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REVIEWS

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UDC 577.217

## Extraribosomal Functions of Bacterial Ribosomal Proteins

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Received March 15, 2011; in final form, March 25, 2011

**Abstract**—Ribosomal proteins (r-proteins) constitute a considerable part of the cell proteome. Although their primary role in the cell is to serve as integral components of protein synthesis machinery, ribosomes, many of them have functions beyond the ribosome (the phenomenon known as moonlighting), acting either as individual regulatory proteins or in complexes with other cell components. Extraribosomal activities of some ribosomal proteins were observed as early as the 1970s–1980s. In recent years, both the list of moonlighting r-proteins and the repertoire of their additional functions beyond the ribosome was greatly expanded, mainly owing to new techniques developed for dissecting RNA/DNA–protein or protein–protein interactions within functional complexes involved in various cell processes. The review surveys information on the extraribosomal functions demonstrated experimentally or presumed for bacterial r-proteins.

**DOI:** 10.1134/S0026893311050025

**Keywords:** bacterial ribosomal proteins, extraribosomal functions, RNA/DNA–protein interactions, protein–protein interactions, regulation of cell processes

### INTRODUCTION

In all organisms, proteins are synthesized on ribosomes, which are intricate ribonucleoprotein complexes and consist of two subunits: small (30S in prokaryotes and 40S in eukaryotes) and large (50S in prokaryotes and 60S in eukaryotes). A ribosome includes three (in prokaryotes) or four (in eukaryotes) ribosomal RNA (rRNA) molecules and several dozens of various ribosomal proteins (r-proteins). Protein synthesis machinery displays a surprising evolutionary conservation. It is thought that a ribosome similar in structure and composition to modern ribosomes formed as early as the last common ancestor of all organisms before they divided into three kingdoms of life (for a review, see [1]). This assumption is based on the fact that conservation of many r-proteins is traceable from bacteria to humans. For instance, 15 small-subunit proteins (S2–S5, S7–S15, S17, and S19 in the bacterial ribosome) and 19 large-subunit proteins (L1–L6, L10–L16, L18, L22–L24, L29, and L30) are universal [2, 3].

It is broadly accepted that the molecular mechanism of polypeptide chain synthesis arose in the RNA world. The peptidyltransferase center (PTC), which consists almost exclusively of RNA, is thought to be a relic of that early step of the evolution of translation machinery [1, 2]. The questions as to the evolutionary step at which r-proteins were added to rRNA and their origin are still a matter of discussion. In view of the surprising structural diversity of r-proteins, it is thought that the most ancient r-proteins coevolved with rRNA to form and/or maintain its active confor-

mation, while younger r-proteins were recruited from other processes to improve the quality and fidelity of translation machinery [1]. Proteins account for one-third to half of the modern ribosome weight and are absolutely essential for translation. Although the genes for certain r-proteins may be deleted without impairing cell viability in bacteria [4], the deletions usually cause various growth defects in certain conditions. The specific functions of r-proteins in protein synthesis were unclear for a long time; it was not until recently that they came to be understood [3, 5].

As structural components of the ribonucleoprotein complex, the majority of r-proteins are capable of RNA binding, and some of them additionally bind DNA, suggesting their cooptation at later evolutionary steps. Within the ribosome, r-proteins interact not only with rRNA but also with their protein partners; i.e., they are capable of protein–protein interactions. These proteins suggest a potential for complexation with other cell components to perform extraribosomal functions, which were demonstrated for several prokaryotic and eukaryotic r-proteins [6, 7].

Extraribosomal activity of several bacterial r-proteins was discovered more than 30 years ago; this relates primarily to the fact that certain r-proteins act as specific translational repressors of their own operons to ensure their self-regulation (for a review, see [8, 9]). The development of new techniques to analyze the intricate functional complexes involved in regulating cell processes showed that the number of r-proteins capable of additional functions in the cell and the variety of these functions were underestimated. In particular,

r-proteins proved to play a role in regulating transcription [10]. Here, we summarize the data on the extraribosomal functions that have been assumed or demonstrated for bacterial r-proteins. The review is composed in the form of a catalog; each moonlighting r-protein is considered in a separate section along with the functions (if known) that the protein performs in the ribosome.

## FUNCTIONS OF RIBOSOME SUBUNITS IN TRANSLATION

The small (30S) subunit of the bacterial ribosome consists of one RNA (16S rRNA) and approximately 20 proteins (depending on the source, e.g., there are 21 proteins, S1–S21, in *Escherichia coli*) and is responsible for recognizing and binding mRNA during translation initiation, decoding information contained in mRNA, and maintaining the reading frame during protein synthesis. The large (50S) ribosome subunit contains two rRNA molecules (23S and 5S) and more than 30 (33 in *E. coli*) r-proteins, has no contact with mRNA, is directly involved in catalyzing the formation of a peptide bond in the PTC, and ensures the exit of the growing polypeptide chain through an exit tunnel.

