Ribosome-messenger recognition: mRNA target sites for ribosomal protein S1

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ABSTRACT
Ribosomal protein S1 is known to play an important role in translational initiation, being directly involved in recognition and binding of mRNAs by 30S ribosomal particles. Using a specially developed procedure based on efficient crosslinking of S1 to mRNA induced by UV irradiation, we have identified S1 binding sites on several phage RNAs in preinitiation complexes. Targets for S1 on Qβ and fr RNAs are localized upstream from the coat protein gene and contain oligo(U)-sequences. In the case of Qβ RNA, this S1 binding site overlaps the S-site for Qβ replicase and the site for S1 binding within a binary complex. It is reasonable that similar U-rich sequences represent S1 binding sites on bacterial mRNAs. To test this idea we have used E. coli ssb mRNA prepared in vitro with the T7 promoter/RNA polymerase system. By the methods of toeprinting, enzymatic footprinting, and UV crosslinking we have shown that binding of the ssb mRNA to 30S ribosomes is S1-dependent. The oligo(U)-sequence preceding the SD domain was found to be the target for S1. We propose that S1 binding sites, represented by pyrimidine-rich sequences upstream from the SD region, serve as determinants involved in recognition of mRNA by the ribosome.

INTRODUCTION
In the regulation of gene expression at the translational level the efficiency by which 30S ribosomal subunits recognize and bind translational initiation regions of mRNAs is a major factor that determines the level of protein synthesis (1,2). The mechanism for specific ribosome-messenger binding is still debated. There are two ways by which a ribosome as a ribonucleoprotein might recognize a nucleotide sequence of mRNA while searching for a translational start: RNA-RNA and RNA-protein interactions. Both kinds of interactions are known to be involved in initiation of translation (1-4). Interaction between the 3'-terminal part of 16S RNA and a purine-rich sequence preceding the initiation codon [the Shine-Dalgarno (SD) interaction] has been proven in many experiments (1-3,5,6). The vast majority of bacterial and phage mRNAs use the SD interaction during translational initiation. But the presence of the 'good' SD domain situated at the proper distance from the initiation codon is not a guarantee that a sequence will be bound by the ribosome with high efficiency. To explain a wide variation in rates of translation of different genes, including different rates for different cistrons within one polycistron, other factors must be taken into account. These could be a secondary structure which masks or on the contrary exposes the initiation signals (2,8-10), or other primary structure recognition determinants. Two additional sites of complementarity between mRNA and 16S-RNA were recently proposed as extra recognition signals (11-12).

In our work we have concentrated on the second type of ribosome-messenger interactions: interactions of mRNA with ribosomal proteins. The possibility that site-specific mRNA-protein interactions take place during translational initiation cannot be excluded and the best candidate to have special targets on mRNAs is ribosomal protein S1. This protein has prominent RNA-binding features with high affinity to pyrimidine-rich sequences (13). Numerous observations suggest that S1 is directly involved in the process of mRNA recognition and binding (13-16). S1 is associated with 30S subunits via its N-terminal globular domain by means of protein-protein interactions (17,18), while its elongated C-terminal part [containing RNA-binding center(s)] lies free within 30S particles in such a manner as to provide binding of mRNA to the ribosome.

Our aim in this work was to localize S1 binding sites on different mRNAs. We have developed a procedure that allowed us to find target sites for S1 on phage RNAs (Qβ and fr) bound to 30S subunits in preinitiation complexes. These sites are situated upstream from the coat protein genes and contain oligo(U)-tracts. To test the idea that similar sequences represent targets for S1 on bacterial mRNAs we have chosen the ssb gene of E. coli which contains an oligo(U)-sequence upstream from the SD-domain (19,20). We have shown that in vitro initiation complex formation between the ssb mRNA and 30S subunits is dependent on S1: free S1 activates the process of mRNA binding by 30S particles lacking S1, and acts as a translational repressor if added in a molar excess over the 30S particles. The target for S1 on the ssb mRNA has been found to be the oligo(U) sequence in the leader of the messenger.

