ISSN 0006-2979, Biochemistry (Moscow), 2008, Vol. 73, No. 6, pp. 652-659. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © A. V. Surdina, T. I. Rassokhin, A. V. Golovin, V. A. Spiridonova, B. Kraal, A. M. Kopylov, 2008, published in Biokhimiya, 2008, Vol. 73, No. 6, pp. 813-821.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM07-345, April 13, 2008.

Selection of Random RNA Fragments as Method for Searching for a Site of Regulation of Translation of *E. coli* Streptomycin mRNA by Ribosomal Protein S7

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Received October 18, 2007
Revision received December 19, 2007

Abstract—In *E. coli* cells ribosomal small subunit biogenesis is regulated by RNA–protein interactions involving protein S7. S7 initiates the subunit assembly interacting with 16S rRNA. During shift-down of rRNA synthesis level, free S7 inhibits self-translation by interacting with 96 nucleotides long specific region of streptomycin (*str*) mRNA between cistrons S12 and S7 (intercistron). Many bacteria do not have the extended intercistron challenging development of specific approaches for searching putative mRNA regulatory regions, which are able to interact with proteins. The paper describes application of SERF approach (Selection of Random RNA Fragments) to reveal regulatory regions of *str* mRNA. Set of random DNA fragments has been generated from *str* operon by random hydrolysis and then transcribed into RNA; the fragments being able to bind protein S7 (serfamers) have been selected by iterative rounds. S7 binds to single serfamer, 109 nucleotide long (RNA109), derived from the intercistron. After multiple copying and selection, the intercistronic mutant (RNA109) has been isolated; it has enhanced affinity to S7. RNA109 binds to the protein better than authentic intercistronic *str* mRNA; apparent dissociation constants are 26 ± 5 and 60 ± 8 nM, respectively. Location of S7 binding site on the mRNA, as well as putative mode of regulation of coupled translation of S12 and S7 cistrons have been hypothesized.

DOI: 10.1134/S0006297908060047

Key words: ribosome biogenesis, S7 protein, streptomycin operon, bacteria, translation regulation, SELEX, SERF

The intensive use of antibiotics in medicine, veterinary, and food industry has resulted in emergence and wide spreading of bacteria resistant to various antibiotics. Because of this, the search for new efficient antibacterial agents requires the detailed investigation of processes that are key for the bacterial cell and factors influencing these processes. Nucleic acid–protein interactions play a key role in many intracellular processes, among which biogenesis of ribosomes occupies a special place. Efficient drugs have already been designed on the basis of effectors of nucleic acid–protein interactions [1, 2].

The ribosome is a multicomponent supramolecular RNA–protein complex capable of self-assembly *in vitro*. Rapidly growing bacterial cells spend about half of their resources for ribosome biogenesis. Because of this, a factor interfering with ribosome biogenesis should have powerful antibacterial properties [3]. In fact, for neomycin and paromomycin, the effect of the ribosome assembly inhibition on bacterial growth is comparable to that of inhibition of ribosome functions *per se* [4]. The biogenesis of ribosomes is rather strictly regulated by RNA–protein interactions [5, 6]. Under unfavorable conditions for bacterial growth, the level of rRNA synthesis decreases. The balance between expression levels of ribosomal proteins

Abbreviations: intercistron) site in *E. coli str* mRNA between cistrons S12 and S7; r6hEcoS7) S7 recombinant *E. coli* protein with N-terminal 6 His; r-proteins) ribosomal proteins; SERF) selection of random RNA fragments; serfamer) generated by SERF RNA fragment capable of protein binding; *str*) streptomycin.

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(r-proteins) and rRNA is provided by a feedback mechanism of protein translation regulation. R-proteins are synthesized from polycistronic mRNA using conjugated sequential cistron translation. In this case, the efficiency of the following cistron translation depends on translation of the preceding one. One of the operon r-proteins is regulatory: if such a protein is accumulated in a free state in a cell, it binds the mRNA and represses both its own translation and that of subsequent cistrons [6].

Biogenesis of *E. coli* small ribosomal subunit is regulated by S7 protein. Together with S4 protein, it initiates the subunit self-assembly [7] by interaction with 16S rRNA [8-10]. S7 protein is expressed from the *rpsG* cistron within the so-called streptomycin (*str*) operon whose name is associated with the exhibition of bacterial resistance to streptomycin after mutation of one cistron (*rpsL*) of this operon. The *str* operon includes also cistrons of ribosomal proteins S12 (*rpsL*) and S7 (*rpsG*) as well as of translation elongation factors EF-G and EF-Tu (Fig. 1) [6, 10-15]. An interesting peculiarity of the *E. coli str* operon is the presence of an extended 96 nt long *str* mRNA region between cistrons S12 and S7. Although these cistrons are separated by a large RNA region, translation of cistron S7 is conjugated with that of the preceding cistron S12, i.e. it happens strictly in succession. In the absence of 16S rRNA synthesis, S7 interacts with the intercistronic region of *str* mRNA and inhibits translation of its own cistron [11, 12]; the possibility of such RNA–protein complex formation was shown *in vitro* [11, 13-15].