Transport RNAs (tRNAs) occupy consecutively A, P, and E sites during a translation cycle. The sites are on both of the subunits. The decoding center of the 30S subunit provides room for the interaction of tRNA anticodons with mRNA, while the 50S subunit accommodates the universal CCA tails of tRNAs carrying an amino acid or the growing protein chain. Owing to high-resolution X-ray analysis and cryoelectron microscopy, it is quite well understood now how r-proteins and rRNA regions are arranged in the most important functional centers, how ligands (tRNA, mRNA, translation factors, and antibiotics) are positioned, and how they interact with components of the ribosome (for a review, see [11] and references therein).

## ORGANIZATION OF THE RIBOSOMAL PROTEIN GENES IN THE BACTERIAL CHROMOSOME

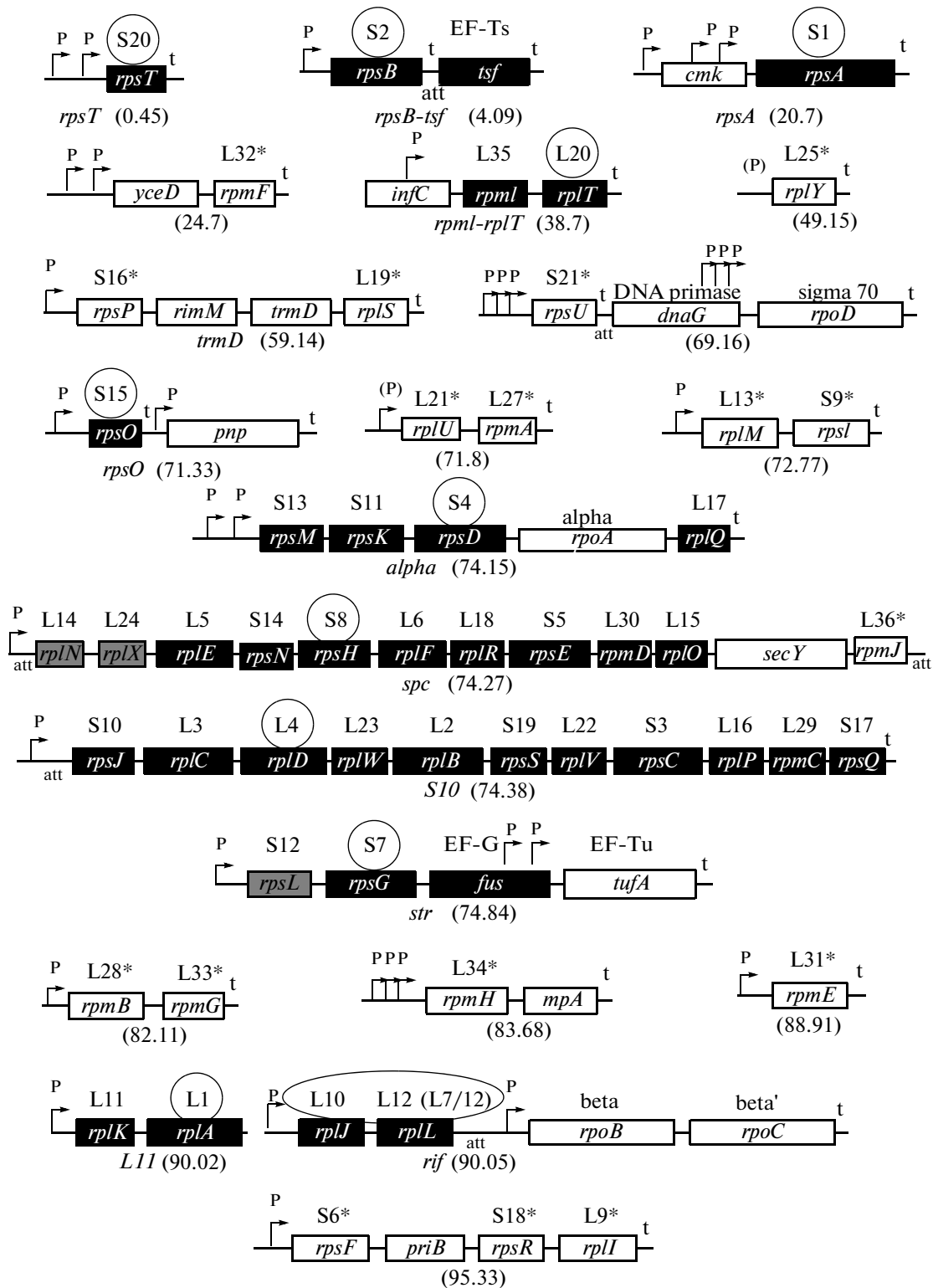
Bacterial r-protein genes are organized in operons, which often include genes coding for nonribosomal proteins, including translation factors (*tsf*, *fus*, and *tufA*), RNA polymerase subunits (*rpoA*, *rpoB*, *rpoC*, and *rpoD*), and components of the replication complex (*dnaG* for primase and *priB* for primosome protein N, which is necessary for replication to restart). This circumstance indicates that the main processes involved in the expression of genetic information are associated and that their coordination is necessary for the bacterial cell. In addition, certain r-protein operons include genes whose products are involved in

tRNA modification and processing (*trmD* and *mpaA*, respectively), rRNA maturation (*rimM*), and transmembrane protein export (*secY*). What the biological significance is of the inclusion of these genes into r-protein operons is still poorly understood because the extraribosomal genes are often regulated independently of the r-protein genes [8]. The structures of r-protein operons and their positions on the *E. coli* chromosome map are shown in the figure.

