A Single Missense Mutation in a Coiled-Coil Domain of *Escherichia coli* Ribosomal Protein S2 Confers a Thermosensitive Phenotype That Can Be Suppressed by Ribosomal Protein S1

Leonid V. Aseev, Anton O. Chugunov, Roman G. Efremov, Irina V. Boni

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia

Ribosomal protein S2 is an essential component of translation machinery, and its viable mutated variants conferring distinct phenotypes serve as a valuable tool in studying the role of S2 in translation regulation. One of a few available *rpsB* mutants, *rpsB1*, shows thermosensitivity and ensures enhanced expression of leaderless mRNAs. In this study, we identified the nature of the *rpsB1* mutation. Sequencing of the *rpsB1* allele revealed a G-to-A transition in the part of the *rpsB* gene which encodes a coiled-coil domain of S2. The resulting E132K substitution resides in a highly conserved site, TKKE, a so-called N-terminal capping box, at the beginning of the second alpha helix. The protruding coiled-coil domain of S2 is known to provide binding with 16S rRNA in the head of the 30S subunit and, in addition, to interact with a key mRNA binding protein, S1. Molecular dynamics simulations revealed a detrimental impact of the E132K mutation on the coiled-coil structure and thereby on the interactions between S2 and 16S rRNA, providing a clue for the thermosensitivity of the *rpsB1* mutant. Using a strain producing a leaderless *lacZ* transcript from the chromosomal *lac* promoter, we demonstrated that not only the *rpsB1* mutation generating S2/S1-deficient ribosomes but also the *rpsA::IS10* mutation leading to partial deficiency in S1 alone increased translation efficiency of the leaderless mRNA by about 10-fold. Moderate overexpression of S1 relieved all these effects and, moreover, suppressed the thermosensitive phenotype of *rpsB1*, indicating the role of S1 as an extragenic suppressor of the E132K mutation.

Ribosomal protein (r-protein) S2 is essential for the translational machinery and is highly conserved across all kingdoms of life (S2 in prokaryotic-type ribosomes, S0 in yeast, and SA in higher eukaryotes), and yet its functions in protein synthesis are still poorly understood. It was suggested that prokaryotic S2 might be involved in stabilizing the Shine-Dalgarno (SD) helix docked in a chamber between the head and the platform (1), as well as in protecting the SD duplex at the early postinitiation step (2). However, this does not explain the vital function of S2 in organisms that do not exploit the SD interactions in translation initiation. S2 is one of the latest components identified in the 30S assembly (3). In *Escherichia coli* and most likely in other Gram-negative organisms, its association with the 30S particle is critical for binding the S1 r-protein that accomplishes the assembly of the 30S subunit that is fully competent in recruiting mRNA (4–6).

Even though the structure of S2 has not yet been resolved for a free protein, it may be extracted from the high-resolution X-ray structure of the ribosome (7, 8). As the ribosome to be crystallized must be depleted of the r-protein S1, S2 (29.3 kDa) appears to be the largest r-protein in the crystal. It is located on the back of the 30S subunit at the hinge between the head and body (Fig. 1). Possessing an elongated bidomain structure, S2 forms several direct contacts with a number of 16S rRNA helices, viz, h35-h37 in the head via the coiled-coil α_2 domain and h26 in the body via the large globular domain (Fig. 1). The strongest interactions between S2 and 16S rRNA are formed between the side of the protruding coiled-coil, which includes both acidic and basic residues, and ribose backbone atoms of the h35-h37 (7). This is consistent with the footprinting data obtained earlier (9). It is also worth mentioning that S2 has been implicated in formation of 100S ribosome dimers, the so-called hibernating ribosomes, in which the 70S monomers interact flexibly via the 30S subunits (10).

Besides its functioning as a ribosomal component, S2 has ex-

traribosomal activities that are different in prokaryotes and eukaryotes. Bacterial S2 is encoded by the first gene of the *rpsB-tsF* operon that also comprises a gene for the Ts elongation factor. In gammaproteobacteria, S2 serves as an autogenous repressor of both genes. It binds within a highly structured 5' untranslated region (5'-UTR) and represses its own translation; this repression interrupts transcription-translation coupling in the operon, thereby decreasing the level of the bicistronic *rpsB-tsF* mRNA and hence the level of the Ts synthesis (11–13). To achieve the efficient autogenous repression, S2 needs a companion, r-protein S1, with which S2 may form a complex outside the ribosome, as revealed by the immunoprecipitation assay (12). Earlier, the stoichiometric S1-S2 complex was isolated during purification of the RNA polymerase from the stationary-phase cultures (14), and very recently, the specific interaction of S1 with S2 as well as with its isolated coiled-coil domain was confirmed by a pulldown assay and other techniques employing the tagged S1 variants (5). At present, how the *rpsB* expression is regulated in species differing from gamma-proteobacteria remains unknown.

During evolution and concurrent with the appearance of the extracellular matrix in higher eukaryotes, the S2 counterpart SA acquired an extraribosomal function as a precursor of the laminin binding receptor (15). The 67-kDa laminin binding protein is a homodimer of the 37-kDa r-protein SA, which is exposed on the cell surface as a receptor for laminin, growth factors, viruses, and

Received 21 July 2012 Accepted 21 October 2012

Published ahead of print 26 October 2012

Address correspondence to Irina V. Boni, irina_boni@ibch.ru.

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doi:10.1128/JB.01305-12

FIG 1 Structure of the S2 ribosomal protein. The inset shows the overall structure of the *E. coli* 30S ribosomal subunit (PDB code 2AVY; 8), encrusted with r-proteins S2 to S20. The main picture shows the domain structure of r-protein S2: a globular domain that interacts with the “body” and an internal coiled-coil domain protruding to the “head” of the 30S subunit. The coiled-coil domain is reminiscent of those of the leucine zipper family proteins; three pairs of leucines and a serine-threonine pair “above” them are shown at the interface of two α -helices. At the beginning of the second α -helix there is a so-called N-terminal capping box motif, 129TKKE132 (consensus S/TXXE), with the residues T129 and E132 capable of forming reciprocal h-bonds.

prions (16). Interaction sites responsible for extracellular functions reside in a C-terminal part of the protein which contains LMWWML, a unique palindromic motif that is absent from prokaryotic S2. Analysis of sequence similarity between prokaryotic S2 and eukaryotic S0/SA revealed another principal difference among them: the protruding coiled-coil α_2 domain that forms the contacts with the 16S rRNA in the head of the prokaryotic small subunit is absent from the eukaryotic S2 counterparts (15, 17).

Two mutated variants of the *E. coli* *rpsB* gene have been previously partly characterized—*rpsB1* (Ts) and *rpsB11* (*rpsB*::IS1) (4, 18). The *rpsB1* mutation confers the temperature-sensitive phenotype, so that the mutant cannot grow above 40°C, and its lethality at high temperature was the first proof of the vital necessity of S2 for bacterial life (4). More recently, the essentiality of S2 was directly confirmed by genetic knockout experiments (19). The *rpsB1* mutant grows slowly at a permissive temperature of 37°C, most likely because S2 is partially inactivated, and the denatured form is either unable to assist in 30S assembly or quickly degraded or both. As a result, a part of 30S appeared to be depleted not only of S2 but also of S1 (4), and the S1/S2-containing ribosomes active in translation accumulate slowly. The *rpsB11* mutation is the insertion of the transposable element IS1 just behind the tandem stop codons, which destabilizes the *rpsB* mRNA, thereby decreasing the level of S2 in a cell (18). Just as in the case of *rpsB1*, a portion of the ribosomes appeared to be depleted of both S2 and S1 and thus incapable of translating common cellular mRNAs. In both mutants, the autogenous control of the *rpsB*-*tsf* expression is destroyed due to the absence of the amount of S2 necessary for repression (12). Even though the S1/S2-depleted ribosomes in the *rpsB* mutants are unable to translate canonical mRNAs, they retain the capability of accommodating the 5'-terminal AUG codon in the P-site during initiation complex formation on leaderless mRNAs (like those encoding the λ c1 repressor or Tn1721 TetR repressor), which are translated in the *rpsB* mutants much

more efficiently than in cells with wild-type (wt) *rpsB* (*rpsB*⁺) (6, 18, 20, 21).