According to a computer search of more than in a hundred sequenced genomes, approximately one third of bacteria have a similar extended intercistronic region in *str* operons. In the other bacteria the intercistronic region is either smaller, about 40 nucleotides, or is completely absent. Using this feature, we have arbitrarily divided bacteria with sequenced genomes into three groups. Studying mechanisms of *str* operon translation regulation in bacteria of different groups is also relevant to applied research because bacterial pathogens important for medicine occur in all three groups.

The development of a method for searching possible regulatory mRNA regions of *str* operons in all three bacterial groups should be carried out for a known operon such as *str* of *E. coli* mRNA. This paper reports selection of random *E. coli str* mRNA fragments having an affinity to recombinant protein S7 [16]. A specific serfamer corresponding to an intercistronic region of *str* mRNA was found. Contrary to authentic intercistron, it contains nucleotide substitutions that are probably the result of erroneous copying and were selected by affinity because they increased that to the protein. The suggested model of RNA–protein interactions for intercistron and S7 protein allowed us to propose a hypothesis concerning a possible mechanism of conjugated translation of S12 and S7 cistrons and its regulation.

METHODS OF INVESTIGATION

Isolation of recombinant *E. coli* S7 protein (r6hEcoS7) from an over-expressing *E. coli* strain. Protein r6hEcoS7 is the recombinant S7 protein containing ten additional amino acids and six His residues at its N terminus [9]. Its gene was cloned into pET28b+ (Novagene, USA) at *Nde*I and *EcoR*I sites. Protein S7 was isolated as described previously [9, 13, 17] with minor modifications. Cells were lysed for 3.5 h at 0°C in Rec-4 buffer: 20 mM Hepes-KOH, pH 7.5, 4 mM $MgAc_2$, 1 M NH_4Cl, 5 mM mercaptoethanol, and 6 M guanidine hydrochloride. The supernatant was applied onto a Ni-NTA column (Promega, USA) that was washed three times with Rec-4, then with Rec-4 containing 20 mM imidazole. The sorbed protein was eluted in Rec-4 with 1 M imidazole. S7 was dialyzed against Rec-4 with 2 M urea, then against Rec-4 with 0.4 M NH_4Cl and stored at −70°C. Protein purity was characterized by standard electrophoresis according to Laemmli. For complex formation, the protein was dialyzed against 20 mM Tris-HCl buffer, pH 7.6, 4 mM $MgAc_2$, 400 mM NH_4Cl, 0.2 mM EDTA, and 4 mM mercaptoethanol.

Generation of a library of random RNA fragments of *E. coli str* operon. The DNA fragment of *E. coli str* operon, chosen for SERF, was obtained by PCR using primers GGCGGGATCGTTGTATATTTCTTGACACC and CAGCATCGCCTGAACACCTTTGTTCTTGAACGC. Seven micrograms of obtained DNA fragment was subjected to partial hydrolysis by 20 units of DNase I (Pharmacia, Sweden) in 200 μl 50 mM Tris-HCl buffer, pH 7.6, 0.5 mM $MnCl_2$ for 40 min at 4°C. To obtain blunt ends, 4 μg of DNA fragments were treated for 10 min at 37°C with 1-5 activity units of DNA polymerase I Klenow fragment in the manufacturer's buffer (Fermentas, Lithuania) containing 50 μM dNTP each. The enzyme was inactivated by heating for 10 min at 70°C.

The library of DNA fragments was cloned at the *Sma*I site into the vector pGEM-3Z (Promega). The library of radioactive RNA fragments was obtained by transcription *in vitro* during 3 h at 35°C in the presence of 6 μg DNA, [α-^{32}P]UTP (Engelhardt Institute of Molecular Biology, Russian Academy of Sciences), 1000 activity units of T7 phage RNA polymerase (Fermentas), 60 units of RNase inhibitor in 40 mM Tris-HCl, pH 7.9, 12 mM $MgCl_2$, 10 mM NaCl, 10 mM DTT, 2 mM spermidine, 2.5 mM NTP.

Selection of RNA library using r6hEcoS7 protein (SERF). Before protein binding, RNA was denatured for 2 min at 95°C in 20 mM Tris-Ac buffer, pH 7.6, 15 mM $MgAc_2$, 200 mM NH_4Cl, 4 mM mercaptoethanol, 0.02% BSA, then placed in ice. RNA and protein were preliminarily incubated separately in the same buffer for 30 min at 37°C, then were mixed and incubated for an additional 30 min at 37°C.

