Nucleic Acids Research, 1993, Vol. 21, No. 4 1007–1011

Intermolecular mRNA–rRNA hybridization and the distribution of potential interaction regions in murine 18S rRNA

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Received September 25, 1992; Revised and Accepted January 20, 1993

ABSTRACT

Intermolecular hybridization experiments show that murine 18S rRNA and 28S rRNA are capable of forming stable hybrid structures with mRNA from genes p53, c-myc and c-mos from the same species. Both 5′-uncoding and coding oncogene p53 mRNA regions contain fragments interacting with rRNA. Computer analysis revealed 18S rRNA fragments complementary to oligonucleotides frequently met in mRNA, which are potential hybridization regions (clinger-fragments). The distribution of clinger-fragments along 18S rRNA sequence is universal at least for one hundred murine mRNA sequences analyzed. Maximal frequencies of oligonucleotides complementary to 18S rRNA clinger-fragments are reliably (2–3 times) higher for mRNA than for intron sequences and randomly generated sequences. The results obtained suggest a possible role of clinger-fragments in translation processes as universial regions of mRNA binding.

INTRODUCTION

Interaction of RNA complementary regions plays an important role in a variety of cellular processes, including those of translation. The existence of complementary regions in ribosomal and prokaryotic messenger RNA and their interaction during initiation, elongation and termination of translation was shown earlier (1–8). The effect of the interaction of Shine–Dalgarno sequence in mRNA with a complementary sequence in 16S rRNA on the translation efficiency in prokaryotes was studied in detail (1–4).

It is believed that mRNA–rRNA complementary interaction during translation also takes place in eukaryotic species, although this supposition has not been experimentally proved (9, 10).

Nucleotide distribution regularities in different mRNA codon positions discovered by Trifonov strongly suggest that mRNA–rRNA interactions are involved in frame-keeping mechanism based on complementary contacts between mRNA and rRNA. The existence of hidden periodical motif 5′(GCU)3′ in eukaryotic mRNA complementary to 18S rRNA fragment supports this notion (7).

In this paper we present results of experimental study of mouse rRNA-mRNA hybridization and computer analysis data on potential regions of their interaction.

MATERIALS AND METHODS

Isolation of poly A-RNA fraction

Total RNA from 3T3 cell culture (mice fibroblasts) was isolated with acid guanidine isothiocyanate and subsequent extraction with chlorophorm-phenol mixture (1:1) as described by Chomczynski and Sacchi (11). The ribosomal RNA fraction was isolated with chromatography on oligo-dT cellulose (12).

Preparation of [P^{32}] labelled mRNA

Labelled [P^{32}] mRNA from mouse genes p53, c-myc, c-mos and 18S rRNA was prepared by in vitro transcription of plasmid constructions containing cDNA fragments of the corresponding genes. RNA synthesis was carried out in the presence of [P^{32}] UTP and SP6 RNA polymerase, as described (13). Triphosphates unincorporated in RNA were removed with gel chromatography on a Sephadex G-50 column.

Intermolecular mRNA–rRNA hybridization in solution and analysis of its products

Unlabelled rRNA and [P^{32}] labelled mRNA were preliminarily heated in 50% formamide at 80°C for 10 min. Then rRNA and mRNA solutions were mixed and incubated in 3×SSC buffer containing 50% formamide at 37°C for 1 h. RNA aliquotes were subjected for agarose gel electrophoresis in TAE buffer, then RNA were transferred on Hybond membrane which was radioautographed.

Blot hybridization

rRNA was subjected for agarose gel electrophoresis under denaturating conditions (14), then rRNA was transferred on Hybond membrane. This membrane with immobilized rRNA on it was prehybridizated during 2 hours and hybridizated with [P^{32}] labelled mRNA (Fig.2-A) or with [P^{32}]labelled 18S rRNA (Fig.2-B) during 16 hours. The same hybridization conditions as for hybridization in solution were used. After hybridization

the membrane was washed in 3×SSC solution during 30 minutes with 2 changes of buffer solution at room temperature and 2 hours at 60°C with one change of washing solution.

