independently of interactions with ribosomal proteins.

Cross-Linking of mRNA Sequences to Ribosomes. To determine whether sequences within naturally occurring mRNAs that are complementary to rRNA were capable of binding to ribosomes by base-pairing to rRNA, a cross-linking experiment was performed by using probes composed of polynucleotide segments found in 5′ untranslated regions (UTRs), 3′ UTRs, and coding regions of mRNA molecules (Table 1 and Fig. 1). Small RNA probes have been used previously to map ribosome interactions (e.g., ref. 19). The results obtained with 14 different probes, other than the 18S and 28S rRNA probes, indicated that all but one probe cross-linked to rRNA within dissociated 60S or 40S subunits (Fig. 3). Some mRNA probes, such as those from heat shock protein 68 mRNA (HSP68) and erythropoietin mRNA (EPO), cross-linked exclusively to the 18S rRNA, whereas other probes also showed a low level of cross-linking to the 28S rRNA. Among this group of probes, AC, containing a segment of adenylyl cyclase type VI mRNA, failed to form detectable cross-links with rRNA within ribosomes. When 35 other mRNA probes with complementarity to various regions of the 18S rRNA (3) were tested for their ability to cross-link to dissociated 60S and 40S subunits, 24 showed patterns similar to those presented in Fig. 3, whereas 11 failed to form detectable cross-links. The failure to cross-link may reflect differences in accessibility within different

Table 1. Description of RNA probes

Probe	Sequence	Complementarity to mouse rRNA	Location in mRNA	Length	Accession code
18S-a	18S rRNA	100% in 86 nt of 18S rRNA (nt 227–312)	—	86 nt	X00686
18S-b	18S rRNA	100% in 139 nt of 18S rRNA (nt 645–783)	—	139 nt	X00686
18S-c	18S rRNA	100% in 15 nt of 18S rRNA (nt 916–930)	—	15 nt	X00686
18S-d	18S rRNA	100% in 57 nt of 18S rRNA (nt 1294–1351)	—	58 nt	X00686
18S-e(α)	18S rRNA	100% in 54 nt of 18S rRNA (nt 1816–1869)	—	54 nt	X00686
28S-G	28S rRNA	100% in 59 nt of 28S rRNA (nt 1767–1825)	—	59 nt	X00525
S15	Ribosomal protein	91% in 22 nt of 18S rRNA (nt 1847–1866)	C, nt 62–83	56 nt	M33330
HSP68	Heat shock protein (HSP68)	88% in 16 nt of 18S rRNA (nt 274–289)	C, nt 147–162	30 nt	M12573
CAL	Calcineurin catalytic subunit	72% in 36 nt of 18S rRNA (nt 251–286)	5′, nt 176–211	36 nt	M81475
EPO	Erythropoietin	100% in 20 nt of 18S rRNA (nt 257–276)	5′, nt 392–411	29 nt	M12930
TROPO	Nonmuscle tropomyosin 5	79% in 14 nt of 18S rRNA (nt 688–701)	3′, nt 1609–1622	27 nt	X53753
HMGI	High mobility group I protein	77% in 34 nt of 18S rRNA (nt 729–762)	3′, nt 530–563	34 nt	X58380
AC	Adenyl cyclase type VI	90% in 20 nt of 18S rRNA (nt 746–765)	3′, nt 3769–3788	44 nt	M93422
UBF	Transcription factor	92% in 13 nt of 18S rRNA (nt 918–930)	3′, nt 2867–2879	29 nt	X60831
DMT	DNA (cytosine-5)-methyltransferase	100% in 11 nt of 18S rRNA (nt 918–928)	C, nt 328–338	29 nt	X14805
GTX	Homeobox protein	79% in 14 nt of 18S rRNA (nt 1118–1131)	5′, nt 133–169	42 nt	L08074
IL-2	Interleukin 2 receptor gamma chain	79% in 24 nt of 18S rRNA (nt 1311–1334)	3′, nt 1484–1507	37 nt	L20048
GTBP	GTP binding protein	84% in 25 nt of 18S rRNA (nt 1481–1505)	5′, nt 36–60	27 nt	U15636
CALM	Calmodulin kinase II	74% in 34 nt of 18S rRNA (nt 1549–1582)	C, nt 1481–1514	34 nt	X14836
mRNA-1c	See ref. 19	—	—	56 nt	—

The 18S and 28S probes listed in the first six rows are based on rRNA sequences. Probes 18S-a, -b, -c, -d, and 28S-G were synthesized as both sense (s) and antisense (α) molecules. The 13 mRNA probes, including S15, are listed below the rRNA probes. C, coding region; 5′, 5′UTR; 3′, 3′ UTR.

regions of the rRNA. This failure may be caused by the secondary structure of the rRNA or masking by ribosomal proteins. Alternatively, the inability of some probes to cross-link may reflect a limitation of the technique, as was discussed above: probes that base-pair to the rRNA may not cross-link depending on the location of the s^4U residue.