The amount of the complex was determined by the radioactive complex binding on nitrocellulose membranes (0.45 μm, Amersham Biosciences Hybond C-Extra; Millipore HA; Schleicher & Schuell BA85) as described elsewhere [9, 13-15]. The radioactivity of the membrane was measured according to Cherenkov in water on a Tracor Analytic scintillation counter (France). Data were processed in Scatchard coordinates following the equation [18]:

$$RP/P_f = (-RP + R_0)/aK_d,$$

where R_0 and P_0 are initial RNA and protein concentrations, respectively; RP, concentration of the complex; $P_f = (P_0 - RP)$, concentration of unbound protein; aK_d, apparent dissociation constant.

Selection was carried out as described previously [16] with some modifications. For gradual enhancement in the selection strictness, the RNA/protein molar ratio was changed from 1 : 1 in the first cycle to 50 : 1 in the ninth cycle. RNA was extracted from the complex on a filter for 1 h at 42°C with 400 μl 10 mM Tris-HCl buffer, pH 8.0, 1 mM EDTA, 8 M urea.

cDNA was obtained by reverse transcription for 1 h at 42°C with 20 units of reverse transcriptase of avian myeloblastosis virus (AMV) and 2 mM of appropriate primers (table) in 50 μl 50 mM Tris-HCl, pH 8.3, 10 mM $MgCl_2$, 50 mM KCl, 10 mM DTT, 0.5 mM spermidine, and 0.2 mM dNTP.

Analysis of serfamers. After nine cycles of selection, serfamers were analyzed by two methods. Using the first method cDNA was amplified with primers 1 and 3 (table), treated with *Eco*RI and *Xba*I, and cloned into vector pBR322* (pBR322 with polylinker pGEM-3Z cloned at *Eco*RI and *Hind*III). DNA of 30 random clones was sequenced with primer 5 (table). Using the second method, cDNA was amplified with primers 1 and 4 (table) to obtain shorter fragments. For three serfamers, isotherms of protein S7 binding were obtained by sorption on nitrocellulose membranes as described above.

RESULTS AND DISCUSSION

Generating a library of random RNA fragments of *E. coli str* operon. To develop a method for searching for sites of RNA binding to S7 protein (as possible candidates for regulatory sites) in *str* operons of different bacteria, an approach called SERF (Selection of Random RNA Fragments) [16] was chosen. Unlike the widely used combinatorial method SELEX [19, 20], one of its variants, SERF, was described for only a single case—searching for RNA–protein complexes forming the structure of the large subunit of *E. coli* ribosomes [16]. In this work regulation of *str* operon translation using mRNA complexes with S7 protein was chosen for further development of this approach as well as for demonstration of the possibility of applying SERF for searching for different types of RNA–protein complexes, in particular, transit regulatory ones.

A set of DNA fragments, termed below as the library of random fragments, was obtained by nonspecific statistical (random) hydrolysis of a significant part of the *str* operon DNA. Its transcription allowed us to obtain the library of random RNA fragments, which had to contain all potential regulatory RNA sites. The library of random RNA fragments was used in cyclic selection for affinity to recombinant protein S7. Since stability of transit regulatory complexes could not be predicted in advance, selection was carried out under conditions able to minimize the loss of target complexes. In this case it was taken into account that due to a high RNA-binding activity of S7 protein, the use of similar conditions will inevitably result in a high level of nonspecific binding and thus, in a low yield of the target fragment.

To obtain the library of random DNA fragments, the 2000 bp long region of *str* operon with coordinates in the *E. coli* K12 genome (GenBank accession No. U00096) of 3472699-3470700 was amplified by polymerase chain reaction. The region incorporated all sites (regions) of supposed regulation of operon translation: promoter zone including leader site of cistron S12, genes of S12 and S7

Primer location within vectors (The pGEM-3Z numbering is given as in http://www.invitrogen.com; the pBR322* numbering begins from the second A (underlined) within the *Hind*III site A<u>A</u>GCTT. Seventeen nucleotides of T7 phage RNA polymerase promoter of the primer 1, *Eco*RI site within primer 1, and *Xba* site within primers 2 and 4 are underlined)

No.	Base sequence of primer	Location within vector	Vector
1	<u>TAATACGACTCACTATA</u>GGGC<u>GAATTC</u>GAGCTCG	2727-17	pGEM-3Z
2	AACAGCTATGACCATG	100-115	pGEM-3Z
3	GATCC<u>TCTAGA</u>CAGGAAACAGCTATGAC	104-121	pGEM-3Z
4	CTGCAGGTCGACTCTAGAGGATCC	26-49	pGEM-3Z
5	GAAAAGTGCCACCTGACG	4241-4258	pBR322*