## MOONLIGHTING R-PROTEINS OF THE 30S RIBOSOME SUBUNIT

**S1** is the largest r-protein (557 amino acid residues in *E. coli*) and is an integral component of translation machinery in all proteobacteria, cyanobacteria (chloroplast precursors), and several other bacterial groups, but it is absent from ribosomes of Gram-positive bacteria with a low GC content [12]. In Gram-negative bacteria, S1 contains six homologous repeats, which consist of 72–75 amino acid residues each and are known as S1 motifs [12]. The S1 motifs are conserved and are found in many RNA-binding proteins of various organisms from bacteria to humans [13]. The two repeats that form the N-terminal domain of S1 lost their RNA-binding function during evolution and acquired the capability of protein–protein interactions, while the four repeats of the central and C-terminal regions are responsible for RNA binding by S1. S1 is added to the 30S subunit via protein–protein interactions of its N-terminal domain to complete the assembly of subparticles active in RNA binding; the protein is arranged so that its extended RNA-binding domain is exposed in solution [12]. The vital function of S1 as a component of the 20S subunit is to bind mRNA at the early steps of translation initiation [14]. The S1 targets are in the 5'-untranslated region (5'-UTR) of mRNA [15]. Although lacking distinct specificity for a particular nucleotide sequence, S1 has higher affinity to A/U-rich single-stranded RNA regions [16].

The capability of protein–protein and RNA–protein interactions underlie numerous functions that S1 plays beyond the ribosome. Various bacteriophages utilize S1 in a number of processes in the infected host cell. S1 acts as one of the four subunits of Q $\beta$  replicase, as well as replicases of other RNA phages, and is absolutely essential for replication of the phage DNA plus strand [17, 18]. Historically, this was the first extraribosomal function demonstrated for r-proteins [17]. S1 interacts with phage  $\lambda$  protein  $\beta$ , which is involved in recombination, and its role in the process cannot be excluded [19, 20]. S1 stimulates activity of phage T4 specific endoribonuclease RegB, which cleaves the Shine–Dalgarno (SD) sequence in certain early phage RNAs when their translation is no longer necessary [21]. It is of interest that the RNA-binding domain of S1 is sufficient for this function, indicating that RegB



Structures of the r-protein operons and their arrangement in the *E. coli* chromosome (updated from [8]). The operon names (if any) and positions on the chromosome map (in centisomes) are indicated according to EcoCyc version 14.6 (<http://biocyc.org/ECOLI>). Proteins regulating the expression of their own operons are encircled; r-proteins without available data on the regulation are asterisked. Genes controlled by the repressor protein are in black; the genes that are not regulated by the relevant r-protein or belong to operons whose regulation is unknown are in white. Alpha, beta, beta', and sigma 70 are the RNA polymerase subunits; P is the promoter; t and att are the transcription terminator and attenuator, respectively.

activity is stimulated via S1 interactions with RNA targets rather than via protein–protein interactions [22].

Acting as an autogenous regulator, S1 inhibits translation of its own mRNA (the *rpsA* mRNA) both in vitro and in vivo when it is produced in an excess relative to the 30S subunits [23, 24]. The ribosome-binding site (RBS) of the *rpsA* mRNA in *E. coli* and related  $\gamma$ -proteobacteria lacks a canonical SD sequence, and the formation of the 30S initiator complex strongly depends on the S1–mRNA interaction [24, 25]. In contrast to other regulatory proteins, S1 does not bind rRNA, and the mechanism of the autogenous regulation is based on competition for mRNA between free S1 and S1 contained in the 30S subunit [24]. The preferential binding of S1 with the *rpsA* mRNA in the presence of other cell mRNAs is most likely due to cooperative interaction of several S1 molecules with A/U-rich regions in the 5'-UTR of the *rpsA* mRNA [24].

It is thought that S1 plays a role in the regulation of the transcription efficiency by increasing the RNA polymerase processivity [26, 27], mRNA polyadenylation [28], and the formation of the antitermination RNA polymerase complex at a specific site (the so-called box A) in the leader regions of rRNA transcripts [10, 29]. However, these assumptions are based on experimental data obtained in vitro. Since S1 preferentially interacts with A/U-rich single-stranded RNA regions, the data on its interaction with A/U-rich box A in vitro [29] may have no biological significance for the formation of the antitermination complex in vivo. Moreover, the same function (binding with box A) is performed by S1 incorporated in the 30S subunit [16]. It was also assumed that S1 is involved in tmRNA binding and mRNA quality control via a *trans*-translation mechanism [30]. The role of S1 in the process is questionable; for instance, such a possibility was ruled out with good reason in [31].

One more function was recently identified for *E. coli* S1. The protein proved to regulate the expression not only of its own operon (*rpsA*) but also of the *rpsB-tsif* operon, which codes for the r-protein S2 and the EF-Ts elongation factor [32].