Computer analysis of intermolecular rRNA–mRNA hybridization potential regions

Computer search of potential regions of intermolecular rRNA–mRNA hybridization was performed by a pair-wise comparison of mRNA and rRNA primary sequences using HYBRID program which revealed complementary oligonucleotides capable of forming stable intermolecular hybrid structures. Free energy of intermolecular hybrid structure was calculated as sum of energies of all its consecutive nucleotide pairs (15). Intermolecular hybrid structures with total free energy lower than −17 kkal/M were considered . Results of HYBRID program work were presented in the dot-matrix form (16), (Fig. 4-A). On the basis of these data, histograms of distribution of complementary mRNA oligonucleotide frequencies along 18S rRNA sequence were created for every sequence pair analyzed. The number of complementary mRNA oligonucleotides for every 18S rRNA sequence fragment was determined. Maxima on histograms of distribution correspond to mRNA oligonucleotides possessing the highest frequency. The same approach was used for intron sequences and randomly generated sequences.

The data obtained were used for construction of summarized distribution histograms for groups of related sequences (i.e.,mRNA sequences, intron sequences, randomly generated sequences), where values of complementary oligonucleotide frequencies for every related sequence group were summed up.

Original algorythms and programs developed in the Institute of Mathematic Problems in Biology were used in the work. Sequences analyzed were from EMBL Data Base, Release 29.

RESULTS AND DISCUSSION

Results of our experiments on hybridization of mouse oncogene p53 with mouse rRNA are shown in Fig.1. Messenger RNA from oncogene p53 formed hybrid structures with both 18S rRNA and 28S rRNA. Transcript from oncogene p53 antisense chain did not form hybrid structures with 18S rRNA and 28S rRNA and did not change its mobility during electrophoretic separation. The ability of p53 oncogene mRNA to form complexes with 18S rRNA and 28S rRNA was also demonstrated in blot hybridization experiments (Fig.2-A). Hybridization between 18S rRNA and 28S rRNA molecules was also observed in these experiments (Fig.2-B). Thus, in the hybridization conditions employed only mRNA-18S rRNA, mRNA-28S rRNA and presumably 18S rRNA-28S rRNA hybrids were formed from all possible variants of intermolecular RNA-RNA hybridization.

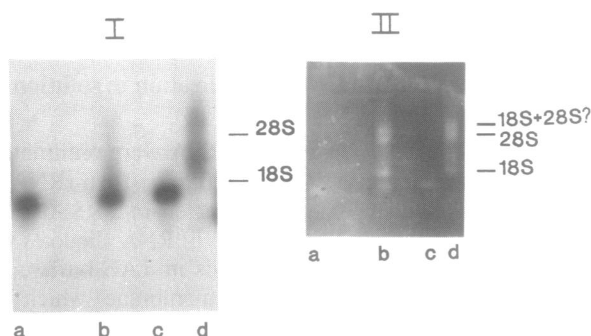

Figure 1. Electrophoregram of mRNA and the antisense chain transcript from in vitro transcribed mouse oncogene p53 after the separation in agarose gel before and after hybridization with mouse rRNA. The 0.5 μg of mRNA or 0.5μg of antisense chain transcript were directly subjected for agarose gel electrophoresis or mixed with 5μg of rRNA in 50μl of hybridization buffer. The mixture was incubated in 37°C during 60 minutes and subjected for agarose gel electrophoresis. I—radioautography of electrophoregram; II—ethidium bromide stained gel. a—antisense chain transcript from oncogene p53; b—antisense chain transcript from oncogene p53 after hybridization with rRNA; c—mRNA from oncogene p53; d—mRNA from oncogene p53 after hybridization with rRNA.

Similar results were obtained in experiments on hybridization of murine ribosomal RNA with mRNA of c-myc and c-mos genes. Messenger RNA of both genes formed hybrid structures with rRNA, and their antisense sequences did not (data are not presented).

