A Functional Interaction between Ribosomal Proteins S7 and S11 within the Bacterial Ribosome*

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In this study, we used site-directed mutagenesis to disrupt an interaction that had been detected between ribosomal proteins S7 and S11 in the crystal structure of the bacterial 30 S subunit. This interaction, which is located in the E site, connects the head of the 30 S subunit to the platform and is involved in the formation of the exit channel through which passes the 30 S-bound messenger RNA. Neither mutations in S7 nor mutations in S11 prevented the incorporation of the proteins into the 30 S subunits but they perturbed the function of the ribosome. In vivo assays showed that ribosomes with either mutated S7 or S11 were altered in the control of translational fidelity, having an increased capacity for frameshifting, readthrough of a nonsense codon and codon misreading. Toeprinting and filter-binding assays showed that 30 S subunits with either mutated S7 or S11 have an enhanced capacity to bind mRNA. The effects of the S7 and S11 mutations can be related to an increased flexibility of the head of the 30 S, to an opening of the mRNA exit channel and to a perturbation of the proposed allosteric coupling between the A and E sites. Altogether, our results demonstrate that S7 and S11 interact in a functional manner and support the notion that protein-protein interactions contribute to the dynamics of the ribosome.

The ribosome is the cellular machinery responsible for protein synthesis in all living organisms. The elucidation of the crystal structure of the prokaryotic ribosome has led to a major progress in understanding its function (1–7). Analysis of the ribosome structure combined with a wealth of biochemical data clearly showed that ribosomal RNA (rRNA) is the key player in the functions of the ribosome, and it is currently assumed that the main task of the ribosomal proteins is to help and stabilize rRNA folding and to facilitate conformational changes in rRNA (8–10). However, a growing list of examples suggests that ribosomal proteins directly participate to protein synthesis. The x-ray structure of the ribosome reveals that S12 is part of the decoding site (Refs. 11 and 12, reviewed in Ref. 13). It also shows that the 30 S proteins S13, S15, and the 50 S proteins L2, L5, L14, L19 are involved in intersubunit bridges, while the 30 S proteins S9, S13, and the 50 S proteins L1, L5, L33 interact with tRNAs, suggesting that they could participate in the translocation step (12). Studies in solution also point to a role for the ribosomal proteins, such as an involvement in mRNA binding for S1 (14–16), a participation in peptidyl transferase activity for L2 (17–19) and the prevention of mRNA slippage for L9 (20).

Several protein-protein interactions were identified in the crystal structure of the 30 S subunit (21, 22) but, so far, only the interaction between proteins S4 and S5 has been directly related to a particular ribosome function, that is the control of translational fidelity. Indeed, mutations that disrupt the S4-S5 interaction decrease translational accuracy (23, 24). Among the other protein-protein interactions observed in the ribosome, one involves S7, a primary binding protein located in the head of the 30 S, which interacts with S11, a tertiary binding protein, positioned on the platform. This S7-S11 interaction encompasses S7 residues 148, 150, 152, 153, and 154 (in Escherichia coli numbering) in the 148–155 region following the C-terminal α-helix (helix 6), and S11 residues 55, 58, 59, 60, and 63 within the loop preceding the first α-helix (helix 1) (Fig. 1A). It is well documented that the 30 S head and platform undergo a series of conformational changes and move one relative to the other during protein synthesis. For example, x-ray and cryo-EM studies clearly showed that such changes occur upon subunit association, binding of aminoacyl-tRNA (aa-tRNA) or factors, and during translocation (5, 12, 24–30). Thus, the S7-S11 interaction, which constitutes a contact between the 30 S head and platform, likely participates in the conformational changes undergone by the 30 S, breaking and re-forming during translation.

