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BASE-PAIRED INTERACTION, IN VITRO, BETWEEN HEN GLOBIN 9S mRNA AND EUKARYOTIC RIBOSOMAL RNAs

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SUMMARY

Hen globin 9S mRNA complexes efficiently with mouse sarcoma 18S rRNA, and to a lesser extent with 28S rRNA, but not with tRNA. The mRNA-18S rRNA complex is dissociated under conditions that lead to disruption of hydrogen bonds, and exhibits a biphasic thermal denaturation curve with Tms at ca. 39° and 58° in 0.15 M NaCl-0.03 M Tris-HCl, pH 7.5. Hen globin mRNA also interacts with 18S rRNAs from various other eukaryotes, and the melting profile and Tm of the complex formed with hen 18S rRNA is very similar to that of the complex formed with mouse sarcoma 18S rRNA.

Interaction between mRNA and rRNA, in vitro, has been observed in prokaryotes (1-3). The recognition of mRNA in E. coli is, at least in part, due to base-paired interaction between the 3' end of 16S rRNA and the initiation regions of bacterial and phage mRNAs (4,5). A comparable base-pairing between mRNA and 18S rRNA has not been demonstrated in eukaryotes. Complexes between globin mRNA and high molecular weight rRNA have been isolated from rabbit (6,7), duck (8), and hen (9) reticulocytes, but the nature of the interaction between mRNA and rRNA in these complexes has not been thoroughly examined. The possibility of non-specific aggregation cannot be overruled. If complementary sequences are present in two different RNA molecules then under appropriate conditions it should be possible to form a specific hybrid between the two in vitro, as has been shown in case of eukaryotic 5S and 18S rRNA (10-13). Specific interaction, in vitro, between E. coli 16S rRNA and 23S rRNA has been implicated in the formation of the 70S ribosomes (14). In this communication we report the formation, in vitro, and some physical properties of reversible base-paired

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complexes between highly purified hen globin 9S mRNA and rRNA from mouse sarcoma 180 Ascites cells and other eukaryotes.

MATERIALS AND METHODS

Mouse sarcoma 180 Ascites cell RNA was labelled with [^{3}H]- nucleosides as described earlier (13), and highly purified 18S rRNA, 28S rRNA and tRNA were prepared from mouse sarcoma cells, hen reticulocytes, rabbit reticulocytes, rat liver, barley and yeast by methods described elsewhere (15). Hen polysomal 9S poly(A)-containing mRNA was prepared from anaemic hen reticulocytes by the method of Naora and Fry (16), and labelled *in vitro* with [^{125}I] (17).

The methods used for RNA-RNA hybridization, and for obtaining thermal denautration profiles were essentially as described earlier (13) and necessary details are given in legends to figures. [^{3}H]- and [^{125}I]-labelled RNA were counted in terric/PPO/POPOP/toluene scintillant in a Beckman LS 100 liquid scintillation counter.

RESULTS AND DISCUSSION

Initial complexing was carried out between equimolar amounts of unlabelled hen globin 9S mRNA and [^{3}H]-labelled 18S rRNA, 28S rRNA and tRNA from mouse sarcoma cells. The extent of complex formation was monitored by oligo (dT) cellulose chromatography on the assumption that any [^{3}H] RNA interacting with poly(A)-containing mRNA should be retained on the column. In the absence of globin mRNA about 2% of [^{3}H] 18S rRNA (Fig. 1A), 4% of [^{3}H] tRNA (Fig. 1C), and 1% of [^{4}H] 28S rRNA (Fig. 1E) bound to the column. In presence of an equimolar amount of unlabelled mRNA about 45% of [^{3}H] 18S rRNA was retained on the column, so that about 43% of the [^{3}H] 18S rRNA must have been retained on the column through interaction with poly(A)-containing mRNA (Fig. 1B). Less than 1% of [^{3}H] tRNA complexed with mRNA when they were present in equimolar amounts (Fig. 1D), and the percentage of interaction did not increase when the amount of [^{3}H] tRNA was increased five-fold in the reaction mixture. These results would suggest that there is specificity in the interaction between globin 9S mRNA and mouse sarcoma 18S rRNA. When equimolar amounts of [^{3}H] 28S rRNA and unlabelled mRNA were present in the reaction mixture, about 18.5% of the [^{3}H] 28S rRNA interacted with mRNA (Fig. 1F). Even though the efficiency of complexing of mRNA with 28S rRNA is much lower than that with 18S rRNA, it is however significant.