In addition to the interactions based on nucleotide sequence complementarity with the rRNA examined in this study, mRNAs also have been postulated to bind in a sequence-independent manner to a series of sites on the ribosome collectively known as the mRNA binding tract (19). Probe mRNA-1c, which has no obvious rRNA-like sequences, has been used in earlier studies to determine the mRNA binding tract in human placental ribosomes (19). When mRNA-1c was cross-linked to mouse P19 ribosomes, the level of cross-linking obtained was close to background (Fig. 3). It should be noted that our experimental conditions differed from those of Graifer *et al.* (19). Under our incubation conditions, the strong cross-linking obtained with rRNA-complementary probes is unlikely to be accounted for by sequence-independent interactions.

Localizing Regions of Cross-Linking with RNase H Digestions. To demonstrate definitively that cross-linking occurred at regions of complementarity, the exact positions of cross-linking were determined by using RNase H digestions of the rRNA. After cross-linking of the 18S-b(α), 18S-d(α), interleukin 2, and S15 RNA probes to dissociated 60S and 40S subunits, rRNA was purified and DNA oligonucleotides complementary to the rRNA were annealed to sequences flanking the region that was complementary to the probe. These oligonucleotides were used to direct cleavage of the rRNA by RNase H, an enzyme that digests the RNA within DNA-RNA hybrids. As shown in Fig. 4, these digestions allowed us to determine whether the cross-linked probes segregated with the rRNA fragments containing the complementary sequence match. The cross-linking of the 18S-b(α) probe was localized to a region of 132 nt, the cross-linking of 18S-d(α) and interleukin 2 probes was localized to a segment of 66 nt, and

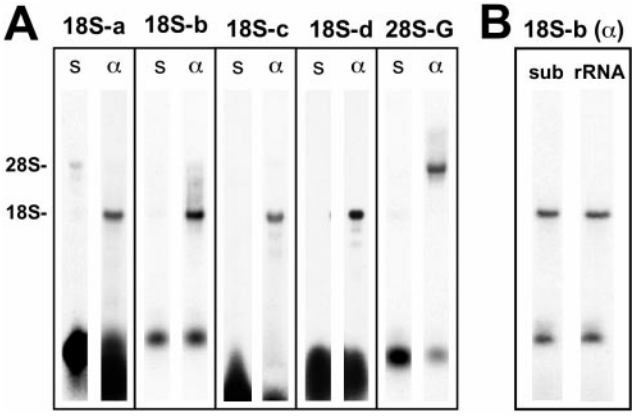

FIG. 2. Cross-linking of rRNA probes to ribosomal subunits. (*A*) The probes included the sense (s) and antisense (α) versions of 18S-a, -b, -c, -d, (see Table 1 and Fig. 1) and of the 28S-GTPase domain (28S-G) (see Table 1). (*B*) Cross-linking of rRNA probe 18S-b(α) to ribosome subunits (sub) or to purified rRNA (rRNA). The positions of the rRNAs were determined by ethidium bromide staining of the agarose gels.

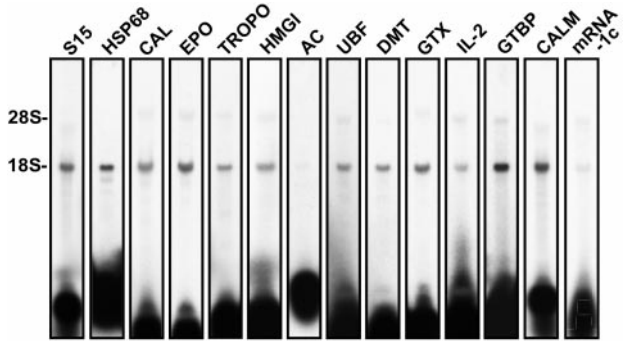

FIG. 3. Cross-linking of various mRNA probes, other than the rRNA probes, to ribosomal subunits. Details of the mRNA probes are provided in Table 1 and Fig. 1. The positions of the rRNAs were determined by ethidium bromide staining of the agarose gels.