Oddly enough, although the *rpsB1* mutant served as a useful tool for studying mechanisms of leaderless mRNA translation (6, 18) or the *rpsB*-*tsf* expression regulation (12), the nature of its thermosensitivity has never been reported. The main goal of this paper was to map and to characterize the *rpsB1* mutation. Sequencing of the *rpsB1* allele revealed a G-to-A transition in the part of the *rpsB* gene which encodes the S2 coiled-coil α_2 domain. The resulting E132K substitution was found to reside in a highly conserved site, a so-called N-terminal capping box (22, 23), at the beginning of the second α -helix. Molecular dynamics simulations showed that this missense mutation in the conserved N-capping TKKE motif (residues 129 to 132) destabilized the coiled-coil structure even at the permissive temperature of 37°C, which would be harmful to the interactions between S2 and 16S rRNA helices in the head of the 30S subunit. Using a specialized strain producing a leaderless *lacZ* transcript from the chromosomal *lac* promoter, we demonstrated that not only the *rpsB1* mutation generating ribosomes depleted of both S2 and S1 but also the *ssyF29* mutation (*rpsA*::IS10) leading to partial deficiency in S1 alone (24) increased the translation efficiency of the leaderless *lacZ* mRNA by 1 order of magnitude. S1 moderately overexpressed from a plasmid relieved all these effects and, moreover, suppressed the temperature-sensitive phenotype of *rpsB1*, acting as an extragenic suppressor of the E132K mutation.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Escherichia coli* strains and plasmids used in this study are listed in Table 1. Construction of the strain producing the leaderless mRNA from the chromosomal *lac* promoter was described earlier (27). In brief, at the first step, the plasmid producing the leaderless transcript was generated on the basis of pES191 (28), which bears downstream from the *lac* promoter/operator the *rpsA* translation initiation region (TIR) fused in-frame with the *lacZ* sequence encoding the alpha peptide. The *rpsA* TIR in this construct comprised the 91-nucleotide (nt)-long 5'-UTR and the first 57 nt from the coding sequence (24, 28). The region between the transcription start and the initiation ATG codon was deleted by using *PfuUltra* II fusion HS DNA polymerase (Stratagene) in PCR with primers flanking the sequence to be deleted (to amplify the whole plasmid except for the region chosen for deletion). The purified PCR product was ligated, treated with DpnI, and used to transform DH5 α for selecting Lac⁺ clones. The resulting plasmid, p5'AUGlacZ, was checked by sequencing and used for transformation of ENSO. In transformed cells, the *lac* promoter and the beginning of the *lacZ* coding sequence were moved from the plasmid onto the chromosome by homologous recombination, resulting in Lac⁺ recombinants selectable on McConkey agar. The resulting strain, KB5'AUGlacZ, produced a leaderless *lacZ* mRNA from the chromosomal *lac* promoter.

The *rpsB1* and *rpsB11* alleles were transferred into the strain by P1 transduction. The Tn10 marker from CAG18436 (26) was cotransduced with the *rpsB* alleles to provide selection of the tetracycline-resistant transductants. The *rpsB1* cells were selected by their Ts phenotype and the *rpsB11* cells by their tiny colonies on agar plates, which were further checked by PCR for the presence of IS1. The *ssyF29* mutation (*rpsA*::IS10) was P1 transduced as described previously (24).

Cell growth and β -galactosidase assay. The strains were routinely grown at 37°C in LB broth supplemented, if necessary, with antibiotics (tetracycline, 12 mg/ml; chloramphenicol, 34 mg/ml). The cells were harvested in exponential phase (optical density at 600 nm [OD₆₀₀], ~0.4 to 0.5) and broken by a repeated thawing-freezing procedure for preparing clarified cell lysates as described previously (31). The protein concentration in a fraction of soluble proteins was determined by a Bradford

TABLE 1 *Escherichia coli* strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s) ^a	Source or reference(s)
Strains		
DH5 α	Cloning host	Laboratory stock
ENSO	<i>his</i> ; formerly HfrG6 Δ 12, the Lac [−] strain lacking the <i>lac</i> promoter and <i>lacZ</i> RBS	25
CAG18436	MG1655 <i>zae</i> -502::Tn10, insertion position on <i>E. coli</i> map 04.75	26
CS240	<i>rpsB11</i> (<i>rpsB</i> ::IS1)	18
RL425	<i>metB</i> HfrP4X λ [−] <i>rpsB</i> ^{ts} (<i>rpsB1</i>)	4
KB5'AUGlacZ	ENSO derivative producing leaderless <i>lacZ</i> mRNA	27
KB5'AUGlacZrpsB ⁺	KB5'AUGlacZ tet ^r (Tn10 at 4.75 min)	This work
KB5'AUGlacZrpsB1	KB5'AUGlacZ tet ^r <i>rpsB1</i> ^{ts}	This work
KB5'AUGlacZrpsB11	KB5'AUGlacZ tet ^r <i>rpsB11</i>	This work
KB5'AUGlacZssyF29	KB5'AUGlacZ tet ^r <i>rpsA</i> ::IS10 (Tn10 at 20 min)	This work
Plasmids		
pEMBL Δ 46	pEMBL8+ derivative lacking <i>lacZ</i> RBS	25
pES191	pEMBL Δ 46 derivative bearing <i>rpsA-lacZ</i> fusion	24, 28
p5'AUGlacZ	pES191 derivative in which transcription starts with the initiator AUG	27
pACYC184	tet ^r , Cm ^r , cloning vector	Laboratory stock
pSP261 (pS1)	pACYC184 derivative expressing the <i>rpsA</i> gene from its own promoters	29
pS2	pACYC184 derivative expressing the <i>rpsB</i> gene from its own promoter	12
pS15	pACYC184 derivative expressing the <i>rpsO</i> gene from its own promoter	30

^a Cm, chloramphenicol; RBS, ribosome binding site; tet, tetracycline.

assay (Bio-Rad). The specific β -galactosidase activities were expressed in nanomoles of ONPG (o-nitrophenyl- β -D-galactopyranoside) hydrolyzed per minute per milligram of total soluble cell proteins.

Identification of the *rpsB1* mutation. The *rpsB* regions from *rpsB*⁺ and *rpsB1* strains were amplified from genomic DNA by using *PfuUltra* II fusion HS DNA polymerase (Stratagene) and primers *rpsBseq*-for (5'-G GGGTCGGTAATATGGGATACG) (positions from −61 to −40 relative to the *rpsB* start codon AUG) and *rpsBseq*-rev (5'-GGAATTCTCGAGC TTGCCGC) (complementary to positions +770 to +789 downstream from the *rpsB* tandem stop codons). The PCR products were purified and sequenced in both directions. Sequencing was performed by Evrogen (Moscow, Russia).

Phylogenetic analysis. The amino acid sequences corresponding to the coiled-coil domain of S2 from different bacterial species covering the whole spectrum of bacterial phyla were extracted from the NCBI database (NCBI/Protein) and aligned by using Align X, a component of Vector NTI Advance 10.1.1.