The crystal structure of the 70 S ribosome complexed with tRNA and mRNA showed that the C-terminal α-helix of S7, close to the region of interaction with S11, contacts the anticodon loop of the E-site-bound tRNA (5, 12). In agreement with the x-ray studies, S7 and S11 were cross-linked to the anticodon loop of the E-site bound tRNA (31, 32). The crystal structure of the 70 S ribosome also showed that the S7-S11 interaction contributes to the formation of the so-called exit channel, a narrow channel, through which passes the mRNA, upstream from the decoding site. The loop of S11 that interacts with S7 is positioned very close to the Shine-Dalgarno helix formed between the mRNA and the 3′-end of 16 S rRNA (Ref. 33, reviewed in Ref. 34). Again, in agreement with the x-ray studies, it was observed that the C-terminal region of S7 could be cross-linked to the Shine-Dalgarno region of mRNA (35).

In this study, we investigated the effect of disrupting the interaction between E. coli S7 and S11 by mutating residues 148 to 155 of S7 and residues 55, 58, 59, 60, and 63 of S11. These mutations were shown not to interfere with the incorporation of the proteins into the 30 S subunit. We found, however, that disruption of the S7-S11 interaction makes the ribosome more error-prone. Also, with toeprinting and filter-binding assays, we observed an enhanced binding of mRNA to 30 S subunits containing mutated S7 or S11. Finally, we found that...
that of the luciferase gene from plasmids pRNALuc2-UGA and pRNALuc2-UGA or pRNALuc2 (40) generating pACYpET-Luc-0, where production of luciferase is dependent upon a −1 frameshift, a stop codon readthrough and a conventional translation, respectively. The beginning of the luciferase genes from plasmids pRNALuc2-UGA, pRNALuc2-UGA and pRNALuc2 was first amplified, using primers designed to revert the Shine-Dalgarno sequence, which is mutated in these plasmids, to a wild-type sequence. The PCR products were digested with BamHI and XhoI and ligated into the appropriately digested plasmid pACYpET-Luc. Plasmid pACYpET-Luc-R218G has a substitution in the active site of luciferase, such that production of active luciferase requires a misincorporation at this site. It was derived from pACYpET-Luc-0 by PCR designed to mutate the first base of codon 218 from an A to a G (Arg to Gly), which impairs luciferase activity (41). The PCR products were digested with EcoRI and Psp5II and ligated into the appropriately digested plasmid pACYpET-Luc-0.

Plasmid pUC18SR32 (42) and pLRCAT (43) were used for the *in vitro* transcription of the T4 gene 32 and the gene coding for chloramphenicol acetyl transferase (CAT), respectively, and the mRNAs produced were used for toeprinting and filter-binding studies.

**Fractionation of Cell Lysates and Assessment of the Incorporation of Plasmid-encoded Proteins S7 and S11 into 30 S Subunits, Ribosomes, and Polysomes—*E. coli* BLR(DEL3)/pLysS cells transformed with plasmid pET-21a (+)−S7, pET-21a (+)−S11 or their mutant derivatives were grown in LB until an OD$_{600}$ of 0.05 was reached. S7 or S11 overexpression was induced by the addition of isopropyl-$\beta$-D-thiogalactopyranoside (IPTG) (Biotek Canada Inc.) at 1 mM for 90 min, followed by lysis of the cells according to a standard procedure (44). The cell lysates were analyzed by centrifugation as described in the Fig. 3 legend. The detection of the plasmid-encoded protein into the free 30 S subunits, 70 S ribosomes, and polysomes was done by Western blot. Ribosomal subunits, 70 S ribosomes and polysomes (2.2 pmol) containing S7, S11 or their mutant derivatives were fractionated by 12% SDS-PAGE, and the proteins were transferred to a nitrocellulose membrane. For wild-type S7 and S7sub148–155, bands were probed with digoxigenin-labeled goat anti-S7 (1:1000) and were resolved with horseradish peroxidase conjugated rabbit-anti-goat antibodies (diluted 1:5000) (Santa Cruz Biotechnology), followed by detection by chemiluminescence. To assess the incorporation of plasmid-encoded S7, S11 and wild-type or mutant S11, the intensity of the bands on a blot detected with an anti-histidine tag antibody coupled to horseradish peroxidase (diluted 1:100) (Santa Cruz Biotechnology) was compared with that of an