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MATERIALS AND METHODS

30S subunits, protein S1, antibodies against S1 (anti-S1 IgG)

E. coli MRE 600 30S ribosomal subunits prepared according to (21) were generously supplied by V.I. Machno and S.V. Kirillow. 30S particles lacking protein S1 were prepared using poly(U)-Sepharose (13). Protein S1 was isolated and purified as described previously (18). Rabbit antiserum against S1 was obtained by a routine procedure (22). Purified IgG fraction was obtained by affinity chromatography on protein A-Sepharose CL-4B (Pharmacia). Immunoblotting of total 30S ribosomal proteins [fractionated according to Laemmli (23)] indicated that our preparation of anti-S1 IgG reacted only with S1. Electrotransfer of proteins onto Schleicher and Schuell BA85 nitrocellulose sheets and development of immunoblots were performed according to Towbin et al. (24).

Messenger RNAs

RNA-containing bacteriophages were gifts of A.B. Chetverin (phage Qβ) and V.M. Berzin (phage fr). Phage RNAs were prepared by the SDS-phenol procedure (4).

Preparation of 30S-phage RNA complexes

30S subunits (40 A260 units, 3 nmol) were incubated with the phage RNA Qβ or fr (about 40 A260 units) at 37°C for 10 min in 1 ml of binding buffer (20 mM Tris-HCl, pH 7.6/10 mM MgCl2/100 mM NH4Cl/6 mM 2-mercaptoethanol) and then cooled on ice. A portion of the mixture was stored as a control, while another part was irradiated at 0°C with stirring by the full light of three low pressure mercury lamps at a dose of 15 quanta per nucleotide (18). The incident light intensity was 2 x 10^7 quanta/cm^2/min (λ = 254 nm). Irradiated and control samples were supplemented with SDS and EDTA to give final concentrations of 1% and 0.02 M respectively. The mixtures were then fractionated on 10-30% linear sucrose gradients in 10 mM Tris-HCl, pH 7.6/0.1% SDS/0.15 M LiCl/10 mM EDTA by centrifugation in Beckman SW-27 rotor at 12°C for 20 h at 26000 rpm. Fractions containing phage RNA were pooled, precipitated with ethanol, pelleted, dissolved in binding buffer and stored at -20°C.

Isolation and identification of phage RNA fragments bound to S1

To isolate phage RNA crosslinked to protein S1 a specific immunoadsorbent was prepared. 500 µl of protein A-Sepharose CL-4B gel swollen in water was shaken with 3.4 mg of purified anti-S1 IgG in binding buffer at 20°C for 1 h, collected by centrifugation and washed in the same buffer. The binding of IgG to the adsorbent was about 100%. Phage RNA isolated from irradiated and control samples (about 3-4 A260 units of each) was incubated overnight at 4°C in binding buffer with 40 µl of the immunoadsorbent gel. Subsequently 20U of RNase T1 were added and samples were kept at 37°C for 30 min to yield partial digestion, sedimented, washed several times, collected by centrifugation and resuspended in kinase buffer (10 mM Tris-HCl,pH 8.0/10 mM Mg acetate/15 mM 2-mercaptoethanol). To label the immobilized fragments, 100 µCi of γ-32P-ATP (Amersham) and 5U of T4 polynucleotide kinase (Boehringer Mannheim) were added and samples were incubated at 37°C for 30 min, washed with water, supplemented with 20 µl of 10% SDS and 40 µl of 10M urea, incubated at 37°C for 2h with shaking, and sedimented. Supernatants were loaded on a 8% acrylamide gel containing 0.1% SDS and 8M urea. The gels were run at 40 mA until the xylene cyanol reached the bottom. Labeled fragments crosslinked to S1 were visualized by autoradiography, eluted from the gel overnight in a solution containing 0.3 M NaCl/0.1% SDS and 10 mg/ml proteinase K (Merck), extracted with phenol, precipitated with ethanol in the presence of carrier tRNA, dissolved in 10 M urea and fractionated on a 15% sequencing gel. Individual fragments visualized by autoradiography were eluted with 12% NaClO4 at room temperature overnight, precipitated with 5 vol. of cold acetone in the presence of 100 µg of Dextran 110000 (Fluka), collected by centrifugation, dried in vacuo, dissolved in water, and subjected to sequence analysis by the enzymatic method of Donis-Keller et al. (25) using RNA sequencing kits and the corresponding protocol of P.L.-Pharmacia.