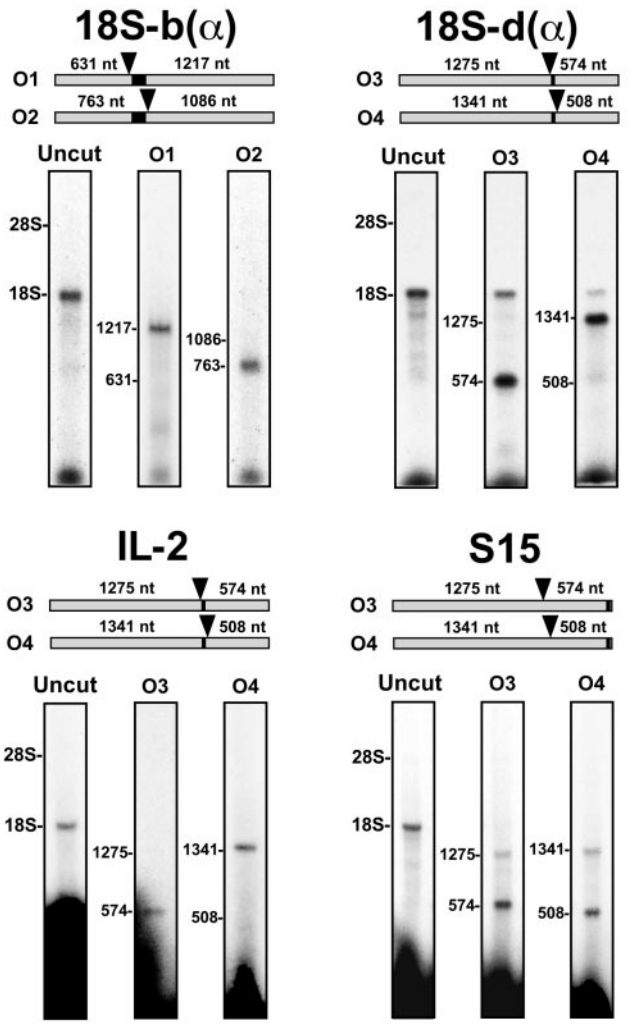

FIG. 4. Mapping the binding sites of four RNA probes by RNase H digestion of the rRNA. 18S-b(α) was localized by using oligonucleotides O1 and O2 to direct RNase H digestion. O1 is complementary to nucleotides 632–652 and O2 is complementary to nucleotides 764–783. 18S-d(α) was localized by using oligonucleotides O3 and O4. Oligonucleotide O3 is complementary to nucleotides 1276–1295, and O4 is complementary to nucleotides 1342–1361. Il-2 and S15 were localized by using oligonucleotides O3 and O4. In the upper section of each panel, the two gray bars represent the 18S rRNA. The black bar indicates the position of complementarity to the probe, and the oligonucleotides (O1-O4) used for the RNase H digestions are represented as arrowheads. The sizes of the rRNA fragments expected with each oligonucleotide are indicated above the 18S rRNA bar. The lower section of each panel shows the results of the RNase H digestions. After cross-linking the probes to ribosomes, as described in *Materials and Methods*, the RNAs were purified away from protein, annealed to one of the oligonucleotides, and the rRNA at this position was digested with RNase H. The positions of the rRNAs were determined by ethidium bromide staining of the agarose gels.

the cross-linking of the S15 probe was localized to a fragment 508 nt from the 3′ end of the 18S rRNA. In all four cases, the localized segments were those containing sequences complementary to the probes. In the case of the S15 probe, cross-linking was predominantly to the small fragment of the 18S rRNA that contains the complementary sequence match. The S15 probe also was cross-linked weakly to another site in the 18S rRNA. It was not possible to map the location of the cross-linked S15 probe to the rRNA by using primer extension because this sequence is located at the extreme 3′ end of the 18S rRNA, which is too short for hybridization to an oligonucleotide primer. The specificity of the interactions with nucleotides 1841–1862 of the 18S rRNA therefore was examined further by competition experiments.