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Fig. 1. Interaction between ribosomal proteins S7 and S11 in the 30 S subunit. A, crystallographic structure of the 30 S ribosomal subunit from *Thermus thermophilus*. The zoomed picture shows the interaction between S7 and S11 with interacting amino acid residues in blue and green, respectively, in *E. coli* numbering. The image was produced using WebLab ViewerPro software version 4 (Molecular Simulations Inc.). B, schematic representation of the *E. coli* S7 and S11 mutants used in this study. The boxed regions represent deleted sequences of S7. Amino acids in red correspond to the mutations introduced in the proteins. The histidine tag sequence at the C terminus of the proteins is not indicated.

**EXPERIMENTAL PROCEDURES**

**Construction of Plasmids Used in This Study**—All the enzymes used in this study were from Amersham Biosciences, unless otherwise specified. All the oligonucleotides were from Biocorp Inc. All PCR amplifications were carried out with the Deep Vent DNA polymerase (New England Biolabs) in a RocheMD 40 from Stratagene. Plasmid pET-21a (+)−S7, which codes for *E. coli* K12A19 S7 under control of a T7 promoter, and mutant pET-21a (+)−S7Δ148–178, with a deletion of the last 31 amino acids of S7, are derivatives of pET-21a (+)−S7Δ148–178, which codes for the C terminus instead of the N terminus of the protein. Mutant pET-21a (+)−S7Δ156–178 was derived from pET-21a (+)−S7 by introducing a stop codon at position 156, using PCR. Mutant pET-21a (+)−S7 subs148–155, where the sequence corresponding to residues 148–155 of S7 was substituted with the sequence AGAGAAAA, was derived from pET-21a (+)−S7 by PCR, using the overlap extension procedure with four oligonucleotides (37). These two constructs also contain a sequence coding for a histidine tag at the C terminus of protein S7. Another construct that was identical to pET-21a (+)−S7Δ156–178 except that the histidine tag is at the N terminus of S7 was also made. The S11 gene was amplified by PCR from *E. coli* K12A19 genomic DNA obtained as described (38), with primers designed to introduce a histidine tag sequence at the C terminus of protein S11. The amplified fragment was digested with NdeI and BamHI, purified with the GFXMD PCR DNA and gel band purification kit (Amersham Biosciences) and ligated into the appropriately digested pET-21a (+), generating pET-21a (+)−S11. This plasmid was subsequently used for the construction of the S11 substitution mutant with mutations R55A, T58A, P59G, F60A, and Q63A (pET-21a (+)−S7 subs55–63), which was produced by overlap extension PCR.

For the construction of the plasmids producing luciferase, we used as a starting point pSDLucNWT (39), a derivative of pBluescriptSK−, containing the luciferase gene with a Shine-Dalgarno sequence under control of a T7 promoter. The origin of replication of this plasmid was replaced by ligating the BglII-Smal fragment containing the luciferase gene to the BglII-Smal fragment of plasmid pACYC177 (New England Biolabs) containing the p15A origin of replication. This generated pACYpET-Luc, which is compatible with the pET-21a (+) plasmids, which have the pBR322 origin of replication. Different derivatives of pACYpET-Luc were constructed to analyze the control of translation fidelity with ribosomes containing mutated S7 or S11. To this end, using PCR, the beginning of luciferase gene in pACYpET-Luc was replaced with that of the luciferase gene from plasmids pRNALuc2-UGA and pRNALuc2-UGA or pRNALuc2 (40) generating pACYpET-Luc-0, pACYpET-Luc-0, and pACYpET-Luc-0, where production of luciferase is dependent upon a −1 frameshift, a stop codon readthrough and a conventional translation, respectively. The beginning of the luciferase genes from plasmids pRNALuc2-UGA, pRNALuc2-UGA and pRNALuc2 was first amplified, using primers designed to revert the Shine-Dalgarno sequence, which is mutated in these plasmids, to a wild-type sequence. The PCR products were digested with BamHI and XhoI and ligated into the appropriately digested plasmid pACYpET-Luc. Plasmid pACYpET-Luc-R218G has a substitution in the active site of luciferase, such that production of active luciferase requires a misincorporation at this site. It was derived from pACYpET-Luc-0 by PCR designed to mutate the first base of codon 218 from an A to a G (Arg to Gly), which impairs luciferase activity (41). The PCR products were digested with EcoRI and Psp5II and ligated into the appropriately digested plasmid pACYpET-Luc-0.