**S2.** Conserved protein S2 (S0 in the yeast ribosome and SA in higher eukaryotes) is a vital component of the ribosome in all organisms, although its exact functions in translation are still unclear. As a component of the bacterial ribosome, S2 is involved in binding the SD duplex during translation initiation [33]; however, this does not explain why S2 is essential for the organisms that do not utilize the SD interactions in mRNA binding. As a free protein, S2 acts as a negative regulator and affects the expression of both of the *rpsB-tsif* operon genes in *E. coli* and other  $\gamma$ -proteobacteria in vivo [32, 34]. Binding to a structural operator region in the 5'-UTR of its own mRNA (*rpsB*), S2 inhibits its translation, thus distorting the transcription–translation coupling for the operon, decreasing the level of

the *rpsB-tsif* bicistronic mRNA, and, eventually, suppressing EF-Ts synthesis [32]. In contrast to the classical regulatory r-proteins (for a review, see [8, 9]), S2 needs S1, which is produced from another operon, for autogenous repression, and the S1–S2 complex is most likely involved in this regulation [32]. This regulation mechanism, which is unusual for r-protein operons, is probably determined by the fact that S2 lacks marked RNA-binding activity, while affinity of the S2–S1 complex for the operator region is high enough to ensure highly efficient repression (our unpublished data).

**S4** is a universal protein (S9 in eukaryotic ribosomes, see [3]) and plays a key role in the biogenesis of the small ribosome subunit. Its interaction with the 5'-terminal domain of the 16S rRNA via the N-terminal protein domain causes the structural changes that are absolutely essential for subsequent assembly of the 30S subunit (see references in [35]). Within the 30S subunit, the r-proteins S4, S3, and S5 form the entrance gates for mRNA and possess helicase activity, which is necessary for melting the secondary structure in the mRNA region entering the mRNA-binding channel during translation [3].

Beyond the ribosome, S4 performs an important regulatory function of an autogenous repressor of the  $\alpha$ -operon in *E. coli* (figure), acting at the translational level. The mechanism of the autogenous control by S4 is described as an entrapment [8, 36, 37]. In contrast to the direct competition mechanism where the binding of a repressor protein to mRNA prevents the binding of the 30S subunit, as, for example, is the case with S1, the interaction of S4 with the 5'  $\alpha$ -mRNA region that includes the RBS of the first cistron (*rpsM*) and forms an intricate pseudoknot allows the 30S subunit to land, but the landing results in a nonproductive complex unable to bind tRNA and, consequently, to form a translationally active initiator complex [36, 37]. The formation of the inactive S4 • mRNA • 30S complex inhibits the synthesis of the r-proteins S13, S11, S4, and L17 from the polycistronic  $\alpha$ -mRNA (figure). It is thought that translation of the cistron coding for RNA polymerase  $\alpha$  subunit is not inhibited in this case, although the cistron is flanked by r-protein cistrons subject to translational suppression; the mechanism of this exclusion is still unclear.

S4 regulates its own synthesis not only in *E. coli* but also in *Bacillus subtilis*, whose S4 gene (*rpsD*) does not form an operon with other r-protein genes but occurs as a separate transcription unit [38]. As in *E. coli*, autogenous regulation takes place at the posttranscriptional level. However, the binding with the S4 repressor in *Bacillus* involves an extended 5'-terminal *rpsD* mRNA region whose secondary structure disagrees with the pseudoknot conformation. The molecular mechanism of *rpsD* mRNA repression was not studied in detail [38]; it is thought that the recognition of the

operator region by the S4 repressor fundamentally differs between *Bacillus* and *E. coli*.

Extraribosomal S4 activity associated with stimulation of rRNA gene transcription is of particular interest [10, 39]. The function of S4 in transcription anti-termination of Rho-dependent terminators is similar to the function of the NusA transcription factor; moreover, S4 is associated with RNA polymerase in vivo, like NusA [39]. Thus, not only does S4 act as a translational repressor of the  $\alpha$ -operon when its level exceeds the level of the free 16S rRNA in the cell but it also stimulates the rRNA synthesis to maintain the balance between rRNA and r-protein syntheses.

**S7** is a universal protein (S5 in eukaryotic ribosomes; see [3]). Like S4, S7 plays a key role in ribosome self-assembly, and its interaction with the 16S rRNA initiates the folding of the 3'-basal domain and the subsequent formation of the head of the 30S subunit. When S7 is synthesized in an excess over the 16S rRNA, the protein utilizes the same RNA-binding determinants to interact with mRNA of the *str* operon and to repress its translation in *E. coli* [40, 41]. The inhibition mechanism includes the interaction of the S7 repressor with the intercistron region between the first (S12) and second (S7) cistrons of the *str* operon to directly repress the S7 synthesis and coupled translation of the downstream *fus* cistron, which codes for EF-G [40, 42]. The expression of the *tufA* (EF-Tu) gene, which is the last in the operon, is inhibited to a far lesser extent because it proceeds from two additional promoters of *fus* (figure). S7 binding to the intercistron region is thought to inhibit translation of the upstream cistron (S12) and also via a retroregulation mechanism because the formation of the repressor complex destabilizes the corresponding mRNA region [40].