Molecular modeling study on the effect of the E132K mutation. To study the probable effect of the E132K mutation on the S2 structure, molecular dynamics (MD) simulations of the coiled-coil domain (residues W95 to G155) were performed for wild-type and mutant variants of this helical pin. The structure was extracted from Protein Data Bank (PDB) entry 2AVY (8); the *in silico* mutation procedure resulting in E132K was performed with PYMOL (The PyMOL Molecular Graphics System, version 1.5.0.4; Schrödinger, LLC). MD simulations were performed with Gromacs 4.5.2 (32), using the following protocol. Either a mutant or a wild-type helical pin was placed into a water box (50 Å by 50 Å by 50 Å; approximately 4,000 water molecules) together with the necessary amount of counterions (Cl[−]) and subjected to energy minimization followed by heating to 310 K (37°C) for 100 ps and 50 ns of unconstrained MD simulations. A GROMOS96 45a3 force field (33) and SPC water model (34) were used. MD simulations were carried out with a time step of 2 fs, under imposed three-dimensional (3D) periodic-boundary conditions, in the isothermal-isobaric (NPT) ensemble with an isotropic pressure of 1 bar and a constant temperature of 310 K. The temperature and the pressure were scaled using a V-scale thermostat (35) and the Berendsen barostat (36) with 0.1- and 1-ps relaxation parameters, respectively. Van der Waals interactions were truncated using a 12-Å spherical cutoff. Electrostatic interactions were treated using the particle-mesh

Ewald summation (real-space cutoff of 12 Å and 1.2-Å grid with fourth-order spline interpolation).

To increase efficiency of conformational sampling, five independent MD trajectories were collected for both wild-type and mutant helical pins. Analysis included visual inspection, calculation of the secondary structure as a function of MD time, and analysis of the hydrogen-bonding pattern in the N-terminal helical capping box motif (T/SXXE) where the studied mutation E132K was mapped.

RESULTS AND DISCUSSION

Identification of the *rpsB1* mutation. The chromosomal regions of the wild-type (*rpsB*⁺) and *rpsB1* (Ts) strains, comprising the *rpsB* gene and its flanking sequences, were amplified by PCR and sequenced in both directions. Comparative analysis of sequences revealed one point mutation in the thermosensitive strain, namely, a G-to-A transition that leads to a single substitution, E132K, in the S2 amino acid sequence corresponding to the coiled-coil domain (Fig. 1). Phylogenetic analysis of the S2 coiled-coil domains from sequenced bacterial genomes belonging to diverse bacterial phyla has shown that this mutation resides in a highly conserved site, KKE, at the beginning of the second alpha helix (Fig. 2). These three amino acids (boxed in Fig. 2) are almost universal in all phyla, with only a few exceptions: e.g., KKQ in *Dictyoglomi*, KKM in *Planctomycetes*, and KRE in several representatives of *Bacteroidetes* (Fig. 2). The high level of conservation may indicate the important role of the KKE sequence in maintaining the functional structure of the coiled-coil domain necessary for ribosome binding (7) or contacts with S1 (5). Alternatively, the E132K mutation can make S2 more prone to degradation by cellular proteases, including the Lon ATP-dependent protease that was shown to specifically degrade S2 in the presence of inorganic polyphosphate (37, 38). These possibilities are not mutually exclusive, since the perturbations in a protein structure provoked by the mutation may expose the disordered regions for proteolysis.

Molecular modeling study on the effect of the E132K mutation. To study how the E132K mutation may affect the structure of

FIG 2 Alignment of amino acid sequences corresponding to the coiled-coil domain of r-protein S2 from various bacterial taxa.

S2, we performed a set of MD simulations for the wild-type (wt) and mutant (E132K) S2 coiled-coil domain (α -helical pin) in an explicitly defined water box (see Materials and Methods). To increase efficiency of conformational sampling, 5 independent MD

trajectories (50 ns each) were collected for the wt and 5 for the E132K variants; in total, 10 MD trajectories were calculated.

In each of five trajectories, the wt-coiled-coil, as controlled by secondary structure analysis and visual inspection, remained sta-