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**Fractionation of Cell Lysates and Assessment of the Incorporation of Plasmid-encoded Proteins S7 and S11 into 30 S Subunits, Ribosomes, and Polysomes—*E. coli* BLR(DEL3)/pLysS cells transformed with plasmid pET-21a (+)−S7, pET-21a (+)−S11 or their mutant derivatives were grown in LB until an OD$_{600}$ of 0.05 was reached. S7 or S11 overexpression was induced by the addition of isopropyl-$\beta$-D-thiogalactopyranoside (IPTG) (Biotek Canada Inc.) at 1 mM for 90 min, followed by lysis of the cells according to a standard procedure (44). The cell lysates were analyzed by centrifugation as described in the Fig. 3 legend. The detection of the plasmid-encoded protein into the free 30 S subunits, 70 S ribosomes, and polysomes was done by Western blot. Ribosomal subunits, 70 S ribosomes and polysomes (2.2 pmol) containing S7, S11 or their mutant derivatives were fractionated by 12% SDS-PAGE, and the proteins were transferred to a nitrocellulose membrane. For wild-type S7 and S7sub148–155, bands were probed with digoxigenin-labeled goat anti-S7 (1:1000) and were resolved with horseradish peroxidase conjugated rabbit-anti-goat antibodies (diluted 1:5000) (Santa Cruz Biotechnology), followed by detection by chemiluminescence. To assess the incorporation of plasmid-encoded S7, S11 and wild-type or mutant S11, the intensity of the bands on a blot detected with an anti-histidine tag antibody coupled to horseradish peroxidase (diluted 1:100) (Santa Cruz Biotechnology) was compared with that of an

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1 The abbreviations used are: CAT, chloramphenicol acetyltransferase; IPTG, isopropyl-1-thio-$\beta$-D-galactopyranoside; ram, ribosomal ambiguity.
**RESULTS**