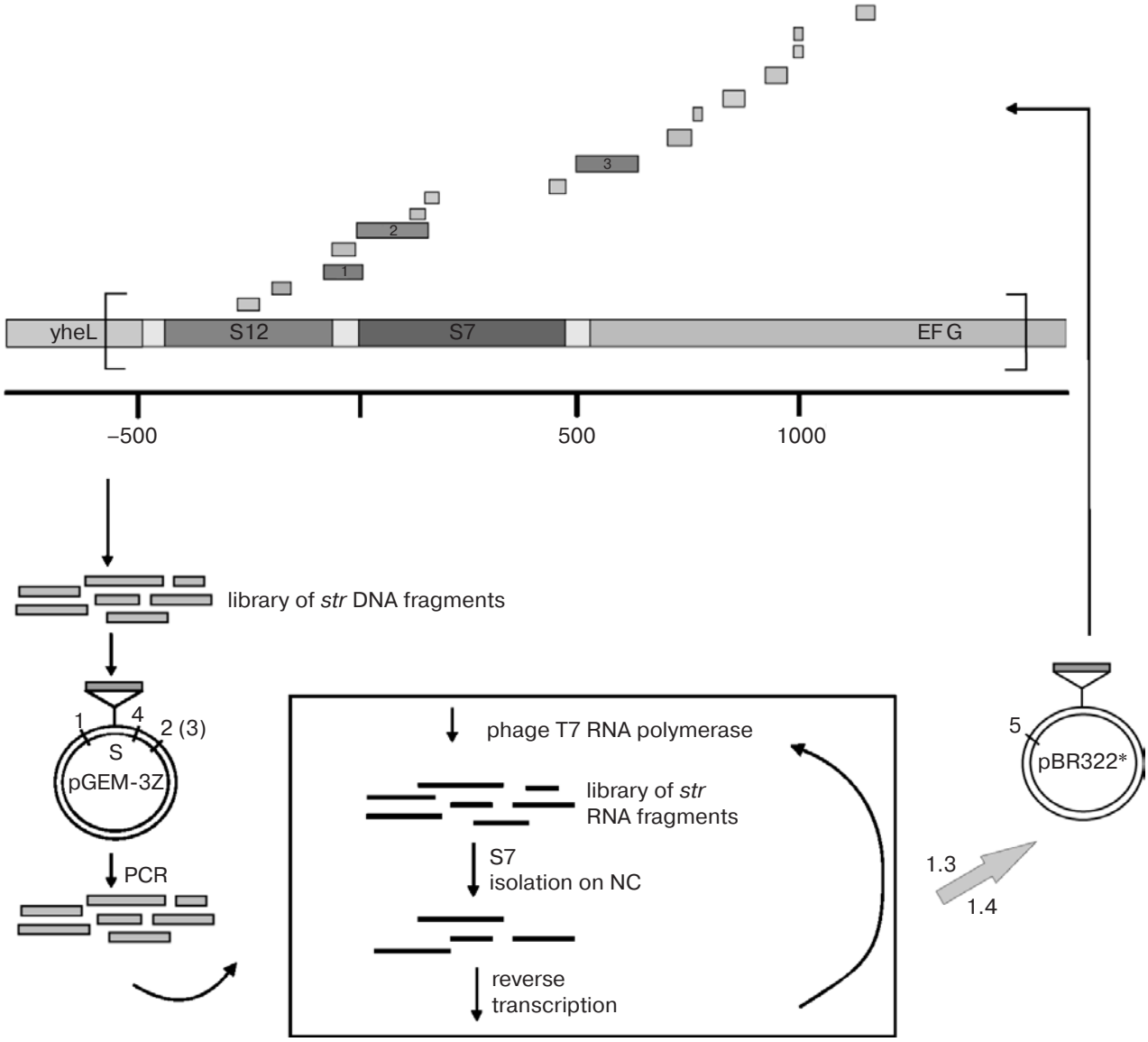

Fig. 1. Outline of SERF experiment with r6hEcoS7 protein and RNA of *E. coli str* operon. The operon region, used for selection, is shown in brackets. The scale is given in nucleotides.

proteins, as well as half of elongation factor EF-G (Fig. 1). To obtain a random set of DNA fragments, the PCR product was hydrolyzed by DNase I in the presence of Mn^{2+}, which resulted in formation of fragments from several tens to several hundreds of base pairs. The library of blunt-ended DNA fragments was cloned into *Sma*I site of pGEM-3Z vector (Fig. 1).

Selection of random RNA fragments of *E. coli str* operon with recombinant protein r6hEcoS7. To obtain a template for transcription of the library of random RNA fragments, PCR was carried out with primers 1 and 2 (table and Fig. 1), which provided for copying both the insert and the vector region including 6 bp on the left of the insert and 76 bp on the right of it.