30S initiation complex formation with the ssb mRNA and its dependence on S1

Initiation complex formation between 30S particles (standard or lacking S1), the prepared in vitro and purified ssb mRNA and uncharged initiator tRNA (Boehringer Mannheim) was tested using toeprinting techniques according to Hartz et al. (26) with minor modifications. A 17-base primer 5'-GGCAACTGCGCC-ACCAT-3' used in primer extension reactions had complementarity to nucleotides +76 to +92 of the ssb mRNA coding sequence (20). Usually each reaction contained in 10 µl of binding buffer 2.5 pmol of 30S subunits, 25 pmol of tRNA^Met and 0.5 pmol of the ssb RNA annealed with the 5'-labeled primer. In experiments on S1 dependence of initiation complex formation,
free S1 in binding buffer or an equal volume of the buffer was added to mRNA prior to addition of 30S subunits. Probes were preincubated with mRNA at 37°C for 10 min, and then incubated for 10 min more with 30S subunits and initiator tRNA. For primer extension AMV reverse transcriptase (USSR preparation, Omutninsk) was added followed by incubation at 37°C for 30 min.

**RNase protection experiments**

Partial digestions of the ssb RNA and its complex with S1 were performed with RNases U2 (A-specific) and T2 (non specific). The ssb mRNA (0.8 pmol per reaction) was preincubated at 37°C for 10 min in binding buffer with increasing amounts of S1 or equal volumes of binding buffer. Subsequently, 3 μg of carrier tRNA and 3 'sequence units' of RNase U2 (Pharmacia sequence grade) or 5 × 10⁻³ U of RNase T2 (Sankyo) were added and digestion allowed to proceed at 37°C for 3 min (U2) or 2 min (T2). Reaction volume was 15 μl. Digestions were stopped by the addition of 15 μl of 0.4 M Na-acetate (pH 4.8)/20 mM EDTA containing 0.5 mg/ml of carrier tRNA, followed by phenol extraction and precipitation with ethanol. Probes were analyzed by primer extension.

**RESULTS AND DISCUSSION**

Site-specific interaction of protein S1 with RNAs from RNA-containing bacteriophages

It is well known that on phage RNAs there is only one 'entry' site for the ribosome—the initiation region of the coat protein gene (27). 30S ribosomal subunits are able to recognize this site even in the absence of an initiator tRNA, but protein S1 appears to be strongly needed for the recognition process (13-16, 27-29). To identify phage RNA regions involved in interaction with S1 during initiation complex formation we have developed a special procedure which is schematized in Fig. 1. The approach is based on the ability of the RNA-binding center of S1 to

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**Figure 2. UV crosslinking of protein S1 to Qβ RNA within 30S-Qβ RNA preinitiation complex.** A. Isolation of labelled fragments crosslinked to S1 (step VII on Fig. 1). Autoradiogram of a 8% acrylamide gel containing 0.1% SDS and 8M urea. UV + and − indicates the lanes with irradiated and nonirradiated control samples. Exposure time—5 min. This step in the procedure gives the same picture for all phage RNAs tested. The position of the labeled material corresponds to that of S1 in the same gel as was revealed by immunoblotting (indicates by an arrow). B. Separation of Qβ RNA fragments specific for S1 after removing of linked protein by proteinase K treatment (step VIII on Fig. 1). Autoradiogram of a 15% sequencing gel. XC—xylene cyanol. Exposure time- 20 min. Q1—the major S1 bound fragment, Q2,Q3—the minor ones which were revealed with longer exposure. C. UV crosslinking of protein S1 to Qβ RNA is sequence specific. Enzymatic sequencing of Q1 fragment (B). Lanes: E—control (− enzyme), L—a ladder prepared by nonspecific cleavage, G—digestion by RNase T1, U+C—Bacillus cereus pyrimidine specific RNase, A+U—RNase Phy M, A—RNase U2. All RNases were from RNA sequencing kit (P.L.-Pharmacia). D. Comparison of sequence patterns for major (lane 1) and minor (lanes 2—6) S1 linked Qβ RNA fragments. A gap in digestion patterns indicated by an arrow might point the region where residual crosslinked peptides are located.
covalently crosslink with bound polynucleotides under UV-irradiation of S1-poly nucleotide complexes (18). This property is observed not only in model systems: S1 is the most prominent protein crosslinked to mRNAs under UV-irradiation of intact E. coli (30).