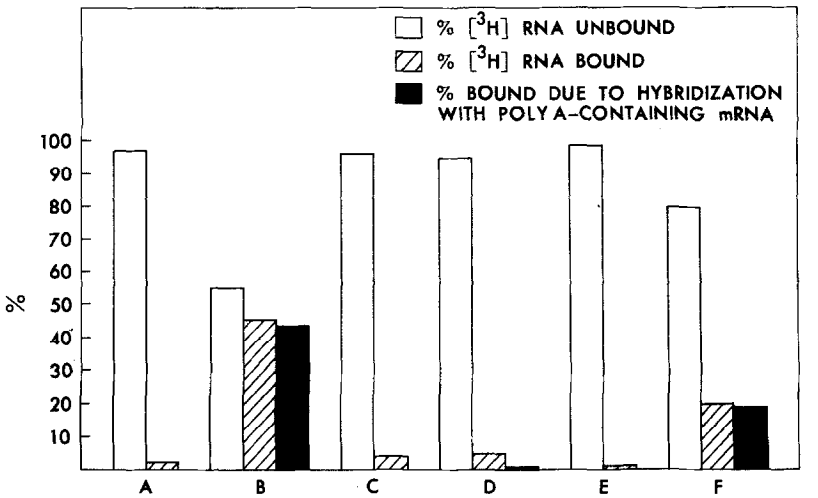

Fig. 1 Oligo (dT) cellulose chromatography of complexes formed between unlabelled hen globin poly(A)-containing mRNA and [^{3}H]-labelled rRNA and tRNA from mouse sarcoma cells. The reaction mixtures contained: A, 1.0 A_{260} unit of [^{3}H] 18S rRNA; B, 1.0 A_{260} unit of [^{3}H] 18S rRNA and 0.3 A_{260} unit of unlabelled mRNA; C, 0.1 A_{260} unit of [^{3}H] tRNA; D, 0.1 A_{260} unit of [^{3}H] tRNA and 0.8 A_{260} unit of unlabelled mRNA; E, 2.0 A_{260} units of [^{3}H] 28S rRNA; F, 2.0 A_{260} units of [^{3}H] 28S rRNA and 0.3 A_{260} unit of unlabelled mRNA. For hybridization the RNA solutions were heated in 0.3 M NaCl at 60° for 10 min. and rapidly cooled. RNA that bound to oligo (dT) cellulose in 0.5 M NaCl-0.01 M Tris-HCl, pH 7.5, was eluted with 0.01 M Tris-HCl, pH 7.5. Unbound and bound RNA radioactivities were determined.

To investigate the nature of interaction between globin mRNA and rRNA, complexes were formed between unlabelled rRNA and mRNA labelled *in vitro* with [^{125}I] (specific activity *ca.* 5 x 10^6 cpm/µg). The solution containing complexed unlabelled 18S rRNA and [^{125}I] mRNA was subjected to sucrose density gradient centrifugation, and the A_{260} (triangles) and radioactive (circles) profiles are shown in Fig. 2A. Although the majority of the radioactivity appeared at the top of the gradient, a distinct radioactive peak slightly heavier than 18S rRNA could be seen. This radioactive peak was not seen when [^{125}I] mRNA subjected to hybridization conditions was centrifuged in a separate gradient (squares), suggesting that this radioactive peak is a specific complex between unlabelled 18S rRNA and [^{125}I] mRNA.

The [^{125}I] mRNA-18S rRNA complex was recovered from the gradient and processed as described in the legend to Fig. 2. Aliquots of the complex in