The library of radioactive RNA fragments was obtained by template transcription using phage T7 RNA polymerase followed by complex formation between total RNA fraction and recombinant S7 protein (r6hEcoS7); RNA–protein complexes were isolated by sorption on nitrocellulose membranes (Fig. 1). RNA fragments were extracted from the membrane-adsorbed complex, and the cDNA copy was obtained, transcribed, and used for the next selection cycle.

Conditions maximally favorable for complex formation were chosen for selection. Nine selection cycles with gradual change of the RNA/protein ratio from 1 : 1 to 50 : 1 were carried out. The increase in the RNA content in the mixture should result in enhanced competition

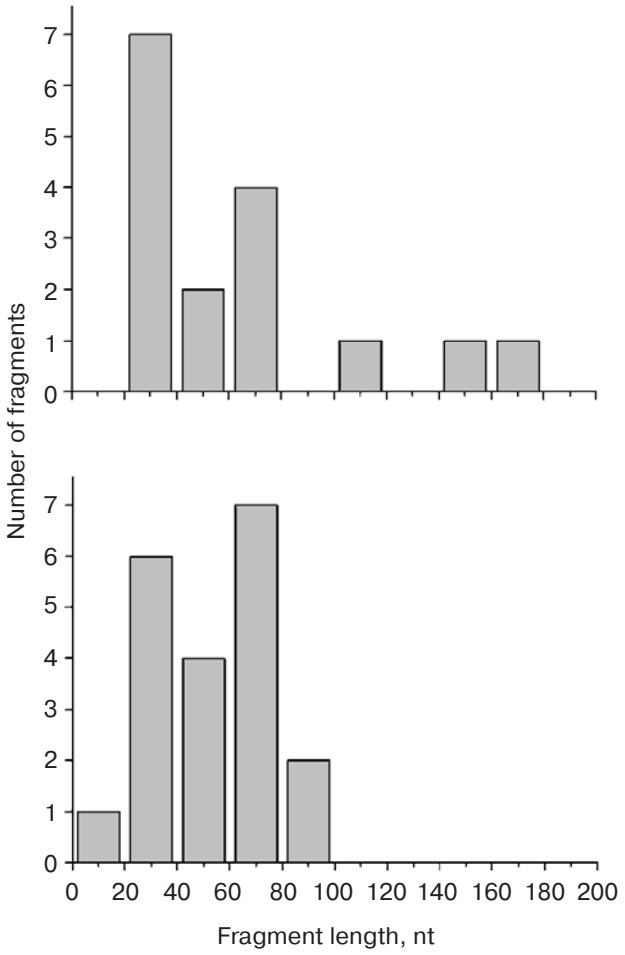

Fig. 2. Histogram of size distribution of selected RNA fragments: above, sense fragments of the *str* operon; below, antisense fragments of the *str* operon are shown. The fragment length is given in nucleotides (nt).

between target fragments for binding to protein, which makes lower the probability of nonspecific complex formation. However, as it seemed impossible to predict in advance the stability of possible transient regulatory complexes, the RNA/protein ratios were changed in the interval from 1 to 50 that is essentially below that in the case of selection of libraries with fully random base sequence in preparation of aptamers (SELEX) [19, 20]. When selection was over, RNA fraction was copied into cDNA using two pairs of primers, 1 and 3 or 1 and 4 (Fig. 1 and table). Both sets of fragments were cloned into polylinker of the pBR322* vector after cDNA treatment with enzymes *Eco*RI and *Xba*. Two obtained clone libraries were screened by sequencing from a primer of five DNA inserts isolated from randomly chosen thirty clones of one clone library and twenty five clones of another clone library (Fig. 1 and table). Twenty fragments corresponded to antisense DNA strand of the *str* operon, whereas sixteen fragments were found in the sense strand. Location of identified sense fragments in the *str* operon is shown in Fig. 1. Not all selected fragments belonged to the *str* operon; in original work on SERF [16] the accumulation of "parasitic fragments" was also registered. Evidently, the emergence of such fragments is caused by aberrations during PCR in complex RNA mixtures. Practically any selection always produces a population of RNA fragments of heterogeneous sequence. In this case, two main factors may be responsible for such situation: none of the methods provides for complete separation of RNA–protein complexes from the excess of the RNA fragment library. Besides, protein S7 is characterized by high affinity to RNA, which inevitably defines a noticeable level of nonspecific binding. In this work SERF produced a heterogeneous fraction of *str* mRNA fragments, just enriched with the target fragment whose search should be continued by different methods. Most of obtained fragments are less than a hundred nucleotides long (Fig. 2). It is reasonable to suppose that fragments with antisense sequences cannot be found among sought target regulatory sites. Therefore, the emergence of antisense fragments less than a hundred nucleotides in size is rather the result of high nonspecific affinity of the S7 protein to RNA. The presence of significant contaminants of original RNA library can be excluded because in their initial distribution by size much longer fragments were prevalent. That is why three sense serfamers more than hundred nucleotides in length were chosen for further analysis (RNA109, RNA158, and RNA169 with numbers corresponding to the serfamer length) (Fig. 1).