We have investigated where S1 binds to phage RNAs within 3OS preinitiation complexes formed in the absence of initiator tRNA in conditions described by Van Duin et al. (28). 3OS subunits used in this experiment contained almost equimolar amounts of S1 and no less than 0.2 mole of IF3 per mole of ribosomes. After irradiation with UV-light (λ = 254 nm) at doses which don’t abolish ribosomal activity (18), the complexes were dissociated by addition of SDS and EDTA and phage RNA was separated from 16S RNA and nonlinked proteins on sucrose gradients (step III, Fig. 1). Full length phage RNA isolated from the gradient fractions was immobilized on the specific immunoadsorbent, protein A-Sepharose containing anti-S1 IgG, and partially digested with RNase T1 in the presence of magnesium ions to conserve the elements of a secondary structure. All protein-free fragments were washed out and as a result only oligonucleotides covalently bound to S1 remained attached. They were labeled at 5'-ends with 32P, eluted from the immunoadsorbent and purified in a SDS and urea containing acrylamide gel. The labeled material was present in the gel only in the case of irradiated complex, indicating that only S1-linked fragments could be isolated by this procedure (Fig. 2A). The products from the gel were blotted onto nitrocellulose sheets and visualized with anti-S1 IgG using the procedure of Towbin et al. (24) (data not shown). This was an additional proof that labeled material in the gel was specific for S1 crosslinked to RNA. Moreover, the region protected by covalently bound S1 against digestion with RNase T1 (the main Qβ fragment identified in our experiments) completely overlaps with the so called S-site of Qβ replicase binding (31) (Fig. 3 C). S1 is known to be an essential component of phage replicases and is responsible for recognition and binding of phage RNA (31,34). Apparently, S1 participates in recognition of phage RNAs by ribosomes and by replicases by similar ways: in both cases S1 recognizes and binds the same region of the phage RNA and thus provides the proper positioning of the RNA on ribosome or replicase surfaces. This

Results of sequencing allow us to determine within the phage RNA primary structure the location of S1-bound fragments. All the fragments, the major and minor ones, belong to a single region of phage RNA sequence which is located upstream from the coat protein gene: in the case of Qβ RNA this region comprises nucleotides 1247–1322, with A of the coat protein initiation codon being at 1344 (31 and personal communication of M. Billeter); in the case of fr RNA nucleotides 1226–1297 and 1336, correspondingly (32) (for fr RNA sequencing gels are not shown). The common features of these Qβ and fr regions are the presence of an oligo(U)-tracts which appear to be the sites of S1-crosslinking (Fig. 3A,B). The site of crosslinking can be identified on sequence lanes of several fragments due to a gap in the RNase cleavage patterns which can be accounted by the presence of residual crosslinked peptides (Fig. 2D).

It is of special interest that in the case of Qβ RNA the region directly involved in S1 crosslinking (1292–1306) coincides with the site bound to S1 in a binary complex S1 Qβ RNA (33). Moreover, the region protected by covalently bound S1 against digestion with RNase T1 (the main Qβ fragment identified in our experiments) completely overlaps with the so called S-site of Qβ replicase binding (31) (Fig. 3 C). S1 is known to be an essential component of phage replicases and is responsible for recognition and binding of phage RNA (31,34). Apparently, S1 participates in recognition of phage RNAs by ribosomes and by replicases by similar ways: in both cases S1 recognizes and binds the same region of the phage RNA and thus provides the proper positioning of the RNA on ribosome or replicase surfaces. This

Figure 3. Nucleotide sequences within Qβ (A) and fr (B) RNAs involved in interaction with protein S1 during translational initiation complex formation. The sites most likely participating in UV crosslinking are underlined. On the Qβ RNA sequence the site involved in binding to S1 within the binary complex (33) is indicated by arrows. C—a secondary structure model proposed for S site of Qβ replicase binding (31). Q1 fragment is indicated by arrows, the site of S1 crosslinking is underlined.