To determine what mRNA regions form complexes with rRNA, we synthesized mRNA fragments corresponding to 5′-uncoding and coding regions of mouse oncogene p53. Results of hybridization of these fragments with rRNA are shown in Fig. 3. It is seen that the both mRNA fragments are capable of

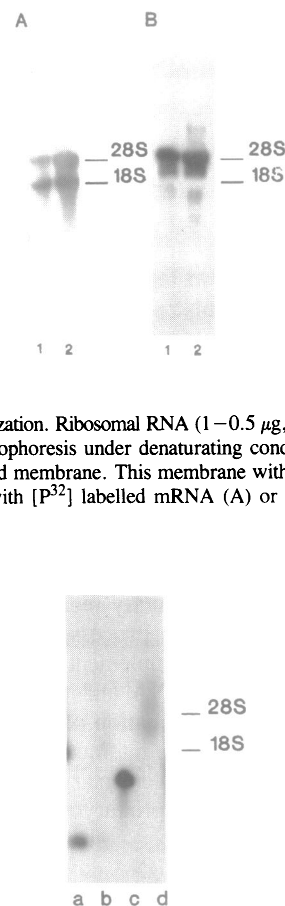

Figure 2. Blot hybridization. Ribosomal RNA (1−0.5 μg, 2 −5 μg) was subjected for agarose gel electrophoresis under denaturating conditions, then rRNA was transferred on Hybond membrane. This membrane with immobilized rRNA on it was hybridizated with [P^{32}] labelled mRNA (A) or with [P^{32}] labelled 18S rRNA (B).

Figure 3. Radioautography of electrophoregram of 5′-noncoding and coding mRNA fragments from in vitro transcribed mouse oncogene p53 after the separation in the agarose gel before and after hybridization with mouse 18S rRNA. The hybridization mixture was contained 0.1mg of mRNA gragment and 5mg of rRNA in volume 50ml. a—5′-noncoding mRNA fragment from oncogene p53; b—5′-noncoding mRNA fragment from oncogene p53 after hybridization with 18S rRNA; c—protein coding mRNA fragment from oncogene p53; d—protein coding mRNA fragment from oncogene p53 after hybridization with 18S rRNA.

forming complexes with rRNA, 5′-uncoding mRNA fragment interacting exclusively with 18S rRNA. These results suggest that both 5′-uncoding and coding mRNA fragments contain regions interacting with rRNA.

It is evident that the ability of rRNA to interact with different mRNAs is due to the existence of complementary regions in these molecules. To reveal potential hybridization regions computer analysis of 18S rRNA (17) and mRNAs from genes p53(18), c-myc(19) and c-mos (20,21) was carried out. Distribution histograms of complementary mRNA sequences from these genes

Table 1. Potential hybridization regions in 18S rRNA corresponding to the most frequent mRNA complementary fragments.

18S rRNA fragment	Nucleotide positions
1. CCCGGGGGGGG	200–210
2. CCCUCCCGGC	250–259
3. CGGCCGGGGGUCGG	262–275
4. CCCGGGG	502–508
5. UCCGCCGCG	707–715
6. CGGGGCC	786–792
7. GCCGCC	839–844
8. GCCGGGGGCA	930–939
9. CCGCCGGGCAGU	1125–1137
10. CCGGGGGGAG	1163–1172
11. GGGUGGUGGUG	1320–1330
12. CCGGGGCU	1512–1519
13. GGCCCCGCCGGGGU	1748–1761

along 18S rRNA sequence are presented in Fig. 4. Maxima on the histograms indicate 18S rRNA sequence regions corresponding to the most frequent (exeeding mean frequency by standard deviation value) complementary mRNA fragments, which are potential hybridization regions. It is seen that the distribution of these regions along 18S rRNA sequence is identical for mRNA from all three genes.

The revealed 18S rRNA complementary regions are universal for different mRNA from different genes. Analysis of distribution histograms for one hundred mRNAs from different mice genes showed that the complementary fragment distribution pattern was identical for all analyzed mRNAs. A typical summarized distribution histogram for twenty mRNAs is presented in Fig. 5-A.