Lentisphaerae	
Victivallis vadensis	RWLGGLTNNVTIRKSTTKMAEIDGTLNS-DAAKSMKKKIIAGLSIRAEKIHRLDGTIAQMKRLE-A
Deinococcus-Thermus	
Deinococcus maricopensis	RWLGGLTNNFTIRMTFDRINBDDMFES-ERINDRFKABRIOLGNREERLRYVGGIRKMTIME-D
Truepera radiovictrix	RWLGGLTNNFTIRSRVERIKETRMFED-ESILRYSKKEQIDGKELDRIOYLGGIKIDYTRLE-D
Marinithermus hydrothermalis	RWLGGLTNNFTIRSAVRNRIEELALFES-EEIKERFKKEQVRUKHLEDRKRYTSGFRRLKRIE-D
Meiothermus ruber	RWLGGLTNNFTITACVNRIAELEALFAS-DEIKERVKIEQVKLQHELERLORNLGGFRKLRRIE-D
Thermus thermophilus	RWLGGLTNNFTISQVRHRIEELALFAS-PEIEERFKKEQVRUKHLEDRKRYTSGFRRLKRIE-D
Tenericutes	
Mycoplasma genitalium	RWLGGLTNNFTKLSISINKNKIVEQQKQ--NANDITKKEINLLSREIERLEKFFGGVKNLKRLE-N
Mycoplasma mycoides	RWLGGLTNNLFTILIRKAIWEIEEEKK-ERLSLRFKKEQIKILKEKTKLEKALGGIKQMKRIE-A
Spiroplasma citri	RWLGGLTNNLFTIHLRVKRIWNTERQEK-N-ELKLIFKKEQMLIKKEKDKLEKFLGGIKQMKRIE-Q
Firmicutes	
Bacillus subtilis	RWLGGLTNNFTITCKRKKRLKDIKKMQEN-ETFDVLFKKEVVOCKKLELERLEKFLGGIKDKRIE-D
Bulleidia extructa	RWLGGLTNNFTITCKSICKLLEIEEAEAT-CAINVYRKKEQALLKKKDRLENFLLGGIKDKRIE-D
Sulfobacillus acidophilus	RWLGGLTNNFTITKRRVKRIAEIEKEQAS-QOWERIEKKEVSAUSQKAKLEKYLGGIEQVRRIE-A
Clostridium botulinum	RWLGGLTNNFTITRPNKLEQLEKMEED-ETFEVLFKKEVILKRNIEKLEKNLGGIKNLDASKLG
Eubacterium rectale	RWLGGLTNNFTIESRUALKAIETMSD-ETFDVLFKKEVIALKKEWBLEKNLGGIKDKRIE-D
Geobacillus sp.	RWLGGLTNNFTITCKRKKRLKDIKKMAE-ETFDVLFKKEVIRIKKEQERLEKFLGGIKDKRIE-D
Syntrophothermus lipocalidus	RWLGGLTNNFTITKRVYRIKLEKMEEE-CAELIEFKKEVARIRAEKLEKFLGGIKDKRIE-G
Thermobacillus composti	RWLGGLTNNFTITCKRDRLEKMEED-ETFEVLFKKEVILKRNIEKLEKFLGGIKDKRIE-S
Staphylococcus aureus	RWLGGLTNNFTISKRKRIEIEKMEED-CLFEVLFKKEVVDEKKEVDRILKFLGGIKDKRIE-Q
Lactobacillus buchneri	RWLGGLTNNFTISQVRKRLKDIKKMST-ETFDIIEFKKEVSLIKQEKLEKFLGGIEQVPIE-D
Selenomonas sputigena	RWLGGLTNNFTITCKRRLKDIKKMEED-ETFDVLFKKEVILKRNIEKLEKFLGGIKDKRIE-G
Synergistetes	
Aminobacterium colombiense	RWLGGLTNNFTIRKRVTRMVETRKMAE-DNWSNIEFKKEVALKKELAKLEYISGIAQMKNVE-D
Jonquetella anthropi	RWLGGLTNNFTILKRVQRMIEIKQIDAD-DDWGEHTKKEISLIRKEKAKLEYISQIRDKRIE-D
Cyanobacteria	
Arthrospira maxima	RWLGGLTNNVTITKRWDRLEKLEARENS-COLDYIEFKKEAAVLRREMKIKYLGGLKQVRRIE-D
Fischerella sp.	RWLGGLTNNVTITKRWDRLEKLEARENS-CALDLIEFKKEASMRRELAQIKYLGGLKQVRRIE-D
Nostoc azollae	RWLGGLTNNVTITKTRAERLEKLEARENS-CALDLIEFKKEGSMRREMTKIKYLGGLKQVRRIE-D
Cyanothece sp.	RWLGGLTNNVTITKRAERLEKLEARENS-CAISRRFKKEAAVLRRELFKIKYLGGLKQVRRIE-D
Synechococcus sp.	RWLGGLTNNVTITKRWDRLEKLEARENS-CALAMRFKKEGSMRRELFKIKYLGGLKQVRRIE-D
Prochlorococcus marinus	RWLGGLTNNVTITKRAERLEKLEARENS-ESIAMRFKKEAAVLRRELFKIKYLGGLKQVRRIE-D
Dictyoglomi	
Dictyoglomus thermophilum	RWLGGLTNNFTITKSRDYMVKLELKN-NYFEKLFKKEANRINRELEKIVVFDGLRCKEIE-D
Dictyoglomus turgidum	RWLGGLTNNFTITKSRDYMVKLELKN-NYFEKLFKKEANRINRELEKIVVFDGLRCKEIE-D
Thermotogae	
Thermotoga maritima	RWLGGLTNNFTIRSRDKLEIELEMEQS-EKLEELFKKEQSRIRVLEKIRKLNLGGIKDKRIE-D
Thermotoga naphthophila	RWLGGLTNNFTIRSRDKLEIELEMEQS-EKLEELFKKEQSRIRVLEKIRKLNLGGIKDKRIE-D
Fusobacteria	
Leptotrichia buccalis	RWLGGLTNNLNTIKTRVKRLKLEEMDADGTLDEAYTKKEAGLRKEMAKSKNLGGIKDKRIE-A
Seibaldella termitidis	RWLGGLTNNLNTIKTRVKRLKLEEMDQNGMLDES YTKKEAGLRKEMAKSKNLGGIKDKRIE-A
Streptobacillus moniliformis	RWLGGLTNNLETIKKRVKLEIELEMEADGTLDEAYTKKEAAIRKEMAKSKNLGGIKDKRIE-A
Spirochaetes	
Borrelia burgdorferi	RWLGGLTNNFTIRKSVQRLKLEKMEVD-ETFDMISKKEISQINREKSKTARNITGIDMETIE-G
Spirochaeta smaragdinae	RWLGGLTNNFTIRKSTHRLKLEKMEVD-ETFESLTKKEIAKINREKSRLEKNLGGIKDKRIE-G
Spirochaeta thermophila	RWLGGLTNNFTIRKSLRLKLEKMEVD-ETFATMSKKEVSRLMKKARTORNLGGIKDKRIE-G
Treponema succinifaciens	RWLGGLTNNFTIRKSLRLKLEKMEVD-ETFDKLTKEKVAAYMKKEITITKNYCGMKDKRIE-G
Aquificae	
Aquifex aeolicus	RWVGGLTNNFTIRKSTIMKLOTLEMEED-ETFDVLFKKEVRNLREKMEKIRKLYNGIRDYKRIE-D
Thermovibrio ammonificans	RWLGGLTNNFTIRKSVFKLEKMEED-ETFDVLFKKEAVRNRKKEKLEERYIGGIENNNRIE-D
Thermocrinis albus	RWVGGLTNNFTIRKSTILKLEKMEED-ETFDVLFKKEVRNLREKMEKIRKLYNGIRDYKRIE-D
Thermodesulfobacteria	
Thermodesulfatator indicus	RWLGGLTNNFTIRRSIEKLEKLEWFFD-ETIERFFKKEIRKLEELKQKLEKNLGGIKDKRIE-Q
Chloroflexi	
Ktedonobacter racemifer	RWLGGLTNNFTITOLRKYRDLERRRDS-EEFERIEFKKEVQRLQDDIARLERILGGIKDKRIE-S
Roseiflexus castenholzii	RWLGGLTNNFTIRKRLRYLINDEQRRAR-EEFNKLTAKATIKRDAEIEKINKILGGIKDKRIE-G
Sphaerobacter thermophilus	RWLGGLTNNFTIRSRRLYKLEERQAAA-ELDTIEFKKEGIRKRELEKIRQTLGGMRDVTCLIE-A
Verrucomicrobia	
Opitutaceae bacterium	RWLGGLTNNFTIRKSTIAKFKFQALETS-EDINKISAKEGAAIKREVMRNKNFAGIVDPEIE-S
Opitutus terrae	RWLGGLTNNFTIRKSTIAKFKYQOMETS-EDMNKISAKEGAAIKREVMRNKNFAGIVDPEIE-S
Pedospira parvula	RWLGGLTNNFTIRKSTIAKFKLEKMETD-EGINQYVKEQSALREHARQFYFDGIRSDKYE-A
Consensus	RWLGGLTNNFTIRSI RLKELE M D G FD LTKKE L L RE EKLEK LGGIKDM RLP D

X	- identical residues (100% conservation)
X	- highly conserved residues
X	- conserved residues
X	- weakly conserved residues
X	- absence of conservation

alpha helix alpha helix

FIG 2 continued

ble (Fig. 3A, B, and D). A reciprocal h-bonding pattern had emerged at the N terminus of the second α -helix of the coiled-coil domain: a side chain of E132 formed an h-bond with the backbone amide of T129 and a side chain of T129 formed an h-bond with the

backbone amide of E132 (Fig. 3B). This motif (TXXE), which is known as an N-terminal capping box, stabilizes N termini of α -helices, thereby playing an important role in the stability and function of proteins (22, 23, 39).

FIG 3 Effect of the E132K mutation on the structure of the coiled-coil domain of S2, as revealed by molecular dynamics simulations. (A) A sample structure of wt-coiled-coil from MD. The structure remains stable, with no disturbance brought to the leucine zipper motif. (B) Zoomed view of the α -helical pin and the N-terminal capping box motif (T129–X–E132). A hydrogen-bonding “lock” is formed by residues T129 and E132 which stabilizes the N terminus of the second α -helix in the coiled-coil domain. (C) Three sample structures of the E132K coiled-coil from MD trajectories 2 (32,300 ps), 4 (36,130 ps), and 5 (31,500 ps). The E132K mutation severely disrupts the α -helical structure. (D) Secondary structure analysis of all 10 MD trajectories in this study. The number of amino acid residues that adopt α -helical conformation is plotted against MD time for all five wt-S2 trajectories (the thick black line with a blue halo shows averages and standard deviations) and individual E132K-S2 trajectories 1, 2, 3, 4, and 5 (red, yellow, magenta, cyan, and green thin lines, respectively). Data are averaged in 50-ps sliding intervals to reduce noise.

In contrast, in three of the five trajectories, the E132K-coiled-coil became unstable, and its overall structure “melted” (Fig. 3C and D). This is because the side chain of K132 is incapable of stabilizing the helix by interacting with T129. Instead, K132 forms a network of h-bonds with the first helix, and this might destabilize the structure of the coiled-coil as well. Apart from losing regular structure, the two α -helices in the mutant change their crossing angle, so that sometimes they appear almost orthogonal, in contrast to the approximately parallel organization in the wild-type structure. In two trajectories of the five, the structure seems to be only slightly disturbed (see the secondary structure analysis for trajectories 1 and 3 in Fig. 3D). This is because the amount of time for the MD simulations was quite limited; we would have to increase sampling and analyze several independent trajectories to reveal possible effects of single-point mutations.