Figure 4. Extension inhibition analysis (toeprinting) of the ssb mRNA. S1 activates and then inhibits toeprints with 3OS subunits lacking S1 (3OS(--S1)). Extension inhibition reactions were performed as described in Materials and Methods. Lane 1: primer extension without addition of 3OS subunits. Lanes 2—6: toeprinting in the presence of 0.25 μM of 3OS(--S1) and 2.5 μM of tRNA_SMet. Concentrations of S1 in reactions are indicated over lanes. S1 at 0.6 μM stimulates ribosome binding, but a 4-fold excess of S1 over 3OS(--S1) (lane 4) is already inhibitory for 3OS initiation complex formation.
SI-dependent binding underlies the repressor function of replicase on coat protein synthesis (27,31).

During initiation of translation SI-messenger interaction not only enhances the affinity of the ribosome to phage RNA [30S subunits lacking SI cannot bind phage RNAs (16)] but also might direct the initiation codon of the coat protein gene to the ribosomal decoding center (see 16). At the same time, S1 is known to be a translational repressor of the coat protein synthesis in vitro when added in molar excess over the ribosome (13,35). From our data this effect can be explained as competition between free SI (see 33) and SI as a part of the 30S subunit for the same site on the messenger. Although this S1-binding site does not overlap the ribosome binding site of the coat protein gene determined in RNase protection experiments (3, 36), the secondary structure of the region (Fig. 3C) is such that the S1 site and the initiation codon are rather close to each other. We propose that the sites containing oligo(U) sequences in Qβ and fr RNAs serve as recognition signals for 30S ribosomes during initiation complex formation.

**Site-specific interaction of S1 with bacterial messengers**

Being an obligatory component of the translational machinery of *E. coli* and other Gram-negative bacteria, protein SI is involved in initiation of translation of almost all cellular mRNA and plays a general regulatory role in protein biosynthesis (13,37). We have proposed that interaction of S1 with bacterial messengers during translational initiation might also be site-specific, as in the case of phage RNAs, with high affinity to U-rich sequences. According to Sherer et al. (38) and Dreyfus (39), the optimal ribosome binding site contains a U-rich region preceding the SD domain. There exist also experimental evidences that U-rich sequences located upstream from the SD signal provide high efficiency of translational initiation both in natural context.