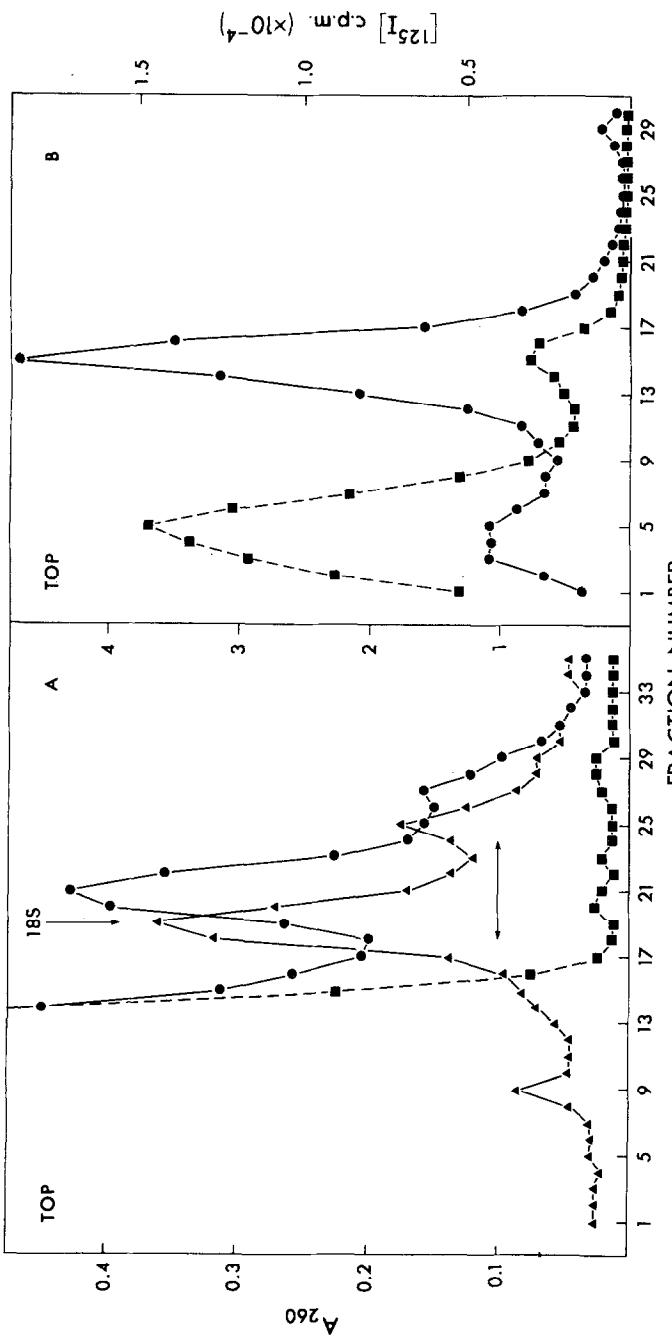

Fig. 2 A. 3.0 A_{260} units of unlabelled 18S rRNA and 0.1 A_{260} unit of [^{125}I] mRNA were annealed as described in legend to Fig. 1, and centrifuged in 5-25% sucrose gradient containing 0.15 M NaCl-0.03 M Tris-HCl, pH 7.5 (gradient buffer) in a Beckman SW27 rotor at 25000 rpm for 18 h at 2°; ▲—▲, absorbance at 260 nm of 1 ml fractions; ●—●, radioactivity of 20 µl aliquots. ■—■, radioactive profile of [^{125}I] mRNA treated similarly and sedimented in a separate gradient. The fractions containing the complex (◀—▶) were pooled, precipitated with ethanol, desalted and dried by washing successively in 67% ethanol, ethanol and ether, and dissolved in 400 µl gradient buffer. B. Aliquots (35 µl) of [^{125}I] mRNA-18S rRNA complex in gradient buffer were heated at 30° (●—●) or 65° (■—■), rapidly cooled, and centrifuged in 5-25% sucrose gradients in a Beckman SW41 rotor at 27000 rpm for 18 h at 2°. Radioactivity in 0.4 ml fractions was determined.