It has been found that α -helices often bear at their N termini either S/T motifs (serine or threonine) or Asx motifs (asparagine or aspartate), which form two characteristic hydrogen bonds to residues located three positions ahead in the sequence (40, 41). The N-capping hydrogen bond interactions have been shown to be essential for maintaining the helix integrity and its thermal stability. Both the first residue (N-cap) and the partner residue (N3) are crucial. Thus, the substitution of either S/T or E/D residues in the N-terminal capping box S/TXXE/D by alanine impeded protein folding and increased thermal lability (42, 43). Molecular dynamics simulation of the mouse prion protein fragment

also revealed that mutations in N-cap and N3 residues destabilized the structure and promoted unfolding of the α -helix (39).

Application of MD simulations to the coiled-coil domain of S2 clearly shows that the E132K substitution in residue N3 of a conserved N-capping TXXE box (see consensus sequence in Fig. 2) causes the loss of the h-bonding partner, disrupting the capping motif and inducing melting of the coiled-coil structure (Fig. 3). As r-protein S2 forms the strongest interactions with the 30S subunit via the coiled-coil domain (7), it is very likely that the melting of its structure caused by E132K impairs interaction with the “head” of the 30S subunit, which may be critical for the S2-ribosome binding. Since the capping motif influences protein thermal stability (reference 42 and this paper), the impact of E132K on the coiled-coil structure should be more harmful at high temperature, leading to the complete inability of the essential S2 protein to associate with the ribosome, which explains the conditionally lethal phenotype of the *rpsB1* mutation.

Revisiting the role of the *rpsB* mutations in translation of leaderless mRNAs. Leaderless mRNAs are devoid of 5' untranslated regions (mRNA leaders) and bear the AUG initiation codon at their 5' terminus or very close to it (reviewed in references 44 and 20). One of the features specific for leaderless mRNAs in *E. coli* is that they can be translated by ribosomes unable to form the initiation complexes with canonical leadered mRNAs, in particular, by the ribosomes devoid of the r-proteins S1 and S2 (6, 18, 21) and even some other r-proteins, as was shown for the cells treated

TABLE 2 Effects of the *rpsB* and *rpsA* mutations and plasmids expressing r-proteins S2 and S1 on the β -galactosidase synthesis from single-copy translational *lacZ* fusions

<i>rpsA/rpsB</i> genes in chromosome	Plasmid	β -Galactosidase activity ^a
5'-AUG-<i>lacZ</i>		
<i>rpsA</i> ⁺ / <i>rpsB</i> ⁺	pACYC184	90 $\pm$ 15
<i>rpsA</i> ⁺ / <i>rpsB1</i>	pACYC184	780 $\pm$ 120
<i>rpsA</i> ⁺ / <i>rpsB1</i>	pS2	110 $\pm$ 20
<i>rpsA</i> ⁺ / <i>rpsB1</i>	pS1	180 $\pm$ 50
<i>rpsA</i> ⁺ / <i>rpsB11</i>	pACYC184	820 $\pm$ 75
<i>rpsA</i> ⁺ / <i>rpsB11</i>	pS2	Nonviable
<i>rpsA::IS10/rpsB</i> ⁺	pACYC184	860 $\pm$ 100
<i>rpsA::IS10/rpsB</i> ⁺	pS1	75 $\pm$ 20
<i>galE-lacZ</i>		
<i>rpsA</i> ⁺ / <i>rpsB</i> ⁺	pACYC184	6,900 $\pm$ 300
<i>rpsA</i> ⁺ / <i>rpsB1</i>	pACYC184	6,500 $\pm$ 650
<i>rpsO-lacZ</i>		
<i>rpsA</i> ⁺ / <i>rpsB</i> ⁺	pACYC184	13,100 $\pm$ 650
<i>rpsA</i> ⁺ / <i>rpsB1</i>	pACYC184	12,600 $\pm$ 800
<i>rpsA-lacZ</i>		
<i>rpsA</i> ⁺ / <i>rpsB</i> ⁺	pACYC184	16,500 $\pm$ 1200
<i>rpsA</i> ⁺ / <i>rpsB1</i>	pACYC184	10,750 $\pm$ 650

^a The specific β -galactosidase activities are expressed in nanomoles of ONPG (o-nitrophenyl- β -D-galactopyranoside) hydrolyzed per minute per milligram of total soluble cell proteins.

with kasugamycin (45). Another peculiarity of the leaderless mRNAs is their capability of forming initiation complexes with 70S ribosomes (21, 46–49), albeit the 30S-mediated initiation pathway cannot be completely ruled out either.

The mechanisms for leaderless mRNA translation in *E. coli* have so far been mainly studied by using either *in vitro* techniques or *in vivo* experiments with plasmids carrying the leaderless translational fusions with the *lacZ* reporter (18, 21, 45–52). Because the copy number of plasmids may vary depending on different factors, for *in vivo* experiments it would be preferable to use a single-copy (chromosomal) genetic construction producing a leaderless transcript. We created KB5' AUGlacZ (Materials and Methods), an *E. coli* strain that meets the requirements, producing a leaderless *lacZ* mRNA from the chromosomal *lac* promoter. Since isogenic strains where *lacZ* expression is driven by diverse translation initiation regions (TIRs) are available (24, 25, 28), a new strain ensures a correct comparison of translational features for leaderless and canonical mRNAs in the β -galactosidase assay.

The β -galactosidase measurements have revealed that translation of the leaderless *lacZ* mRNA in *E. coli* is in fact very inefficient compared to translation of the leadered mRNAs (Table 2). Thus, the deletion of the 5' untranslated region (5'-UTR) from the SD-less *rpsA* TIR decreased the translation yield by more than 2 orders of magnitude (compare data for the construct *rpsA-lacZ* and its derivative 5' AUGlacZ in Table 2). Given that the leaderless λ c1 and Tn1721tetR mRNAs (related to *E. coli*) encode the repressor proteins for which just a few molecules per cell suffice for the dedicated repression, one may suppose that translation of leaderless mRNAs in *E. coli* is programmed to be inefficient under conditions when most ribosomes are homogenous and contain a complete set of r-proteins. This is most likely true for other representatives of members of the *Proteobacteria*. Recently, it has

been shown that the *acuR-acuI-dddL* mRNA transcript of *Rhodobacter sphaeroides* is leaderless and that the first gene, *acuR*, which encodes a TetR-type transcriptional repressor, is translated at a far lower level than the downstream genes whose translation is driven by canonical SD-dependent ribosome binding sites (53). The presence of analogous leaderless transcripts in other proteobacteria was predicted (53).

In the presence of the *rpsB* mutations, *rpsB1* and *rpsB11*, the efficiency of the leaderless *lacZ* mRNA increased by about 8- to 10-fold (Table 2), indicating the same tendency as was shown previously for λ c1 mRNA *in vivo* (18). This is a specific effect, as translation yields for canonical leadered mRNAs, e.g., *galE-lacZ* or *rpsO-lacZ*, do not change noticeably in mutant strains (Table 2). This means that the conditions in mutant cells became more or less equally unfavorable for translation of all leadered mRNAs because of the decreased rate of accumulation of S1/S2-containing ribosomes (see the introduction). At the same time, a subpopulation of ribosomes deficient in S1/S2 and able to translate only leaderless mRNAs enlarges in mutants, thus creating a unique chance for leaderless mRNAs to escape competition with canonical mRNAs. In the presence of pS2, a plasmid producing the wild-type S2 and thereby suppressing the *rpsB1* thermosensitivity, translation efficiency of the leaderless *lacZ* mRNA drops to the level typical of the *rpsB*⁺ background (Table 2). It is worth mentioning that for the *rpsB11* cells, pS2 is lethal. As was shown previously (12), the IS1 insertion in the intercistronic *rpsB-tsf* region significantly decreases the level of EF-Ts due to a polar effect of IS1 on the *tsf* expression, even though the polarity is not 100%. In the presence of pS2, the Ts level should be further reduced because of resuming S2-mediated repression, making the cell nonviable. Consistently, the lethal effect of pS2 is suppressed in the presence of a compatible second plasmid that expresses the *tsf* gene, thus supplying Ts necessary for survival (12).