**Table 1.** Examples of *Escherichia coli* genes which contain oligo(U)-stretches in 5'-untranslated regions.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Genes Sequence</th>
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<tbody>
<tr>
<td>ara BAD</td>
<td>ACCCGUUUUUUGGGAUGGAGGAACGUG</td>
</tr>
<tr>
<td>ara FGH</td>
<td>UCAAUUCAGUUUUUGCUCACAAACGACACUAAAGCUGGAGAGCAGG</td>
</tr>
<tr>
<td>ara C</td>
<td>UGAAGCUUAAUAGUACCACACUACUGCUUAAACGUGGAGGAGCAGG</td>
</tr>
<tr>
<td>atp E</td>
<td>UUUAACCACACCACUACUGCUUAAACGUGGAGGAGCAGG</td>
</tr>
<tr>
<td>bio A</td>
<td>ACCUAACACUAAUUUGGGAACGUGGAAGU</td>
</tr>
<tr>
<td>deo C</td>
<td>AAAACUCGCAAUGGAGAUGGAGAAUG</td>
</tr>
<tr>
<td>dna A</td>
<td>GGGUCUUUUCGAUGGACUGCAAAACUUG</td>
</tr>
<tr>
<td>eco R1 methilase</td>
<td>UAAGGGGAACUUG</td>
</tr>
<tr>
<td>fol A</td>
<td>AUUUUUUUUAUGGUGAAACUG</td>
</tr>
<tr>
<td>gal E</td>
<td>AUUUUAUUGUGGGAUGGAGGAAUG</td>
</tr>
<tr>
<td>his S</td>
<td>GGGUCUUUUGGGAUGGAGGAAUG</td>
</tr>
<tr>
<td>met S</td>
<td>UAAACCCACUAAAGGAUGGAGG</td>
</tr>
<tr>
<td>mut H</td>
<td>AAUAUAUGGGAACAGUAAAGU</td>
</tr>
<tr>
<td>omp A</td>
<td>AUAAUCAUGGGAUGGGAUGGAGG</td>
</tr>
<tr>
<td>pho A</td>
<td>GUUUUUAUAGUACUAGGGAAGG</td>
</tr>
<tr>
<td>rhn</td>
<td>GUUUAACAGGUAUACGAGGGAAGG</td>
</tr>
<tr>
<td>RF1</td>
<td>GUCAACUAGGGGAUGG</td>
</tr>
<tr>
<td>RF2</td>
<td>GAAUGAGAUUGGCAG</td>
</tr>
<tr>
<td>rpl J</td>
<td>GAAGUGAGAUUGGCAG</td>
</tr>
<tr>
<td>rps B</td>
<td>CAACUAUAGGGAUGG</td>
</tr>
<tr>
<td>rps G</td>
<td>UUUUGGACAAUGCAGUAAAGG</td>
</tr>
<tr>
<td>rps J</td>
<td>UAAUCUAUAGGGAUGG</td>
</tr>
<tr>
<td>rps M</td>
<td>UUUUGUUCAGGGAUGG</td>
</tr>
<tr>
<td>ssb</td>
<td>GGUUACCACUAUUG</td>
</tr>
<tr>
<td>tar</td>
<td>GAGUGAAGCUG</td>
</tr>
<tr>
<td>tra D</td>
<td>UUUUUUCAUAGGGAUGG</td>
</tr>
</tbody>
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Table 1. Examples of *Escherichia coli* genes which contain oligo(U)-stretches in 5'-untranslated regions.
Table 2 5'-Untranslated regions of operons regulated by attenuation mechanism.

<table>
<thead>
<tr>
<th>Genes</th>
<th>AUG</th>
</tr>
</thead>
<tbody>
<tr>
<td>his G</td>
<td>UUUUUUAUGCCGCUUGAUAAACGCUAGAAGUAAAGAAUAACAA</td>
</tr>
<tr>
<td>leu A</td>
<td>UUUUUUAUGCCGCUUGAUAAACGCUAGAAGUAAAGAAUAACAA</td>
</tr>
<tr>
<td>phe A</td>
<td>CCUUUUUAUGCCGCUUGAUAAACGCUAGAAGUAAAGAAUAACAA</td>
</tr>
<tr>
<td>thr A</td>
<td>UUUUUUAUGCCGCUUGAUAAACGCUAGAAGUAAAGAAUAACAA</td>
</tr>
<tr>
<td>trp E</td>
<td>UUUUUUAUGCCGCUUGAUAAACGCUAGAAGUAAAGAAUAACAA</td>
</tr>
<tr>
<td>pyr B</td>
<td>CUUUUUUAUGCCGCUUGAUAAACGCUAGAAGUAAAGAAUAACAA</td>
</tr>
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</table>

(40-42) and when placed before other genes (9,42-44). We believe that this U-rich enhancer sequences serve as additional recognition signals and are targets for ribosomal protein S1 during initiation of translation. We have noticed that 5'-untranslated regions of many E. coli genes contain oligo(U) stretches: among these there are many highly expressed in a cell—those of ribosomal proteins, translational factors etc. (Table 1). Promoter proximal genes of operons controlled by an attenuation mechanism, where oligo(U) sequences are parts of P-independent terminators, can also be regarded as containing such features (Table 2). Here it should be added, that the S1 requirement for the synthesis of anthranilate synthetase, encoded by two first genes of the E. coli trp operon, was shown in direct biochemical experiments (45), and crosslinking of S1 to the trp mRNA was observed as a result of UV-irradiation of intact E. coli cells (30).