**S8** is a universal protein (S22 in yeast ribosomes and S15a in higher eukaryotes; see [3]) and plays an important role in assembly of the 30S subunit by binding imperfect helix H21 of the 16S rRNA. In addition, S8 acts as an autogenous expression regulator of the *spc* operon, which codes for 11 r-proteins and the SecY component of the protein export apparatus [8, 43]. The mechanism of this regulation is similar in many features to the repression of the *str* operon by S7 (see above) because the repressor binds in the vicinity of the translation start of the third cistron *rpLE* (figure), which codes for L5, rather than upstream of the first cistron of the operon, as is the case with the majority of r-protein operons subject to autogenous regulation [8]. Binding to the *spc* mRNA, the S8 repressor blocks translation of the *rpLE* cistron directly and translation of the downstream r-protein cistrons by disturbing translational coupling [44]. Translation of the two upstream cistrons (L14 and L15) is inhibited via retroregulation, which destabilizes the mRNA [45]. Data on the regulation of the two most distant cistrons, *secY*

and *rpmJ* are unavailable. The crystal structure of the S8 complex with the operator mRNA region was solved to a high resolution (2.8 Å). It was observed that the two RNA targets of S8 (the operator mRNA region and hairpin H21 of the 16S rRNA) are structurally similar (molecular mimicry) and that the same RNA-binding site is involved in both of the functions [46].

**S9.** The genes of S9 (*rpsI*) and L13 (*rpLM*) form an operon (figure), whose regulation is unknown as of yet. The role of S9 (S16 in higher eukaryotic ribosomes) in the ribosome function is related to the positioning of peptidyl-tRNA in the P site of the 30S subunit, and the interaction between the C-terminal region of S9 with the anticodon loop of tRNA is important for maintaining the mRNA reading frame [47]. The moonlighting function of S9 was thought to have nothing to do with translation because a study of the protein-protein interactions involved in SOS repair identified UmuC, one of the key components of the process, as an interaction partner of S9. Moreover, S9 was found to accelerate UmuC renaturation after its partial denaturation in vitro, supporting the functional significance of their interaction [48]. Recently, the complex of UmuC and UmuD' was identified as SOS-specific DNA polymerase V, which is responsible for replication at sites of DNA lesions [49], but a possible involvement of S9 in the process was not mentioned.

**S10** (S20 in eukaryotes; see [3]) is a universal component of the small ribosome subunit. Its specific function in the ribosome was poorly understood until recently, while its extraribosome activity was the focus of research. S10 was the first bacterial r-protein found to play a role in regulating transcription [10, 50]. As a transcription factor (NusE), S10 is involved in assembly of the antitermination complex to prevent a transcription arrest on Rho-dependent terminators in rRNA operons or the phage  $\lambda$  chromosome. The S10–NusB complex binds to the box-A sequence in the *nut* sites of phage  $\lambda$  DNA and in regions upstream of the 16S and 23S rRNA genes in their RNA transcripts [10, 51]. The S10 conformation in complex with NusB is the same as in the 30S subunit [52, 53]. Structurally, S10 has a globular domain and an extended loop; the latter is strongly essential for S10 incorporation in the ribosome, but its absence does not affect antitermination activity [53]. Although different domains of the protein are involved in translation and transcriptional regulation, S10 cannot bind with NusB and the ribosome simultaneously. S10 plays a key role in the formation of the antitermination complex because S10 overproduction suppresses the *nusB* null mutation [53].

Transcription and translation are tightly coupled both temporally and spatially in bacteria, but the coupling mechanism was unknown until recently. Recent studies provided direct evidence that the two processes are associated physically [54] and that the key role in

determining the transcription rate is played by the ribosome that follows RNA polymerase and translates the mRNA during its synthesis [55]. The physical association of the two supramolecular machineries is due to the NusG transcription factor, whose N-terminal domain binds with RNA polymerase, while the C-terminal domain binds with S10 on the ribosome [54]. S10 competes with the Rhos termination factor for the C-terminal domain of NusG, preventing translation termination on the transcript. Thus, the function of S10 in the ribosome during transcription–translation coupling is similar to its function in the antitermination complex, where S10 (NusE) is bound with RNA polymerase through NusG and competes with Rho to prevent termination on Rho-dependent terminators of rRNA operons and the phage  $\lambda$  chromosome [55].

**S15** acts as a primary protein during assembly of the 30S subunit in vitro. Its interaction with the 16S rRNA stimulates the binding of the r-proteins S6, S11, S18, and S21 with the central domain of the 16S rRNA to generate the platform of the 30S subunit. It is surprising that, in spite of this, S15 is not a vital protein, and *rpsO* deletion mutants are viable, although they express a cold-resistant phenotype [56, 57]. This means that ribosomes may be assembled even in the absence of S15 in vivo in favorable temperature conditions.