Increased efficiency of the leaderless mRNA translation in the *rpsA* mutant. Interestingly, an increase in the efficiency of leaderless mRNA translation comparable with the effect of the *rpsB* mutations can be observed in *rpsB*⁺ cells bearing the *ssyF29* (*rpsA::IS10*) mutation that significantly slows down the accumulation of the S1-containing ribosomes (and hence the cell growth) due to the instability of the *rpsA* mRNA caused by the IS10 insertion (24). The *rpsA::IS10* mutant produces a truncated S1 that retains much of its activity in protein synthesis despite the lack of the last 92 amino acids (24). The efficiency of the leaderless mRNA translation in the *rpsA::IS10* mutant increased by as much as 1 order of magnitude (Table 2), indicating that the deficiency of ribosomes in both S2 and S1, as is the case of the *rpsB* mutants, is not a mandatory requirement for translation enhancement and that the deficiency in S1 alone is sufficient for this effect. This is consistent with the fact that the S1-depleted 70S ribosomes are able to form a tight, crystallizable complex with a short mRNA analog, 5' AUGUUU, in the presence of the anticodon stem-loop of the initiator tRNA (54). Analysis of polysomes isolated from the *rpsA*⁺ and *rpsA::IS10* exponential cultures (Fig. 4) showed a significant decrease in polysome fractions and an increased amount of the 70S couples in the mutant cells. Accumulation of 70S ribosomes correlates with the enhanced translation of the leaderless *lacZ* mRNA in the *rpsA* mutant (Table 2), thus supporting the alternative 70S pathway for the translation initiation on leaderless mRNAs (21, 46, 48). As expected, in the presence of pS1, the plasmid expressing the wild-type *rpsA* gene from its own pro-

FIG 4 Sedimentation analysis of polysomes isolated from exponential cultures of *rpsA*⁺ (right) and *rpsA::IS10* (*ssyF29*; left) strains growing in LB at 37°C. Cell extracts were prepared and analyzed essentially as described in reference 57. Polysome profiles in 10% to 40% sucrose gradients show a decreased level of polysomes and accumulation of 70S ribosomes in mutant cells.

motor system, the efficiency of the leaderless mRNA translation returned to the low level observed in the *rpsA*⁺ strain (Table 2).

The negative effect of S1 on the leaderless mRNA translation is most likely explained by the binding of this protein as a component of the 30S subunit to the nascent mRNA transcripts during translation initiation. In the case of canonical mRNAs bearing the 5'-UTR, this binding facilitates mRNA recruitment and formation of the 30S initiation complex on the downstream start codon, while in the case of leaderless mRNA, the S1-nascent mRNA interaction should prevent accommodation of the 5'-AUG codon in the P-site.

Suppression of the *rpsB1* mutation in the presence of pS1.

Surprisingly, the expression of the *rpsA* gene from a low-copy-number pS1 plasmid not only corrected the *rpsA::IS10* phenotype but also suppressed the *rpsB1* (Ts) mutation, so that in the presence of pS1, the *rpsB1* strain restored an ability to grow at an elevated temperature of 42°C (Fig. 5). Given that the S2 coiled-coil domain where the *rpsB1* mutation was mapped provides interaction both with the head of the 30S subunit and with S1, the aforementioned detrimental impact of E132K on the coiled-coil structure might impair these essential contacts. The suppression of *rpsB1* by moderate overexpression of S1 suggests that the E132K mutation does not prevent the S1 binding. Binding of excess S1 to mutated S2 corrects the growth defect of the *rpsB1* mutant (Fig. 5), indicating that most of ribosomes regained their competence in translating cellular messengers. In parallel with the restored translational capacity due to S1/S2 reassembly, the ribosomes lost an ability to translate leaderless mRNAs. Indeed, the efficiency of the leaderless *lacZ* mRNA translation in *rpsB1*/pS1 cells dramatically decreased (Table 2). The idea that in the presence of pS1 many more ribosomes became able to translate canonical mRNAs is supported by changes in growth rate: while the doubling time at 37°C in LB for *rpsB1*/pACYC184 cells was about 55 min, it was 43 min in the presence of pS1, which is almost the same as for wt cells bearing pS1 (40 min). The likely explanation is that S1 helps S2 to maintain the structure needed for interaction with the 30S subunits even at elevated temperatures; this chaperon-like effect is

FIG 5 Suppression of the thermosensitive phenotype of the *rpsB1* mutant by overexpression of r-proteins S2 and S1 from low-copy-number plasmids pS2 and pS1. Cloning vector pACYC184 and its pS15 derivative expressing r-protein S15 served as specificity controls. The *rpsB1* cells transformed with plasmids were streaked on LB agar plates containing 34 mg/ml chloramphenicol and incubated overnight at 37°C or 42°C.

consistent with the recently defined interaction of S1 with the S2 coiled-coil domain (5). It is also possible that due to S1 binding, the mutated S2 is protected against cellular proteases which attack the misfolded region. The moderate S1 overexpression from pS1 is necessary for this suppression effect, which may be accounted for by a slight (35%) decrease in the *rpsA* TIR translation efficiency in the *rpsB1* background (Table 2).

S1 appears to be a second extragenous suppressor of the thermosensitive mutation in the essential r-protein S2. Earlier, suppression of the *rpsB* Ts mutations by the DEAD box RNA helicase CsdA was reported (6, 55). It was proposed that CsdA could induce a certain conformational change in 16S RNA, facilitating binding of mutated S2 (6). However, CsdA has been shown to be involved in the biogenesis of the 50S rather than the 30S subunits, and the *csdA* gene deletion did not change the amount of S1/S2 in 30S preparations (56). These data do not support the proposed model, and the mechanism for the CsdA-mediated suppression of *rpsB1* still remains to be elucidated.

Concluding remarks. In this work, we identified for the first time the nature of the thermosensitive *rpsB1* mutation in a gene encoding r-protein S2. The mutation consists of a single E132K substitution in the coiled-coil α_2 domain and resides in a highly conserved site, TKKE, reminiscent of the so-called TXXE N-capping box (23), at the beginning of the second alpha-helix. The MD simulation data clearly demonstrate a detrimental impact of this single mutation on the coiled-coil structure even at the permissive temperature of 37°C (Fig. 3). Since S2 is very loosely attached to the 30S ribosomal subunit and since the strongest interactions between S2 and 16S RNA are formed between the side of the coiled-coil and h35–h37 helices in the head of 30S (7), the destruction of the coiled-coil organization would make the S2-30S interaction impossible at high temperatures. This provides a clue for understanding the conditionally lethal thermosensitive phenotype of the *rpsB1* mutant. The high level of conservation of the

TKKE N-capping motif (Fig. 2) suggests that other conserved residues also contribute to the functional structure of S2, thus opening prospects for future studies.