To test the idea that oligo(U)-stretches in leader parts of bacterial genes represent targets for S1, we have studied the ssb gene (see Table 1). 30S initiation complex formation and its dependence on S1 were examined in toeprint (extension inhibition) experiments (Fig. 4). As was shown for other mRNAs, the 30S particle bound to the messenger in the presence of initiator tRNA inhibits reverse transcription from a primer annealed downstream on the template that leads to appearance of a toeprint signal at +15 position from the first base of the initiation codon (2,26). When 30S subunits completely devoided of S1 were tested by this method for their ability to bind to the ssb mRNA, no toeprint signal was observed (Fig. 4). The addition of free S1 produced a significant stimulation of the mRNA binding ability of the 30S subunit that led to appearance of the toeprint at +15 position. Increasing of S1 concentration in reactions resulted in disappearance of the toeprint, reflecting an inhibition of initiation complex formation. We have obtained similar effects with other messengers (will be published elsewhere).

Thus S1 can act both as a translational 'activator' and repressor, depending on its molar ratio to 30S subunits. It is reasonable to ask how an excess of free S1 prevents binding of 30S subunits to the ssb mRNA. As in the case of Qβ RNA (see above), this effect is expected if free S1 binding to the mRNA occurs at the same site as does binding of S1 when it is a part of 30S subunit. This simple competition mechanism can explain the function of S1 as gene-nonspecific translational repressor in vitro. According to this model, in order to identify the target for S1 within translation initiation complexes, it is sufficient to find the S1 binding site on the messenger within a complex between mRNA and pure S1.

To identify the S1 binding site on the ssb mRNA we have used two methods: enzymatic footprinting and UV-induced crosslinking. Partial digestions of the free ssb RNA with RNases U2 and T2 followed by primer extension revealed that S1 protected a region comprising an oligo(U)-tract preceding the SD domain and some upstream nucleotides (A-22,A-25,26). No pronounced protection of other mRNA parts within translational initiation region was observed.

![Figure 5. Footprinting of protein S1 bound to the ssb mRNA. Lanes 1,2,3—partial RNase U2 digestion of the ssb mRNA (0.8 pmol) incubated without (lane 1) or with increasing amount of S1: 8 pmol (lane 2) and 16 pmol (lane 3). Lanes 4,5—partial digestion of the ssb mRNA with RNase T2 without S1 (lane 4) and in the presence of 4 µM S1 (lane 5). Below—a part of the leader sequence of the ssb mRNA with indication of nucleotides protected by S1 against RNase U2 (small arrows) and RNase T2 (large arrow).](image)
Sl-dependent stop is also observed (Fig. 6). At the present time that all U-residues in a cluster can participate in crosslinking while favorable.

Broadening of the signal probably means photoreactions: photomodification of uracil residues and RNA-oligo(U) sequence in the control experiment without addition of SI appears most likely due to the UV-induced modification of most of the bases more than 20 nucleotides upstream from the SD domain could be reactive in the initiation process not simply because the absence of secondary structure would expose the initiation signals as was proposed in (10), but because they might serve as recognition signals bound by components of translational machinery such as ribosomal protein SI.

CONCLUDING REMARKS

Examples of the genes containing in their 5'-untranslated regions U-clusters are presented in the Tables 1 and 2. As one can see these clusters are situated at different distances from the initiation codon thus being a 'drifting' element of mRNA primary structure. Our proposal that these elements work in a process of ribosome-mRNA recognition and binding via interaction with protein SI. The results also suggest that unstructured regions upstream from the SD domain could be reactive in the initiation process not simply because the absence of secondary structure would expose the initiation signals as was proposed in (10), but because they might serve as recognition signals bound by components of translational machinery such as ribosomal protein SI.

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REFERENCES