Like many r-proteins interacting with rRNA at early steps of ribosome assembly, S15 autogenously regulates the expression of its own gene at the level of translation initiation [8]. The mechanism regulating the S15 synthesis in *E. coli* was studied in detail; the relevant RNA sites were identified; and the amino acid residues involved in recognizing the two targets, the 16S rRNA and *rpsO* mRNA, were determined [57]. The *rpsO* expression is regulated via ribosome entrapment [8], as is the case with the regulation by S4 (see above). Structurally, the 5'-UTR of the *rpsO* mRNA occurs in two equilibrium conformations, one of which is a pseudoknot. Free S15 binds to the 5'-UTR occurring in the pseudoknot conformation and fixes it to allow the binding of the 30S subunit. In this complex, however, the AUG initiator codon cannot occupy its P-site position essential for tMet-tRNA<sup>Met</sup> binding and the subsequent formation of a productive initiator complex; this was directly demonstrated by cryoelectron microscopy of the *rpsO* mRNA·S15·30S ternary complex [58].

There is no apparent structural analogy between the operator mRNA region (a pseudoknot) and the S15-binding site of the 16S rRNA (a junction of three hairpins), but the two RNA targets are recognized by the same set of amino acid residues of S15, and the recognition site is G-U/G-C in both of the cases. This circumstance suggests a limited similarity for the two recognition sites (limited molecular mimicry; see [57]).

The autogenous regulation of the *rpsO* expression at the translational level was demonstrated for *Thermus*

*thermophilus* [59] and *B. stearothermophilus* [60], which are phylogenetically distant from *E. coli*. It is of interest that the structured operator regions of the 5'-UTR of the *rpsO* mRNA do not form a pseudoknot in these cases, rather occurring as a combination of three hairpin structures [59, 60]. As in *E. coli*, S15 utilizes the same RNA-binding site to bind to the operator region as is used to recognize the 16S rRNA during assembly of the 30S subunit. Thus, the autogenous regulation of the S15 synthesis is highly conserved in evolution, but its fine molecular mechanisms may differ among different phylogenetic groups. The entrapment mechanism functions in *E. coli*, while the competition mechanism is used in the other cases, where repressor binding to mRNA prevents ribosome landing [60].

**S16** is essential for assembly of active 30S subunits in bacteria because its binding induces the conformational changes in the 16S rRNA that stabilize a pseudoknot in the decoding center [61]. The S16 gene belongs to the *trmD* operon, which includes not only r-protein genes (S16 and L19) but also genes responsible for 30S maturation (*rimM*) and tRNA modification (*trmD*) [62]. There are no data on the regulation of the operon by r-proteins. S16 was found to possess DNA-binding and deoxyribonuclease activities; the protein is capable of recognizing cruciform DNA structures and introduce point specific breaks in one of the strands, in particular, downstream of the adenine of the 5'-AGTT-3' sequence in the *oriC* region of *E. coli* [63]. The biological significance of DNase activity of S16 is unclear; this activity is possibly a rudiment. Since S16 is not universal [1, 3], it is possible to assume that the protein was recruited from processes associated with DNA metabolism at a certain evolutionary step.

**S20** acts as a primary protein during assembly of the bacterial 30S subunit. S20 binds with at least two sites of the 16S rRNA, bringing the 3'-minor and 5' domains together [64]. Although cells knocked out in the *rpsT* (S20) gene do not lose their viability [4], their growth is dramatically slowed down because ribosomes are defective in mRNA binding and subunit association [65]. The *rpsT* gene occurs as a separate operon and has two promoters and a terminator (figure). There is evidence that free S20 autogenously regulates the *rpsT* expression at the translation initiation level and may compete with the 30S subunit for binding the initiator region of the *rpsT* mRNA but only in the presence of the UUG rare natural start codon [66].

## EXTRARIBOSOMAL FUNCTIONS OF 50S-SUBUNIT PROTEINS OF THE BACTERIAL RIBOSOME

**L1** is a universal protein (L10 in higher eukaryotes) and interacts with the 23S rRNA to form a side protrusion, which is known as the L1 stalk of the 50S subunit.

This mobile structure guides the tRNA movement through the ribosome during translation and is responsible for the release of deacylated tRNA from the E site [67]. RNA-binding activity of L1 underlies its dual function in bacteria and archaea [68, 69]. L1 acts as a translational autogenous repressor, regulating the L11 and its own syntheses in *E. coli* (figure) and the syntheses of L11, L1, L10, and L12 in archaea (for a review, see [69]). The L1 complex with mRNA is less stable than with the 23S rRNA in all cases; consequently, when free 23S rRNA appears in the cell, L1 binds to it, leaving mRNA and allowing its translation to be resumed [69]. Crystal structures of the L1 complexes with the operator mRNA region and the 23S rRNA fragment interacting with L1 within the 50S subunit demonstrated a structural similarity of the two RNA targets [68]. However, the 23S rRNA region is more intricate structurally and its interaction with L1 involves additional contacts, which determine a higher stability of the L1–rRNA complex and ensure the binding preference that underlies the L1-dependent regulation [69].