Competition Experiments Demonstrate Specificity of S15 Binding. To demonstrate that the cross-linking of the S15 probe to the 18S rRNA was specific and involved binding to nucleotides 1841–1862 of the 18S rRNA, we attempted to compete the binding and cross-linking of the S15 probe with unlabeled RNA complementary to this region of the 18S rRNA. Ribosomal subunits were incubated with a 10-fold molar excess of S15 probe and with a 100-fold molar excess of potential competitor. The RNA tested as a specific competitor in this experiment [18S-e(α)] is complementary to nucleotides 1816–1869 of the 18S rRNA, a stretch that spans the regions of complementarity found in the S15 probe. To determine whether 18S-e(α) would bind to the 18S rRNA, it was tested as a probe and cross-linked to the 40S and 60S ribosomal subunits. As shown in Fig. 5*A*, this probe cross-linked specifically to the 18S rRNA. When 18S-e(α) was tested as a potential competitor for S15 binding, it effectively blocked cross-linking of the S15 probe (Fig. 5*B*). This effect appeared to be specific because a similar molar excess of poly(C) RNA did not have any effect on the cross-linking of the S15 probe (Fig. 5*B*). These results corroborate the previous RNase H localization results and further indicate that S15 specifically binds to its region of complementarity in the 18S rRNA.

Cell-Free Translation of S15-Luciferase Fusion RNAs. The mRNA for the S15 ribosomal protein was chosen as a first example to examine whether rRNA sequence complementarity within an mRNA molecule can affect translation. The S15 mRNA is complementary to the 18S rRNA with a match of 20 of 22 nucleotides (Fig. 6). This complementarity is in a region of the S15 mRNA encoding amino acids 11–18. The effect of this polynucleotide segment on translation of the polypeptide product was evaluated by using construct S15-W, encoding the amino terminal 21 amino acids of S15 in-frame with the firefly luciferase gene. A number of variations were introduced into S15-W that decreased complementarity to the 18S rRNA to varying degrees (Fig. 7*A*). To avoid changes in luciferase activity that were unrelated to the degree of complementarity to the 18S rRNA, these mutations were designed so that the amino acid sequences of the S15-luciferase fusion proteins were identical to that produced by the wild-type construct S15-W. In addition, the mutations were designed by using codon usage tables for mouse (20), so that the codon preference for this segment of the S15 mRNA was not altered relative

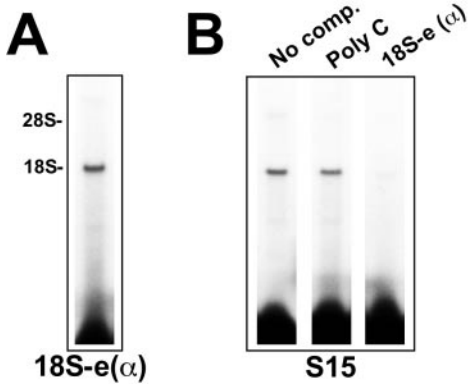

FIG. 5. (*A*) Cross-linking of 18S-e(α) RNA probe to ribosome subunits. (*B*) Competition of cross-linking of S15 RNA probe with various unlabeled RNAs. The first lane (No comp.) is in the absence of competitors, the second lane is in the presence of polyC as a nonspecific competitor, and the third lane is in the presence of 18S-e(α), an RNA complementary to the same region of the 18S rRNA as the S15 mRNA. Competitors were used at a 10-fold molar excess over probe. The positions of the rRNAs were determined by ethidium bromide staining of the agarose gels.

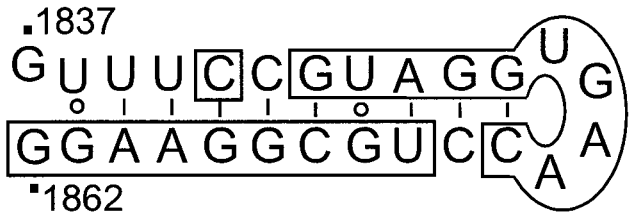

FIG. 6. Complementarity of S15 mRNA to 18S rRNA. The secondary structure of the 18S rRNA at nucleotides 1835–1865 is represented as determined *in situ* by Holmberg *et al.* (21). Vertical lines indicate base-pairing, and ○ represents GU base-pairing. The positions at which the S15 mRNA (nucleotides 62–83) is complementary are indicated by the boxed nucleotides.

to the codon preference of the wild-type sequence (20). These two constraints did not allow the degree of complementarity to the 18S rRNA to be decreased to less than 54%, or increased to more than the wild-type sequence (91%). The mRNA constructs with the poorest matches, S15-M1 and S15-M2, were 54% complementary to the 18S rRNA. S15-M3 was 77% complementary, S15-M4 was 86% complementary, and wild-type S15-W was 91% complementary to the 18S rRNA.