Computer analysis data for mRNA family sequences were compared with that for nucleotide sequences of different functional nature (introns, randomly generetad sequences, spacers between genes). Summarized distribution histogram for twenty randomly generated sequences of the same length is presented in Fig. 5-B. It is seen that randomly generated sequences differ in complementary fragment distribution pattern, their maximal complementary fragment frequencies are two-three times lower than that for mRNA family. Similar results were obtained for intron sequences and spacers between genes (data are not shown).

Thus, mouse mRNA family is characterized with the definite pattern of complementary fragment distribution along 18S rRNA sequence, the frequency of fragments complementary to

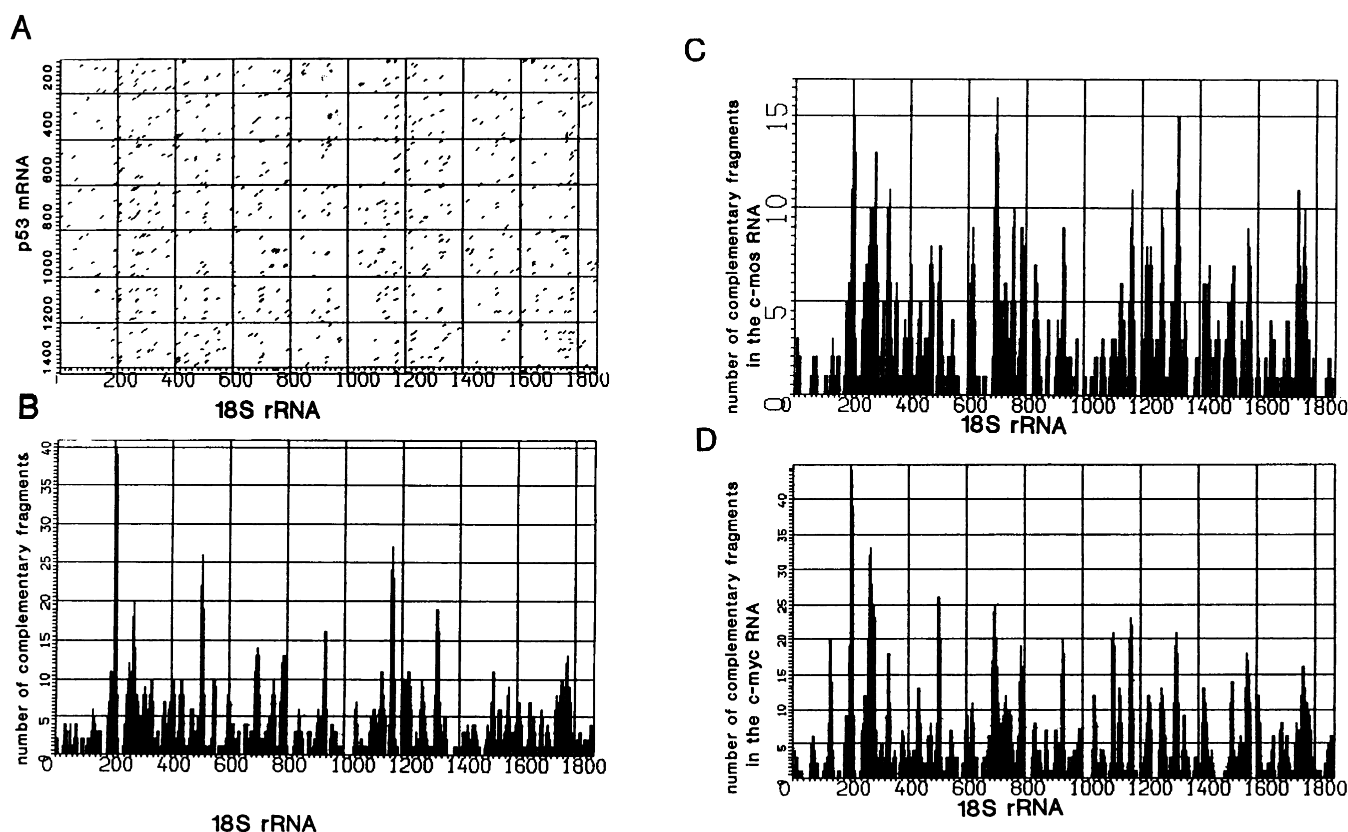

Figure 4. Dot-matrix and distribution histograms of complementary mRNA fragments along 18S rRNA sequence from mouse. A—dot-matrix for p53 mRNA and 18S rRNA complementary fragments; B—distribution histogram for p53 mRNA and 18S rRNA complementary fragments; C—distribution histogram for c-myc mRNA and 18S rRNA complementary fragments; D—distribution histogram for c-mos mRNA and 18S rRNA complementary fragments.