**L2** is a conserved r-protein (L8 in higher eukaryotes; see [3]) that occurs in the vicinity of the PTC in the 50S subunit and is essential for translational activity of the ribosome. Reconstructed in the absence of L2 in vitro, 50S subparticles are incapable of association with 30S subparticles because of the lack of contacts with S20, which produces a highly stable complex with L2 [70]. Moreover, L2 is involved in tRNA binding in the A and P sites, and a mutation of conserved His229 disturbs the PTC function [70]. It was not until recently that L2 proved to form functional complexes beyond the ribosome [71, 72]. A search for protein partners of the  $\alpha$  subunit of *E. coli* RNA polymerase was performed to identify the potential transcriptional modulators, and L2 was found among the interaction partners [71]. Since r-proteins often produce a nonspecific background in functional proteomics because of their high concentrations in the cell, independent tests were performed both in vitro and in vivo to verify the biological significance of the finding. It was shown that L2 specifically interacts with the  $\alpha$  subunit and that the interaction selectively increases P1 promoter activity in rRNA operons. Other r-proteins (L1, L3, L20, and L27) were incapable of modulating transcription. Thus, L2 acts not only as a vital component of the ribosome but also as a transcriptional activator of rRNA operons that contributes to the regulation of a balanced synthesis of ribosome components [71].

In addition, L2 forms a complex with the HtpG chaperone (an analog of eukaryotic Hsp90) and increases its activities in ATP hydrolysis by more than one order of magnitude [72]. The physiological significance of this interaction is still unclear.

**L4** is a universal r-protein and is important for assembly and activity of the large ribosome subunit in all organisms [3]. A globular domain of L4 occurs on the surface of the 50S subunit, while an extended non-structured loop (a tentacle) reaches into the subunit core and forms many contacts with the 23S rRNA in the vicinity of the PTC in the narrowest region of the nascent peptide exit tunnel (see references in [3]). This region of the 50S subunit provides a binding site for erythromycin and other macrolide antibiotics, and mutations of the L4 loop result in resistance to these antibiotics. There is presumably no direct contact between erythromycin and L4, and antibiotic resistance is due to mutation-induced structural changes in the 23S rRNA [73].

In the free form, L4 specifically regulates the expression of its own operon *S10* (figure) and acts as a unique regulator because, in contrast to other regulatory r-proteins, L4 affects not only translation but also transcription of *S10* to cause its premature termination [8]. The L4 repressor binds within the extended nonstructured 5'-UTR of mRNA in both of the cases, and the sites involved in regulating translation and transcription partly overlap [8]. Transcription termination resulting from L4 binding to the mRNA leader presumably involves NusA, although NusA is not necessary for L4 binding [8, 74]. While L4 is highly conserved, the structure of the mRNA operator region and the L4-dependent autogenous repression mechanisms display only a limited phylogenetic conservation in the subclass of  $\gamma$ -proteobacteria, suggesting their relative recent evolutionary origin [75]. A 23S rRNA fragment corresponding to the primary L4-binding site is capable of reducing L4 activity in the regulation of the *S10* mRNA; this circumstance points to a competition between the two RNA targets. A structural analysis of the minimal L4-binding sites of the 23S rRNA and the 5'-UTR of the *S10* mRNA confirmed their structural similarity [74].

In addition to the RNA-binding sites involved in the interactions with rRNA and mRNA, L4 has a region potentially capable of interacting with other proteins [76]. This assumption was verified recently [77, 78]. In the ribosome, L4 was found to contact RNA helicase SrmB, which is involved in assembly of the 50S subunit in vivo [77]. Beyond the ribosome, L4 binds with the C-terminal domain of RNase E to inhibit its activity toward certain mRNAs, whose higher expression is necessary in various types of stress [78]. It is thought that L4-dependent modulation of RNase E activity is a part of the cell strategy ensuring the cell survival in adverse conditions [78].

**L10 and L7/12.** L12 (L7/L12) is an exclusion from the common rule of an equimolar proportion of ribosome components because two dimers of the protein occur within the 50S subunit in the majority of bacteria and even three occur in *Thermotoga maritima* [79].

The L7/L12 dimers interact with L10 to produce a tight complex, which is bound with L11 and a 23S rRNA region in the 50S subunit. The components together form an extended ribosome stalk, which performs important functions of recruiting the GTP-binding translation factors (IF2, EF-Tu, EF-G, RF3), stabilizing their conformations, and stimulating GTP hydrolysis (see [80] and references therein). Functional analogs of L7/L12 are found in ribosomes of all organisms [3].

In *E. coli*, L10 and L7/L12 are encoded by genes of one operon, *rplJLrpoBC* (figure). The free pentameric complex L10(L7/12)<sub>4</sub> acts as an operon-specific translational repressor, and its binding site is within the extended 5'-UTR upstream of the L10-coding cistron *rplJ* [8, 81]. The operon additionally includes *rpoB* and *rpoC* for the  $\beta$  and  $\beta'$  RNA polymerase subunits (this circumstance determined the operon name, *rif*, because mutations of these genes confer rifampicin resistance), but the *rpoB* and *rpoC* expression is regulated independently of the translational regulation by the L10(L7/12)<sub>4</sub> complex [8]. A key role in recognizing the operator mRNA region is played by L10, which recognizes the same kink-turn motif as in the 23S rRNA region within the ribosome [81]. A solid argument in favor of this assumption is that mutations in similar positions of the kink-turn motifs of rRNA and mRNA similarly affect the RNA-protein interactions [81]. Thus, the mRNA structure at the binding site for the repressor complex mimics the complex landing site of the 23S rRNA. The specific mechanism that sustains the translational inhibition resulting from L10(L7/12)<sub>4</sub> binding is still poorly understood because the repressor landing site is distant from the RBS so that a direct competition with the ribosome is unlikely.