Cell-free translation of *in vitro*-transcribed RNAs was performed by using lysates prepared from the P19 cell line, and luciferase activity was used as a measure of translation (Fig. 7*B*). The amount of translation of mRNAs from constructs with more complementarity to the 18S rRNA was up to 50% less than that of the constructs with less complementarity to the 18S rRNA. Moreover, there was a correlation between the degree of complementarity to the 18S rRNA and translation: decreasing complementarity led to an increase in translation.

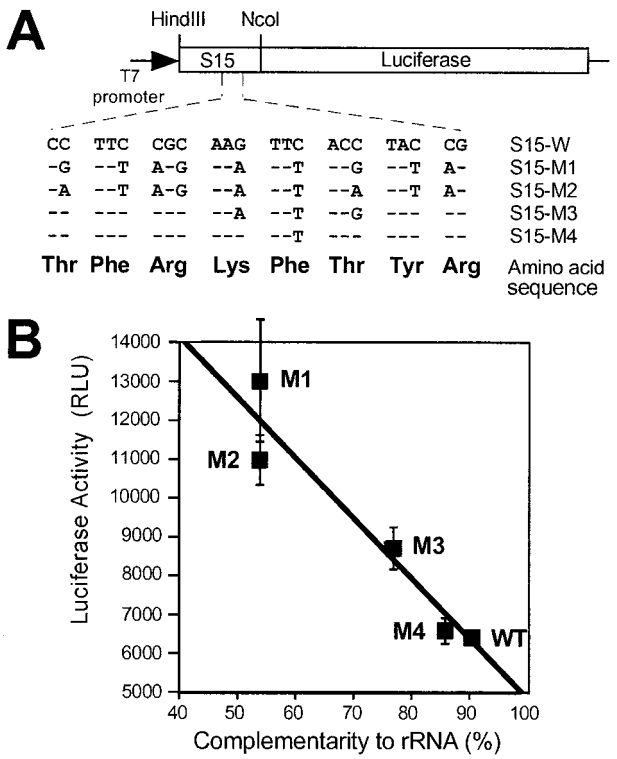

FIG. 7. Cell-free translation of S15-luciferase fusion RNAs. (*A*) Schematic representation of the reporter plasmids indicating the wild-type (S15-W) and mutated S15 sequences (S15-M1 to S15-M4). The amino acid sequences encoded by S15-W and mutations S15-M1 to S15-M4 are listed below the nucleotide sequences. The S15 and luciferase sequences are shown as open bars, and the T7 promoter is indicated as an arrow. (*B*) Effect of complementarity between S15 mRNA and 18S rRNA on translation of S15-luciferase fusion mRNAs. Luciferase activity is expressed as relative light units (RLU). The luciferase activity for each construct was calculated from four independent experiments, and the average was plotted ± SEM.

DISCUSSION

In this study, we have found that RNA sequences with complementarity to rRNA, such as those found in naturally occurring mRNAs, can bind ribosomes by base-pairing to the rRNA. We also have presented an example illustrating how these interactions can affect translation. The specificity of binding was demonstrated in three ways. Probes exactly complementary to the 28S or 18S rRNAs cross-linked to the corresponding rRNA, whereas probes lacking complementarity did not. Specificity also was shown by localization experiments using RNase H to digest the 18S rRNA at sequences flanking regions of complementarity and, in the case of the S15 mRNA, by competition with a specific competitor RNA.

The accessibility of different regions of the rRNA to various RNA probes may appear surprising inasmuch as a considerable portion of the rRNAs are base-paired (21), and rRNAs are associated with approximately 82 ribosomal proteins (22). However, it has been shown previously that particular segments of the rRNAs are accessible to very short DNA oligonucleotides (e.g., ref. 23). This accessibility is also consistent with the results of a recent structural study of the 50S subunit, indicating that the rRNA is crisscrossed over the entire large subunit (24). The accessibility of short segments within the rRNA may be sufficient for some RNAs, such as the probes used in the present study and various mRNAs, to base-pair to the rRNA. In other cases the RNAs may base-pair to a double-stranded segment of rRNA by displacing one strand of the rRNA. Such a mechanism of strand displacement actually may proceed very quickly, as was demonstrated in a recent study that involved dissociation of a 56-bp double-stranded RNA by a single-stranded RNA (25). A similar mechanism of strand displacement may function in the interactions of particular mRNAs with rRNAs.