This study focuses on ribosomal proteins S7 and S11 from *E. coli*. The interaction between the two proteins was observed in the crystal structure of the ribosome from a thermophilic bacterium (21, 22). About 140 sequences of S7 and 90 sequences of S11 from different organisms in all three major evolutionary kingdoms were obtained, using the Swiss-Prot protein data base (us.expasy.org/sprot), and compared. Fig. 2 shows representative examples of S7 and S11 sequences illustrating this comparison. It can be seen that the region of interaction between S7 and S11 is extremely well conserved in bacteria. In eukaryotes and archaeabacteria, the region corresponding to the 148–155 region of bacterial S7 is missing whereas region 55–63 of S11 is present although slightly less conserved. One can thus predict that the interaction between S7 and S11 observed within the ribosome of a thermophilic bacterium (21, 22) is conserved in the ribosome of a thermophilic bacterium. In *E. coli*, the interaction between the two proteins was observed in the crystal structure of the ribosome from a thermophilic bacterium (21, 22). About 140 sequences of S7 and 90 sequences of S11 from different organisms in all three major evolutionary kingdoms were obtained, using the Swiss-Prot protein data base (us.expasy.org/sprot), and compared. Fig. 2 shows representative examples of S7 and S11 sequences illustrating this comparison. It can be seen that the region of interaction between S7 and S11 is extremely well conserved in bacteria. In eukaryotes and archaeabacteria, the region corresponding to the 148–155 region of bacterial S7 is missing whereas region 55–63 of S11 is present although slightly less conserved. One can thus predict that the interaction between S7 and S11 observed within the ribosome of a thermophilic bacterium is maintained in eukaryotes and archaeabacteria. In *E. coli*, we, therefore, decided to investigate whether the disruption of this interaction affects the function of the *E. coli* ribosome. Plasmid pET-21a(+)−S7 and pET-21a(+)−S11, which contain, respectively, *E. coli* K S7 and S11 gene with a histidine tag at the C terminus under control of a T7 promoter, were used for the construction and expression of mutants of S7 and S11. In one S7 mutant, the sequence 148-AGAGAAAA-155 region of bacterial S7 is missing whereas region 55-63 of S11 is present although slightly less conserved. One can thus predict that the interaction between S7 and S11 observed within the ribosome of a thermophilic bacterium is maintained in *E. coli*. We, therefore, decided to investigate whether the disruption of this interaction affects the function of the *E. coli* ribosome. Plasmid pET-21a(+)−S7 and pET-21a(+)−S11, which contain, respectively, *E. coli* K S7 and S11 gene with a histidine tag at the C terminus under control of a T7 promoter, were used for the construction and expression of mutants of S7 and S11. In one S7 mutant, the sequence 148-AGAGAAAA-155 region of bacterial S7 is missing whereas region 55-63 of S11 is present although slightly less conserved. One can thus predict that the interaction between S7 and S11 observed within the ribosome of a thermophilic bacterium is maintained in *E. coli*. We, therefore, decided to investigate whether the disruption of this interaction affects the function of the *E. coli* ribosome. Plasmid pET-21a(+)−S7 and pET-21a(+)−S11, which contain, respectively, *E. coli* K S7 and S11 gene with a histidine tag at the C terminus under control of a T7 promoter, were used for the construction and expression of mutants of S7 and S11. In one S7 mutant, the sequence 148-AGAGAAAA-155 is replaced by a 155 region of bacterial S7 is missing whereas region 55-63 of S11 is present although slightly less conserved. One can thus predict that the interaction between S7 and S11 observed within the ribosome of a thermophilic bacterium is maintained in *E. coli*. We, therefore, decided to investigate whether the disruption of this interaction affects the function of the *E. coli* ribosome. Plasmid pET-21a(+)−S7 and pET-21a(+)−S11, which contain, respectively, *E. coli* K S7 and S11 gene with a histidine tag at the C terminus under control of a T7 promoter, were used for the construction and expression of mutants of S7 and S11. In one S7 mutant, the sequence 148-AGAGAAAA-155 is replaced by a
deleted. The E. coli K S7 contains an additional 23 amino acid extension at its C terminus, compared with the E. coli B S7, and mutant S7\textsuperscript{\textDelta 156–178} thus corresponds to E. coli B S7 protein. One S11 substitution mutant was produced by making mutations in the loop that precedes the first a-helix and interacts with S7. To minimize any perturbation in the structure of this a-helix, only the residues in the 55–63 region that interact with S7 in the crystal structure of the 30 S subunit were mutated as follows: R55A, T56A, P59G, F60A, and Q63A, generating plasmid pET-21a(+)–S11\textsubscript{sub55–63}. The different mutant proteins used in this study are summarized in Fig. 1B.