Serfamer RNA109 is a mutant variant of intercistronic mRNA fragment, and two other serfamers cor-

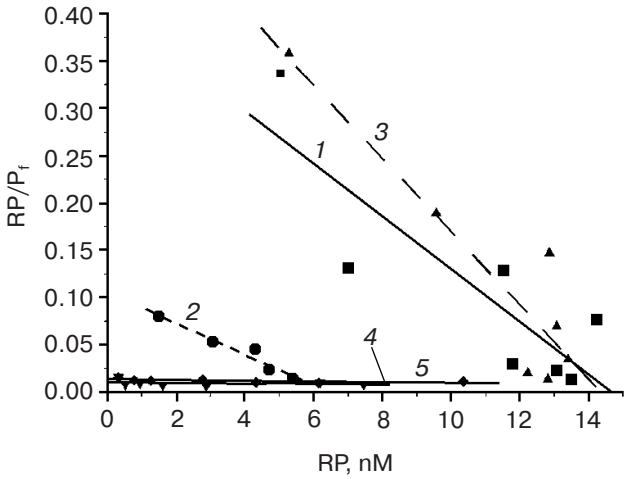

Fig. 3. Scatchard plots for the r6hEcoS7 protein binding to RNA fragments: *1*) 16S rRNA fragment (compare with [8-10]), aK_d = 36 ± 10 nM; *2*) intercistronic fragment of *str* operon (compare with [10-13]), aK_d = 60 ± 8 nM; and for binding to serfamers: *3*) RNA109, aK_d = 26 ± 5 nM; *4*) RNA158, aK_d > 1 μM; *5*) RNA169, aK_d > 1 μM. RP, complex concentration; P_f, unbound protein concentration; RNA concentration was 20 nM.

respond to initiation sites of cistrons EF-G and S7, respectively. Positions of these three RNA regions in the *str* operon suggest their possible participation in the *str* operon translation regulation by binding to S7 protein. Because of this, the interaction with S7 protein for all three serfamers was characterized using binding isotherms. Figure 3 shows Scatchard plot for binding of protein S7 to three serfamers and to authentic fragments of the *str* operon intercistronic fragment and 16S rRNA. Among the three serfamers, only RNA109 specifically binds S7 protein. In this case binding efficiency of serfamer RNA109 is approximately double that of the authentic intercistronic fragment.

Serfamer RNA109 is a derivative of the full-sized intercistronic mRNA region (Fig. 4). While its left end is located in the structural part, not far from terminating codon of S12 cistron; the position of the right end is quite specific. The 3′-terminus of RNA109 serfamer corresponds to the end of a fragment formed upon spontaneous hydrolysis of intercistronic RNA fragment [13]. Evidently, formation of such a stable fragment is defined by peculiarities of the RNA secondary structure, namely by formation of a stable duplex by the Shine–Dalgarno sequence. The directed deletion analysis of the *str* mRNA intercistronic fragment has shown that the minimal fragment able to bind S7 protein (Rassokhin, unpublished) has 3′ terminus identical to that of serfamer RNA109. Seemingly, this position of the 3′ terminus may determine both structural stability of the RNA structure and its protein-binding ability.

A very interesting peculiarity of serfamer RNA109 is the presence of mutations. Such mutations could emerge upon multiple cycles of polymerase copying, whereas the process aimed at selection of the most stable complexes with protein fixed such mutations in the population of selected RNA. In fact, the efficiency of binding of serfamer RNA109 to r6hEcoS7 is twice as high as that of authentic intercistronic RNA fragment, their apparent