Role of rRNA Complementarity in Translation. Although it has been speculated that base-pairing might be involved in the translation of eukaryotic mRNAs (e.g., refs. 26 and 27), or might be the basis for some forms of translational regulation (discussed in ref. 3), there has been little experimental evidence to support this idea. An example suggested to involve base-pairing interactions with the 18S rRNA is that of viral mRNAs that contain an internal ribosome entry sequence (IRES) in the 5′ UTR (reviewed in ref. 28).

In prokaryotes, there is evidence that sequences resembling rRNA can function as translational enhancers and repressors. These sequences include the downstream boxes (reviewed in ref. 29) and upstream enhancer elements such as the epsilon sequence (reviewed in ref. 30). A particularly interesting prokaryotic example, because of its possible implications for eukaryotic translational regulation, is the Q_L RNA (31). This RNA contains a segment that is complementary to the 16S rRNA, appears to interact directly with the 16S rRNA, and functions in trans to enhance translation of the *prgB* mRNA. Although translational regulation in prokaryotes cannot be directly compared with that in eukaryotes, our results raise the possibility that rRNA complementarity in mRNAs also may be the basis for some mechanisms of translational control in eukaryotes. In this regard, it will be particularly informative to examine whether the complementary sequences tested in this study also can interact with the rRNA in the context of the full-length mRNA molecules.

Effects of rRNA Complementarity on Translation. The S15 mRNA contains a sequence within its coding region that is complementary to a hairpin structure at the 3′ end of the 18S rRNA that is one of the most highly conserved sequences within the small subunit rRNA. This region of the 18S rRNA has been investigated before, and it had been noted that a

number of mRNAs contained short antisense matches to this segment of the 18S rRNA (e.g., ref. 32). In the cell-free translation experiments of the present study, this complementarity resulted in a lower translation efficiency, with a strong negative correlation between the degree of complementarity and the level of luciferase activity generated. We speculate that, in this system, base-pairing of the S15-luciferase mRNA to the rRNA sequesters it, decreasing the availability of the mRNA for translation. The inhibition of S15 mRNA translation as a consequence of direct binding to ribosomes is consistent with the results of a study in *Tetrahymena* in which antisense sequences were inserted into a region of the 28S rRNA that was known to be on the surface of the large subunit (33). Alternative explanations for the present cell-free translation results are that the sequence complementarity causes stalling or pausing during elongation, or that binding of the specific RNAs to ribosomes blocks interactions with other molecules or actually changes the structure of the ribosome in such a way as to affect translation. It will be informative to analyze other mRNAs with complementarity to the same region of the 18S rRNA as the S15 mRNA (3). In particular, mRNAs with this same complementary sequence in the 5′ or 3′ UTRs will be useful to determine how this sequence functions when present in different locations of the mRNA.

Possible Implications for the Control of Gene Expression. Although the importance of rRNA-like sequences in the regulation of mRNA expression currently is unknown, the results of this study and a database analysis (3) suggest that a large number of mRNAs have the potential to interact with ribosomes. In the present study, the focus was on rRNA complementarity, but there are also a large number of mRNAs with matches that are similar to the rRNA in the sense orientation. It may be informative to explore whether some of these mRNAs interact with ribosomal proteins and to examine the significance of these interactions for translation.

If complementarity-based interactions between mRNAs and rRNAs play a role in the translational regulation of selected mRNAs, as is suggested by the results of the present study, then the translation efficiency of particular mRNAs might be sensitive to the presence of other mRNAs in the cell that compete for the same binding sites on the rRNA. A number of conditions therefore might be expected to modulate translation efficiency within the cell. For example, changes in mRNA or ribosome concentrations that alter ribosome saturation might differentially affect the translation of groups of mRNAs. Specific sites on ribosomes might be blocked or made accessible by ribosomal or nonribosomal proteins (34, 35) or by RNAs that associate with ribosomes. Translation also might be regulated by any changes in ribosomes that alter the accessibility of their rRNAs or proteins to sequences on different groups of mRNAs. In addition to the implications for translational regulation, it also might be possible to use specific antisense rRNA fragments to manipulate the expression of groups of mRNAs that contain similar sequence complementarities.

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