**L14.** Data on the specific function of the universal r-protein L14 (L23 in eukaryotes) in translation are unavailable, but L14 was mentioned as a probable candidate for a role in DNA replication in the earliest review on the extraribosomal functions of r-proteins [6]. It was found that L14 induces a 40-fold increase in activity of ATP-dependent DNA helicase Rep, which is involved in replication of cell and phage DNAs in *E. coli* [82]. L14 is capable of binding with both RNA and DNA. DNA-binding activity is thought to mediate Rep stimulation. When the role of Rep in the cell was unclear, the biological significance of L14-mediated stimulation of Rep activity was questioned. It was not until recently that a study in vivo elucidated the functions of helicases Rep, DinG, and UvrD [83]. A combination of at least two helicases proved essential for solving a conflict between replication and transcription when the replication fork proceeds through actively transcribed chromosome regions [83]. Progress in studies of the biological roles of DNA helicases makes it possible to think that the role of heli-

case-stimulating factors, such as L14, will be better understood in the future.

**L20** is absolutely essential during early assembly of the bacterial 50S subunit, and a deletion of *rplT*, which codes for L20, is lethal [84]. In *E. coli*, L20 acts as an autogenous translational repressor of its own operon *rpmI-rplT* [85]. Although *infC* for the IF3 translation initiation factor is upstream of *rpmI* (figure), its expression is not regulated by L20, but is rather controlled by IF3 itself. Moreover, the transcriptional promoter of the *rpmI-rplT* operon is within the *infC* coding region in *E. coli*. Binding to the operator mRNA region upstream of the *rpmI* cistron, L20 directly represses the L35 synthesis, and this leads to inhibition of its own synthesis because of the alteration of translational coupling [85, 86]. The extended (450-nt) operator for the L20 repressor is rather intricate in structure; the interaction between distant regions forms a pseudoknot-like structure. As a repressor, L20 interacts with two sites in the operator, and both of them are important for repression in vivo and are structurally similar to the L20 landing site on the 23S rRNA [85]. Translational inhibition is thought to proceed via a competition mechanism because the ribosome also preferentially binds to the pseudoknot [86].

It is of interest that, in *B. subtilis*, *infC-rplI-rplT* form an operon that is transcribed from a promoter located upstream of *infC* [87]. The operon is similarly controlled by L20, but, in contrast to *E. coli*, it is transcription, rather than translation, that is subject to the regulation. Two alternative structures may form in the mRNA leader upstream of the *infC* translation initiation site, and one of the structures acts as a transcription terminator. L20 binding provokes the formation of the terminator structure. Although the L20-dependent regulatory mechanisms fundamentally differ between *E. coli* and *B. subtilis*, a structural similarity of the binding sites on mRNA and the 23S rRNA underlies the regulation in either case [87].

**Ribosomal chaperones.** Some of the r-proteins facilitate RNA folding to prevent the formation of misfolded, functionally inactive structures; i.e., they act as RNA chaperones. This r-protein activity does not require ATP hydrolysis, and, theoretically, may be involved in both ribosome assembly and the formation of the active conformations of nonribosomal RNAs. Among the 30S-subunit proteins, S12 has the most distinct RNA chaperone activity [88]. S12 lacks nucleotide sequence specificity and has affinity for nonstructured RNA. It was shown that S12 facilitates the splicing of group I introns of bacteriophage T4 in vitro and that the protein is not involved in the splicing proper but is necessary only for the optimal RNA structure because it may be removed using proteolytic enzymes before the splicing reaction is initiated by adding GTP [87]. Similar RNA-chaperone functions can be performed by up to one-third of all 50S-subunit



proteins [89], and L1 has the highest activity. The function is conserved phylogenetically and is characteristic of L1 homologs of all three kingdoms of life, although there are exceptions [90]. L15, L16, L18, and L19 act as chaperones for both RNAs and proteins, and their chaperone activity toward proteins is comparable with that of the classical Hsp90 chaperone [91]. This function of r-proteins may be necessary in stress, when only a minor portion of ribosomes is used to synthesize stress proteins, while “extra” r-proteins act as chaperones to maintain their active structure and, thereby, to facilitate the cell viability.

## CONCLUSIONS

The above data clearly demonstrate that r-proteins are capable of functioning beyond the ribosome to modulate various cell processes (including phage-specific ones): translation (the r-proteins acting as autogenous translational repressors), transcription (S10, S4, S1, L2, and L4), the regulation of the mRNA stability (L4 and S1), DNA repair and replication (S9 and L14), and phage RNA replication (S1). These additional functions are due to the RNA/DNA-binding properties of r-proteins and their capability of interacting with other cell proteins. Certain r-proteins (S1, S4, and L4) of the bacterial ribosome perform several additional functions, acting as multifunctional cell components. There are a lot of grounds for believing that the list of extraribosomal functions of r-proteins is far from complete and that new and even unexpected activities of structural components of the ribosome will be discovered as a global picture is constructed for cell component interactions, whose description is the aim of interactomics.

## ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project nos. 06-04-48353a and 09-04-01014a).

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