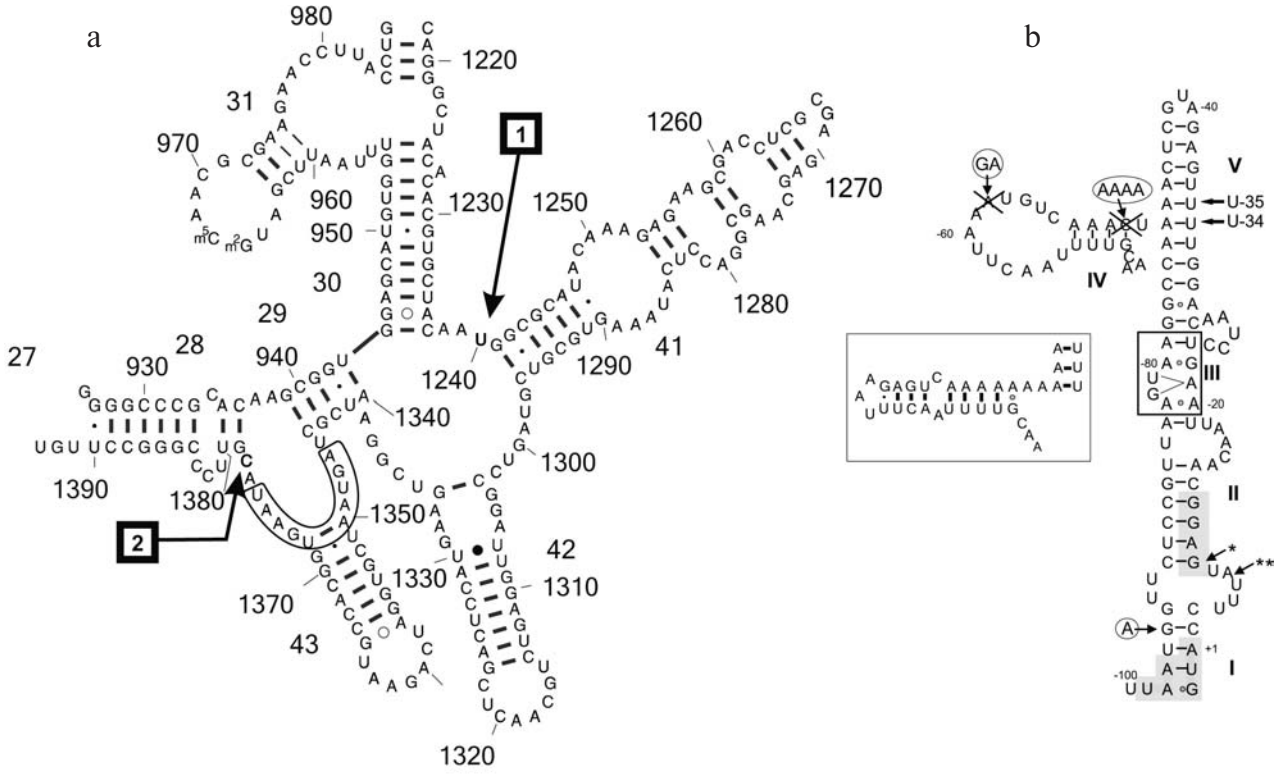

Fig. 4. a) Model of putative secondary structure of the main 3′ terminal domain of 16S rRNA fragment (S7 protein binding site) [9, 27]. The UV-induced RNA–protein cross-links for S7 protein within 30S subunit of *E. coli* ribosome are shown by arrows: *1*) cross-link with U1240; *2*) cross-link with C1378. The frame shows the 16S rRNA region identical to intercistronic fragment of *str* mRNA [10]. b) A putative secondary structure of *E. coli str* operon fragment between S12 and S7 cistrons. A modified variant of earlier proposed structure [13] is shown: secondary structure of region III is arranged identically to that of corresponding region of 16S rRNA [24]. Numbering of double-stranded regions is given as described by Saito and Nomura [11]. Numbering of nucleotides begins from A of the initiation codon AUG of S7 protein (it is taken as +1). The frame includes a part of region III identical to 16S rRNA [10]. Termination codon S12, Shine–Dalgarno sequence, and initiation codon S7 are shown in gray. Nucleotide substitutions in serfamer are shown in ovals; the supposed secondary structure of the serfamer mutant hairpin IV is given in the insert. Arrows for U(−34) and U(−35) show that they form the UV-induced cross-link with r6hEcoS7 protein in a binary complex [13]. The arrow with an asterisk points to the bond spontaneously hydrolyzed by RNase [13] and to the 3′ end of minimal mRNA fragment able to bind S7 (Rassokhin, unpublished). The arrow with two asterisks points to the 3′ end of serfamer RNA109; the 5′ end of the serfamer is not shown.

dissociation constants being 26 ± 5 and 60 ± 8 nM, respectively (Fig. 3). Most likely, the enhanced affinity of the RNA to the protein is caused by emergence of mutations in hairpin IV (Fig. 4). The appearance of AAAA block instead of UC in position (−49, −50) and AG substitution for UA in position (−57, −58) may stabilize the structure of a new variant of hairpin IV* (see the insert in Fig. 4). The stabilization of hairpin IV* may in turn stimulate folding of adjacent RNA sites (like double-stranded region III) that will result in enhanced efficiency of binding of this RNA structure to the protein.

Structure of the site of binding of the *str* mRNA intercistronic fragment to EcoS7 protein. Two variants of RNA structure were proposed earlier for the site of S7 protein binding to the intercistronic fragment of *str* mRNA. This proposition was based on the identity of the *str* mRNA and 16S rRNA primary structures (Fig. 4). Saito and Nomura [11] supposed that the double-stranded region between III and V (−76)CCA(−74)/3′-(−30)AGGUU(−34)-5′ in *str* mRNA was identical to double-stranded region 42 (1327)CCA(1329)/3′-(1311)AGGUU(1307)-5′ in 16S rRNA. However, this supposition was not substantiated experimentally.