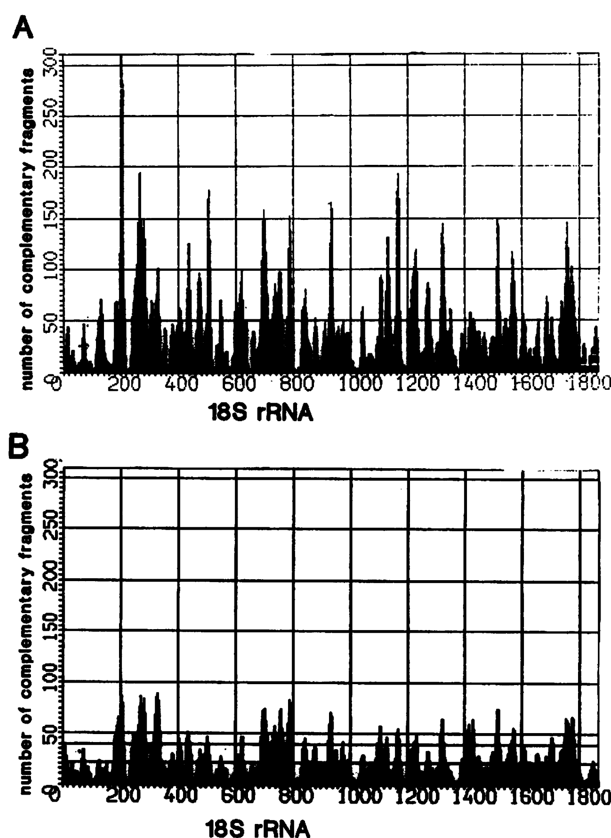

Figure 5. Summarized distribution histograms of complementary mRNA fragments from nucleotide sequences of different functional nature along mice 18S rRNA sequence. Abscissa and ordinate are the same as in Fig. 4. A—mouse messenger RNAs (20 sequences); B—randomly generated sequences (20 sequences of the same length as mRNAs).

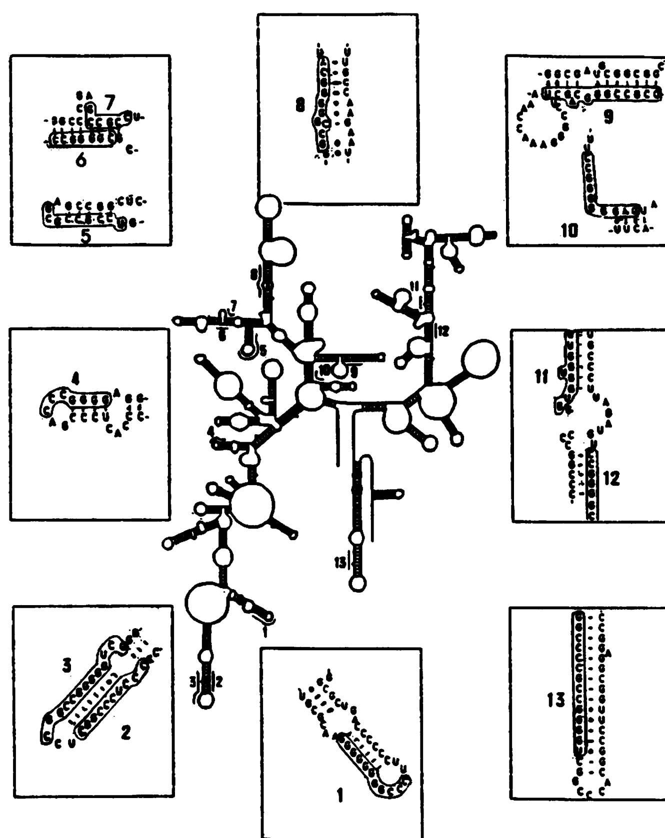

Figure 6. Location of clinger-fragments in computer-predicted secondary structure model of rodent 18S rRNA (22).