0.15 M NaCl-0.03 M Tris-HCl, pH 7.5, were heated and quickly cooled and then analyzed by sucrose density gradient centrifugation (Fig. 2B). When the complex was heated at 30° (circles), most of the radioactivity sedimented to the position of the complex, but when heated at 65° (squares) most of the [^{125}I] mRNA was dissociated from the complex and appeared at the top of the gradient. There was negligible degradation of the unlabelled 18S RNA under these conditions suggesting that the mRNA-18S rRNA complex is dissociated on heating. Very similar results were obtained with [^{125}I] mRNA-28S rRNA complex. The mRNA-rRNA complexes could also be substantially dissociated in 70% formamide and by aqueous denaturation at room temperature. The above treatments are known to disrupt hydrogen bonds, suggesting that globin mRNA and rRNA interact by base-pairing. The thermal denaturation profiles of mRNA-18S rRNA complexes are shown in Fig. 3. In 0.15 M NaCl-0.03 M Tris-HCl, pH 7.5, the complex shows a biphasic denaturation curve (Fig. 3A) with Tms at ca. 39° and 58°. In a buffer containing 0.01 M $MgCl_2$, 0.1 M Tris-HCl (pH 7.5), 0.05 M NH_4Cl and 1% SDS, a two-component thermal denaturation profile is also evident (Fig. 3B) with Tms at ca. 41° and 61°. This suggests that there is some heterogeneity in the mRNA-18S rRNA population, with 25-30% of the complex dissociating at the lower temperature and the major part of the complex dissociating at higher temperature. In 0.1 M NaCl, the Tm of the major component is less than 50° (data not shown). Thus, the Tm for the dissociation of mRNA from the mRNA-18S rRNA complex is dependent upon ionic strength as would be expected for base-paired intermolecular hybrids.

Hen globin mRNA was also able to interact with 18S rRNAs from hen reticulocytes, rabbit reticulocytes, rat liver, yeast and barley (data not shown). The melting profile and Tm of the homologous in vitro mRNA-18S rRNA complex from hen reticulocytes (Fig. 3C) is very similar to that of the complex formed between hen globin mRNA and mouse sarcoma 18S rRNA (Fig. 3A), suggesting that the sequence(s) in 18S rRNA to which hen globin mRNA binds is probably conserved. The melting profile of the complex formed between hen globin mRNA and mouse sarcoma 28S rRNA (not shown) is very similar to the mRNA-18S rRNA

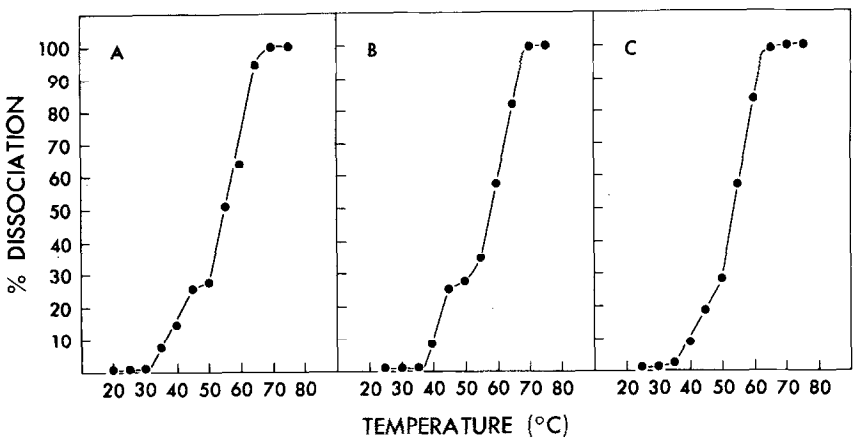

Fig. 3 Thermal denaturation profiles of: A. Hen globin mRNA-mouse sarcoma 18S rRNA complex in 0.15 M NaCl-0.03 M Tris-HCl, pH 7.5; B. The complex in 0.01 M $MgCl_2$-0.1 M Tris-HCl, pH 7.5, -0.05 M NH_4Cl and 1% SDS; C. Hen globin mRNA-hen 18S rRNA complex in 0.15 M NaCl-0.03 M Tris-HCl, pH 7.5. The [^{125}I] mRNA-18S rRNA complex was processed as described in legend to Fig. 2A and dissolved in the appropriate buffer. Aliquots were heated at a series of increasing temperatures, quickly cooled and then centrifuged in 5-25% gradients (Fig. 2B). The proportions of [^{125}I] mRNA released from the complex as a function of temperature were determined.

complex. It remains to be determined whether hen globin mRNA binds to similar sequences in 18S and 28S rRNA, or the apparent complexing between mRNA and 28S rRNA is due to contamination of 28S rRNA preparation with aggregated 18S rRNA. Complexes between globin mRNA and 18S rRNA have been isolated from rabbit (6,7) and hen (9) reticulocytes.