Another variant of RNA structure for the site of S7 protein recognition was proposed by Robert and Brakier-Gingras [10]. The *str* mRNA binding site consists of three RNA fragments: (−84)AGUAA(−79), 3′-(−19)UAAGU(−23)-5′, and (−30)ACAAU(−26) (Fig. 4). The identical site in 16S rRNA consists of an imperfect duplex in the basement of hairpin 43 (1346)AGUAA(1350)/3′-(1376)UAAGU(1372)-5′ and of a single-stranded region between basements of hairpins 30 and 41 (1236)ACAAU(1240). Contacts of these 16S rRNA regions with S7 protein were identified experimentally using UV-induced cross-links for 30S subunit of *E. coli* ribosome in solution. In the presence of iminothiolane, nucleotides C1378 [21, 22] and U1240 [21-23] were cross-linked to S7 protein, the second cross-link being the chief one upon UV irradiation in the absence of cross-linking agent (Fig. 4). Both nucleotides are in contact with S7 protein in the crystalline structure of small ribosomal subunit of *T. thermophilus* (PDB 1FJF) [24]. The interaction of the protein with the third site (ACAAU), identical for both RNAs can be excluded for spatial reasons.

Evidently the main site for binding of S7 protein on intercistron of *str* mRNA is located in the region of bifurcation of hairpins III, IV, and V (Fig. 4). Binding of S7 protein requires partially untwisted conformation of double-stranded region V, which makes possible direct interaction of heterocyclic bases U(−34) and U(−35) with the protein [11, 13].

The efficiency of binding of S7 protein to the region of bifurcation depends on stability of the adjacent hairpin IV. The A-U pair substitution with U-A in the hairpin IV (−51/−69) results in more than double inhibition of conjugated translation by S7 protein, which points indirectly to the enhancement of the affinity of this mutant RNA to the protein [11]. The A-U pair inversion to U-A eliminates the uncertainty of authentic complementarity for hairpin IV, which corresponds to its folding and formation of the adjacent site III and, thus, of the S7 protein binding site as a whole.

In this work, the mutant serfamer was the result of the PCR error and selection of RNA variants characterized by enhanced affinity to S7 protein. The supposed secondary structure of the serfamer is shown in the insert to Fig. 4. The enhanced serfamer affinity to S7 protein can be explained, like in the previous example, by the higher stability of hairpin IV compared to the authentic one.

Hypothesis on the mechanism of coupled translation of the S12 and S7 cistrons of *str* mRNA. We supposed earlier [13] that after termination of S12 cistron translation the ribosome "hops" (a short distance diffusion) to the initiation codon of S7 cistron. The efficiency of such reinitiation is defined by the neighboring of two codons in the intercistron structure, where hairpin V plays the structure-forming role (Fig. 4). Taking into account a possible mechanism of S7 protein interaction with intercistron, it is possible to propose the following mechanism of coupled translation. Normally, upon termination of S12 cistron translation (in the absence of free S7 protein), double-stranded regions I and II of mRNA undergo unwinding, because a small ribosomal subunit interacts with ten mRNA nucleotides on the right of the codon in the P-site [25]. Thus, spatial closeness of terminating ribosome and initiation codon S7 is defined only by the stability of hairpin V.

The inhibition by S7 protein of coupled translation of S12 and S7 cistrons can be explained by two scenarios. According to the first, interaction with S7 protein results in fixation of RNA structure in the region of bifurcation of hairpins IV, V, and III, thus making it difficult for ribosome termination of the S12 cistron translation (Fig. 4). The emerged translation break may launch the mechanism with participation of transport-template RNA [26], which will disturb coupled translation. According to the other scenario, interaction with S7 protein results in destabilization of structure-forming hairpin V which positions terminating ribosome at the distance of 87 nucleotides from the S7 cistron initiation codon; the probability of local diffusion will significantly decrease, thus eliminating the coupling between translation termination and initiation.

Therefore SERF was able to fish 109-nucleotide-long RNA serfamer out of *str* operon, which is capable for specific and high affinity binding to recombinant protein S7. Thus, SERF can be used to search for transient regulatory complexes in different operons of ribosomal proteins. Also, suppositions were put forward concerning localization of the site of binding of S7 protein to the

intercistronic region in *str* mRNA, and a possible regulatory mechanism of coupled translation of S12 and S7 cistrons.

Authors are grateful to A. Barta, A. Bogdanov, and A. Mankin for reading the manuscript and valuable comments.

The work was supported by the Russian Foundation for Basic Research (grants 04-04-48942 and 07-04-01034), RFBR-NWO (grant 03-04-89001 (047.015.018)), and RFBR-NSFC (grant 04-04-39014).

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