ribosomal RNA in mRNA molecules is considerably higher than that in nucleotide sequences of a different functional nature. A possibility of RNA–RNA duplex formation depends on complementary fragment frequencies in the interacting molecules, so there is reason to believe that 18S rRNA sequence regions corresponding to the most frequent mRNA complementary fragments participate in mRNA–rRNA interaction. We shall call these 18S rRNA regions 'clinger-fragments'. Nucleotide positions and primary structures of these fragments are presented in Table 1. It is seen that all of them have high G+C-content. Eight of the thirteen clinger-fragments contain G blocks (at least four neighbor G), which means that the corresponding mRNA complementary fragments are enriched with C and C+U blocks.

The location of these sequences in computer-predicted secondary structure model of rodent 18S rRNA (22) is presented in Fig.6. It is seen that clinger-fragments are located mainly on boundaries of double-helical and single-stranded regions. However, it is not clear which of clinger-fragments may be present on the surface of intact ribosome. We suggest that interaction of clinger-fragments with mRNA is possible due to cooperative processes, transient on-off base pairing. Fipro and Dahlberg (1990) suggested that the transient on/off base-pairing may result in a relative increase in the concentration of mRNA in the region of the 30S subunit, thus enhancing the potential for this mRNA to be translated (23).

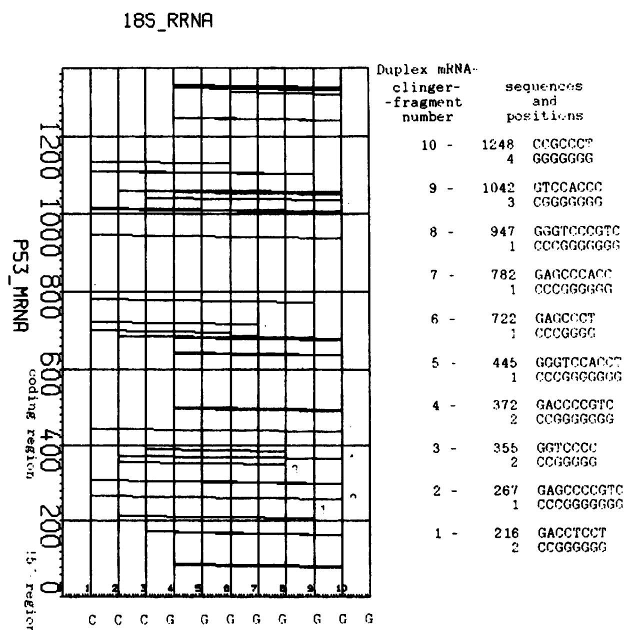

Figure 7. Dot-matrix revealing p53 oncogene mRNA regions complementary to 18S rRNA clinger-fragment CCCGGGGGGGG (200–210bp).

Computer analysis showed that both 5′-uncoding and coding mRNA regions contain fragments with different levels of complementarity to 18S rRNA clinger-fragments. An example of this for clinger-fragment CCCGGGGGGGG (200–210bp) is presented in Fig.7. These results are in agreement with data of our experiments on hybridization of 5′-uncoding and coding regions of mouse oncogene p53 mRNA with 18S rRNA.

We suggest that the function of clinger-fragments consists in universal binding of mRNA molecules in translation processes, although not all the revealed clinger-fragments may be available for complementary interaction with mRNA molecules in vivo due to the secondary rRNA structure formation and rRNA packing into the ribosome. The universal mRNA binding ability may be a unique property of rRNA molecules necessary for ribosome functioning.

ACKNOWLEDGEMENTS

We thank Dr A.S.Kondrashov for creating the program Hybrid, Dr N.A.Spiridonov and Dr P.M.Chumakov for helpful discussions and critical comments, Dr R.R.Khunafina and Dr N.N.Nazipova for technical assistance in program development.

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