The observed enhancement of translation efficiency of the leaderless mRNA in the *rpsB* and *rpsA* mutants compared to the wild-type *E. coli* strain and its suppression in the presence of S1 in *trans* allow us to suppose that translation of leaderless mRNAs in *E. coli* (and related bacteria where S1 is essential) is naturally programmed as inefficient since the translational apparatus has evolved in an S1-dependent way. However, this is true only for growth under normal conditions when the ribosome population is mostly homogenous. The *rpsA/rpsB* mutations (references 6 and 18 and this paper) or stressful conditions, such as kasugamycin treatment (45) or MazF activation (49), generate a subpopulation of ribosomes that are unable to translate canonical leadered mRNAs and may perform only leaderless mRNA translation, which may be important for cell survival. It is worth emphasizing that S1 expressed in *trans* is able to suppress not only the *rpsA* mutation but also the thermosensitive phenotype of the *rpsB1* mutant. As the coiled-coil domain of S2 has been shown to interact not only with 30S but also with S1 (5), the ability of S1 to suppress the E132K mutation destroying the coiled-coil organization indicates that S1 may act as a chaperon, maintaining the coiled-coil structure and restoring the S2-30S interaction. In addition, the S1-S2 interaction may protect the mutated S2 from attacks of cellular proteases at elevated temperature. This finding brings new insight into the intimate relationship between these essential components of the bacterial translational machinery.

ACKNOWLEDGMENTS

We are grateful to Mathias Springer and Max Gottesman for sharing strains for the P1 transduction of the *rpsB1* and *rpsB11* mutations and to Isabelle Iost for her valuable advice and aid in polysome preparation and analysis. Access to computational facilities of the Joint Supercomputer Center RAS (Moscow) is gratefully acknowledged.

This work was supported by RFBR grant 09-04-01014 and by the Program of the Presidium of RAS "Molecular and Cellular Biology" (I.V.B.), by RFBR grant 10-04-01217 to R.G.E., and by the Ministry of Education and Science of the Russian Federation (contract 07.514.11.4127) to R.G.E. and A.O.C.

REFERENCES

- Kaminishi T, Wilson DN, Takemoto C, Harms JM, Kawazoe M, Schlutzen F, Hanawa-Suetsugu K, Shirouzu M, Fucini P, Yokoyama S. 2007. A snapshot of the 30S ribosomal subunit capturing mRNA via Shine-Dalgarno interaction. *Structure* 15:289–297.
- Yusupova G, Jenner L, Rees B, Moras D, Yusupov M. 2006. Structural basis for messenger RNA movement on the ribosome. *Nature* 444:391–394.
- Culver GM. 2003. Assembly of the 30S ribosomal subunit. *Biopolymers* 68:234–249.
- Bollen A, Lathe R, Herzog A, Denicourt D, Lecocq JP, Desmarez L, Lavallé R. 1979. A conditionally lethal mutation of *Escherichia coli* affecting the gene coding for ribosomal protein S2 (*rpsB*). *J. Mol. Biol.* 132:219–233.
- Byrgazov K, Manoharadas S, Kaberdina AC, Vesper O, Moll I. 2012. Direct interaction of the N-terminal domain of ribosomal protein S1 with protein S2 in *Escherichia coli*. *PLoS One* 7:e32702. doi:10.1371/journal.pone.0032702.
- Moll I, Grill S, Gründling A, Bläsi U. 2002. Effects of ribosomal proteins S1, S2 and the Dead/CsdA DEAD-box helicase on translation of leaderless and canonical mRNAs in *Escherichia coli*. *Mol. Microbiol.* 44:1387–1396.
- Brodersen DE, Clemons WM, Jr, Carter AP, Wimberly BT, Ramakrishnan V. 2002. Crystal structure of the 30S ribosomal subunit from *Thermus thermophilus*: structure of the proteins and their interaction with 16S RNA. *J. Mol. Biol.* 316:725–768.
- Schuwirth BS, Borovinskaya MA, Hau CW, Zhang W, Vila-Sanjurjo A, Holton JM, Cate JHD. 2005. Structure of the bacterial ribosome at 3.5 Å resolution. *Science* 310:827–834.
- Powers T, Stern S, Changchien L-M, Noller HF. 1988. Probing the assembly of the 3' major domain of 16S RNA. Interactions involving ribosomal proteins S2, S3, S10, S13 and S14. *J. Mol. Biol.* 201:697–716.
- Ortiz JO, Brandt F, Matias VRF, Sennels L, Rappsilber J, Scheres SHW, Eibauer M, Hartl FU, Baumeister W. 2010. Structure of hibernating ribosomes studied by cryoelectron tomography in vitro and in situ. *J. Cell Biol.* 190:613–621.
- Aseev LV, Boni IV. 2011. Extraribosomal functions of bacterial ribosomal proteins. *Mol. Biol. (Mosk)* 45:739–750. (In Russian.)
- Aseev LV, Levandovskaya AA, Tchufistova LS, Skaptsova NV, Boni IV. 2008. A new regulatory circuit in ribosomal protein operons: S2-mediated control of the *rpsB-tsF* expression in vivo. *RNA* 14:1882–1894.
- Aseev LV, Levandovskaya AA, Skaptsova NV, Boni IV. 2009. Conservation of the regulatory elements implicated in the control of the *rpsB-tsF* operon expression in gamma-proteobacteria. *Mol. Biol. (Mosk.)* 43:111–118. (In Russian.)
- Sukhodolet MV, Garges S. 2003. Interaction of *Escherichia coli* RNA polymerase with the ribosomal protein S1 and the Sm-like ATPase Hfq. *Biochemistry* 42:8022–8034.
- Ardini E, Pesole G, Tagliabue E, Magnifico A, Castronovo V, Sobel ME, Colnaghi MI, Menard S. 1998. The 67-kDa laminin receptor originated from a ribosomal protein that acquired a dual function during evolution. *Mol. Biol. Evol.* 15:1017–1025.
- Nelson J, McFerran NV, Pivato G, Chambers E, Doherty C, Steele D, Timson DJ. 2008. The 67 kDa laminin receptor: structure, function and role in disease. *Biosci. Rep.* 28:33–48.
- Kazmin DA, Chinenov Y, Larson E, Starkey JR. 2003. Comparative modeling of the N-terminal domain of the 67 kDa laminin-binding protein: implications for putative ribosomal function. *Biochem. Biophys. Res. Commun.* 300:161–166.
- Shean CS, Gottesman M. 1992. Translation of the prophage λ cI transcript. *Cell* 70:513–522.
- Bubunenkov M, Baker T, Court DL. 2007. Essentiality of ribosomal and transcription antitermination proteins analyzed by systematic gene replacement in *Escherichia coli*. *J. Bacteriol.* 189:2844–2853.
- Moll I, Grill S, Gualerzi CO, Bläsi U. 2002. Leaderless mRNAs in bacteria: surprises in ribosomal recruitment and translational control. *Mol. Microbiol.* 43:239–246.
- Moll I, Hirokawa G, Keil MC, Kaji A, Bläsi U. 2004. Translation initiation with 70S ribosomes: an alternative pathway for leaderless mRNAs. *Nucleic Acids Res.* 32:3354–3363.
- Aurora R, Rose GD. 1998. Helix capping. *Protein Sci.* 7:21–38.
- Harper ET, Rose GD. 1993. Helix stop signals in proteins and peptides: the capping box. *Biochemistry* 32:7605–7609.
- Boni IV, Artamonova VS, Dreyfus M. 2000. The last RNA-binding repeat of the *Escherichia coli* ribosomal protein S1 is specifically involved in the autogenous control. *J. Bacteriol.* 182:5872–5879.
- Dreyfus M. 1988. What constitutes the signal for the initiation of protein synthesis on *Escherichia coli* mRNAs? *J. Mol. Biol.* 204:79–94.
- Singer M, Baker TA, Schnitzler G, Deischel SM, Goel M, Dove W, Jaacks KJ, Grossman AD, Erickson JW, Gross CA. 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. *Microbiol. Rev.* 53:1–24.
- Komarova AV, Chufistova LS, Aseev LV, Boni IV. 2005. An *Escherichia coli* strain producing a leaderless mRNA from the chromosomal *lac* promoter. *Bioorg. Khim.* 31:557–560. (Article in Russian.)
- Boni IV, Artamonova VS, Tzareva NV, Dreyfus M. 2001. Non-canonical mechanism for translational control in bacteria: synthesis of ribosomal protein S1. *EMBO J.* 20:4222–4232.
- Skov J, Schnier J, Rasmussen MD, Subramanian AR, Pedersen S. 1990. Ribosomal protein S1 of *Escherichia coli* is the effector for the regulation of its own synthesis. *J. Biol. Chem.* 265:17044–17049.
- Le Derout J, Boni IV, Regnier P, Hajnsdorf E. 2010. Hfq affects mRNA levels independently of degradation. *BMC Mol. Biol.* 11:17.
- Tchufistova LS, Komarova AV, Boni IV. 2003. A key role for the mRNA leader structure in translational control of ribosomal protein S1 synthesis in γ -proteobacteria. *Nucleic Acids Res.* 31:6996–7002.
- Hess B, Kutzner C, Van der Spoel D, Lindahl E. 2008. GROMACS 4:

- algorithms for highly efficient, load-balanced, and scalable molecular simulation. *J. Chem. Theory Comput.* 4:435–447.
33. Schuler LD, Daura X, van Gunsteren WF. 2001. An improved GROMOS96 force field for aliphatic hydrocarbons in the condensed phase. *J. Comput. Chem.* 22:1205–1218.
 34. Berendsen HJC, Postma JPM, van Gunsteren WF, Hermans J. 1981. Interactions models for water in relation to protein hydration, p 331–342. *In* Pullman P (ed), *Intermolecular forces*. Dordrecht Reidel Publishing Company, Boston, MA.
 35. Bussi G, Donalio D, Parrinello M. 2007. Canonical sampling through velocity rescaling. *J. Chem. Phys.* 126:014101.
 36. Berendsen HJC, Postma JPM, van Gunsteren WF, DiNola A, Haak JR. 1984. Molecular dynamics with coupling to an external bath. *J. Chem. Phys.* 81:3684.
 37. Kuroda A, Nomura K, Ohtomo R, Kato J, Ikeda T, Takiguchi N, Ohtake H, Kornberg A. 2001. Role of the inorganic polyphosphate in promoting ribosomal protein degradation by the Lon protease in *E. coli*. *Science* 293:705–708.
 38. Nishii W, Suzuki T, Nakada M, Kim YT, Muramatsu T, Takahashi K. 2005. Cleavage mechanisms of ATP-dependent Lon protease toward ribosomal S2 protein. *FEBS Lett.* 579:6846–6850.
 39. Iovino M, Falconi M, Petruzzelli R, Desideri A. 2001. Role of the helix capping in the stability of the mouse prion (180–213) segment: investigation through molecular dynamics simulations. *J. Biomol. Struct. Dyn.* 19:237–246.
 40. Wan WY, Molner-White EJ. 1999. A natural grouping of motifs with an aspartate or asparagine residue forming two hydrogen bonds to residues ahead in sequence: their occurrence at alpha-helical N termini and in other situations. *J. Mol. Biol.* 286:1633–1649.
 41. Wan WY, Molner-White EJ. 1999. A recurring two-hydrogen-bond motif incorporating a serine or threonine residue is found both at alpha-helical N termini and in other situations. *J. Mol. Biol.* 286:1651–1662.
 42. Dragani B, Stenberg G, Melino S, Petruzzelli R, Mannervik B, Aceto A. 1997. The conserved N-capping box in the hydrophobic core of glutathione S-transferase P1-1 is essential for refolding: identification of a buried and conserved hydrogen bond important for protein stability. *J. Biol. Chem.* 272:25518–25523.
 43. Lanigan MD, Tudor JE, Pennington MW, Norton RS. 2001. A helical capping motif in ShK toxin and its role in helix stabilization. *Biopolymers* 58:422–436.
 44. Janssen GR. 1993. Eubacterial, archaeobacterial, and eukaryotic genes that encode leaderless mRNA, p 59–67. *In* Baltz RH, Hageman GD, Scatrud PL (ed), *Industrial microorganisms: basic and applied molecular genetics*. American Society for Microbiology, Washington, DC.
 45. Kaberdina AC, Szaflarski W, Nierhaus KH, Moll I. 2009. An unexpected type of ribosomes induced by kasugamycin: a look into ancestral times of protein synthesis? *Mol. Cell* 33:227–236.
 46. Balakin AG, Skripkin EA, Schatsky IN, Bogdanov AA. 1992. Unusual ribosome binding properties of mRNA encoding bacteriophage λ repressor. *Nucleic Acids Res.* 20:563–571.
 47. O'Donnell SM, Janssen GR. 2002. Leaderless mRNAs bind 70S ribosomes more strongly than 30S ribosomal subunits in *Escherichia coli*. *J. Bacteriol.* 184:6730–6733.
 48. Udagawa T, Shimizu Y, Ueda T. 2004. Evidence for the translation initiation of leaderless mRNAs by the intact 70S ribosome without its dissociation into subunits in eubacteria. *J. Biol. Chem.* 279:8539–8546.
 49. Vesper O, Amital S, Belitsky M, Byrgazov K, Kaberdina AC, Engelberg-Kulka H, Moll I. 2011. Selective translation of leaderless mRNAs by specialized ribosomes generated by MazF in *Escherichia coli*. *Cell* 147:147–157.
 50. Delvillani F, Papiani G, Dehò G, Briani F. 2011. S1 ribosomal protein and the interplay between translation and mRNA decay. *Nucleic Acids Res.* 39:7702–7715.
 51. Giliberti J, O'Donnell S, Van Etten WJ, Janssen GR. 2012. A 5'-terminal phosphate is required for stable ternary complex formation and translation of leaderless mRNA in *Escherichia coli*. *RNA* 18:508–518.
 52. Krishnan KM, Van Etten WJ, III, Janssen GR. 2010. Proximity of the start codon to a leaderless mRNA's 5' terminus is a strong positive determinant of ribosome binding and expression in *Escherichia coli*. *J. Bacteriol.* 192:6482–6485.
 53. Sullivan MJ, Curson AR, Shearer N, Todd JD, Green RT, Johnston AW. 2011. Unusual regulation of a leaderless operon involved in the catabolism of dimethylsulfoniopropionate in *Rhodobacter sphaeroides*. *PLoS One* 6:e15972. doi:10.1371/journal.pone.0015972.
 54. Berk V, Zhang W, Pai RD, Cate JHD. 2006. Structural basis for mRNA and tRNA positioning on the ribosome. *Proc. Natl. Acad. Sci. U. S. A.* 103:15830–15834.
 55. Toone WM, Rudd KE, Friesen JD. 1991. *deaD*, a new *Escherichia coli* gene encoding a presumed ATP-dependent RNA helicase, can suppress a mutation in *rpsB*, the gene encoding ribosomal protein S2. *J. Bacteriol.* 173:3291–3302.
 56. Charollais J, Dreyfus M, Iost I. 2004. CsdA, a cold-shock RNA helicase from *Escherichia coli*, is involved in the biogenesis of 50S ribosomal subunit. *Nucleic Acids Res.* 32:2751–2759.
 57. Charollais J, Pflieger D, Vinh J, Dreyfus M, Iost I. 2003. The DEAD-box RNA helicase SrmB is involved in the assembly of 50S ribosomal subunits in *Escherichia coli*. *Mol. Microbiol.* 48:1253–1265.