It has recently been shown (18-20) that sequences present in the 5' non-coding regions of large numbers of eukaryotic mRNAs are complementary to a very highly conserved purine-rich sequence (region B) near the 3' end of eukaryotic 18S rRNA (Fig. 4A). A base-paired complex that can be formed between region B of eukaryotic 18S rRNA and a sequence present in the 5' noncoding region of hen β globin mRNA (22) is shown in Fig. 4B. This base-paired structure has a free energy (23) of -16.8 kcalories and is expected to be quite stable. The probability of finding such complementarity (Fig. 4B) between region B of 18S rRNA and a sequence of 9 contiguous bases in hen β globin mRNA (ca. 650 nucleotides) is only 0.25% (24). It remains to be seen whether the base-paired

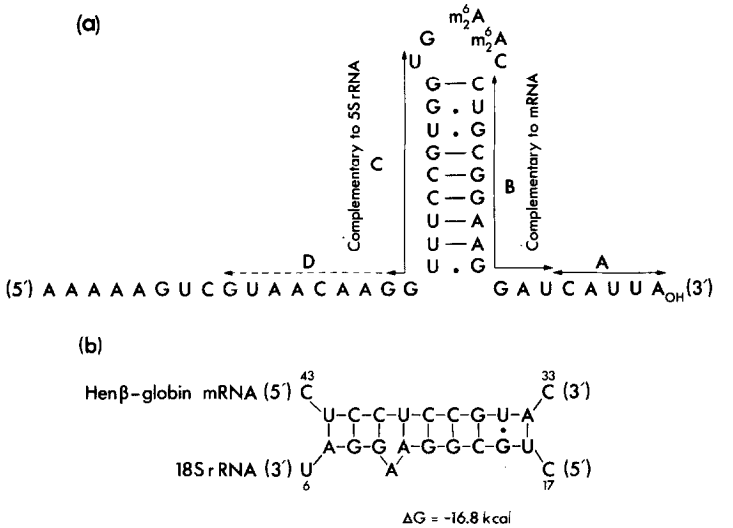

Fig. 4 A. The structure of the 3' end of yeast 18S rRNA (21). Regions have been designated A-D for descriptive purposes (see text). B. A base-paired hybrid that can be constructed between region B of eukaryotic 18S rRNA and the 5' noncoding region of hen β globin mRNA (22). The 18S RNA residues are numbered 3'→ 5' from the 3'-OH terminus, and mRNA residues are numbered 3'→ 5' from the AUG codon. Free energy of the hybrid was determined by Tinoco's improved method (23).

structure shown in Fig. 4B is involved, at least in part, in the *in vitro* interaction between hen globin mRNA and 18S rRNA reported in this paper. The hexanucleotide CUUPyUG, present in the 5' noncoding region of α and β globin mRNAs from rabbit, human and mouse (20), is also complementary to region B of 18S rRNA.

The sequences in the 5' noncoding regions of eukaryotic mRNA, that are complementary to region B of eukaryotic 18S rRNA are present at variable distances from the initiator codons and may not be protected by 80S ribosomes. Therefore, the interaction between the 5' noncoding region of mRNA and the 3' end of 18S rRNA in eukaryotes may not have a function analogous to that in *E. coli* (4,5). Regions C of rat liver (25) and yeast 18S rRNA (21) are complementary to sequences present near the 3' end of eukaryotic 5S rRNAs (Azad, A.A., to be submitted elsewhere). Efficient and selective hybridization *in vitro* has been demonstrated between eukaryotic 5S and 18S rRNA (10-13), and it has been proposed (10) that specific base-paired interaction between 5S RNA in the large subunit and 18S RNA

in the small subunit may be involved in the reversible association of ribosomal subunits. Since regions B and C of 18S rRNA are probably base-paired in a hairpin stem (Fig. 4A), and since mRNA and 5S rRNA are complementary to region B and C, respectively, the following scheme may be operative in the formation of the 80S initiation complex. The hairpin structure in 18S rRNA may be opened up by helix-unwinding proteins, similar to the prokaryotic ribosomal protein S1 (26), and the 5' noncoding region of mRNA may bind to region B of 18S rRNA, thereby leaving region C of 18S rRNA free to base-pair with 5S RNA in the large ribosomal subunit, and leading to the union of the ribosomal subunits. Specific ribosomal and factor proteins would be expected to play an important role in the formation, stabilization, and destablization of such RNA-RNA base pairs.

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