Effect of Mutating S7 and S11 on Their Incorporation into 30 S Subunits and on Ribosome Profiles—For the assays described below, we used E. coli BLR(DE3)/pLysS as a host cell for the pET-derived plasmids encoding S7 or S11 under control of the T7 promoter, except when otherwise mentioned. This strain carries the T7 RNA polymerase under control of the lac promoter in its genome and also contains plasmid pLysS, which allows a stringent control of the expression of the cloned genes in the T7 promoter (47, 48). The host cells were transformed with pET-21a(+)–S7 or pET-21a(+)–S11 or their mutant derivatives. Addition of IPTG to the cell culture induced transcription of the ribosomal protein genes. We first examined whether the overexpression of the wild-type and mutant proteins affects cell growth by measuring the optical density at 600 nm of the cell culture at different times (data not shown). As previously reported, overexpression of wild-type S7 leads to a major bacterial growth defect (49, 50). This effect results from the repression of translation of the polycistronic chromosomal \textit{str} mRNA by S7. This mRNA encodes S12 and elongation factors EF-G and EF-Tu in addition to S7, and repression of its translation likely reduces the number of ribosomes actively engaged in translation (51). Overexpression of mutant S7\textsuperscript{\textDelta 156–178} and S7\textsubscript{sub148–155} interfered with bacterial growth like wild-type S7, while overexpression of mutant S7\textsuperscript{\textDelta 148–178}, as previously observed, affected growth rate to a larger extent (50). Neither overexpression of S11, which is not a repressor protein, nor that of its mutant affected the cell growth.

The incorporation of the plasmid-encoded proteins into the 30 S, 70 S ribosomes and polysomes was investigated by Western blot after overexpression of S7, S11 or their derivatives for 90 min. First, a fractionation of the cell lysate by centrifugation through a sucrose gradient containing 10 m M magnesium was performed (Fig. 3). Overexpression of wild-type S7 and S7\textsuperscript{\textDelta 156–178} were both seen to cause an increase in the amount of the ribosomal subunits relative to the ribosomes and polysomes (profiles 2 and 3, respectively), an effect related to the repression of the translation of the \textit{str} mRNA, as explained above. Interestingly, when either S7\textsuperscript{\textDelta 148–178} or S7\textsubscript{sub148–155} was overexpressed, the relative increase in the amount of free ribosomal subunits was larger (profiles 4 and 5, respectively), suggesting that the 30 S subunits with S7 that lacks the region of interaction with S11 have an association defect. Contrasting with S7 and its derivatives, overexpression of S11 or its mutant did not affect the ribosome profile (profiles 6 and 7, respectively). Next, the incorporation of plasmid-encoded S7, S11, and their mutant derivatives in the subunit, ribosome and polysome fractions was analyzed by Western blot. To this end, we used an anti-S7 antibody for the wild-type and mutant S7\textsubscript{sub148–155} (Fig. 4A), that are longer than the chromosome-encoded \textit{E. coli} B S7 by 23 amino acids, which enables us to readily discriminate between plasmid-encoded and chromosome-encoded S7. The other S7 mutants (S7\textsuperscript{\textDelta 156–178} and S7\textsuperscript{\textDelta 148–178}), and the plasmid-encoded wild-type and mutated S11 cannot be discriminated from the chromosome-encoded proteins on the basis of their size. The incorporation of plasmid-encoded \textit{versus} chromosome-encoded protein was assessed by comparing the intensity of the bands detected with an anti-histidine tag antibody, which reveals exclusively the plasmid-encoded protein, to that of the bands detected with an anti-S7 or S11 antibody, which reveals both the plasmid-encoded and chromosome-encoded proteins. It was observed that about 80% of S7, S11 or their derivatives present in the 30 S subunits originate from the plasmid, indicating that neither the mutations nor the presence of the histidine tag prevent the incorporation of the proteins into the 30 S subunit. In the 70 S ribosomes and polysomes, the level of incorporation was about 75% for the
plasmid-encoded wild-type S7 and S7/H9004, 156–178, and for the plasmid-encoded wild-type or mutated S11. However, the proportion of S7/H9004148–178 and S7subs148–155, which lack the sequence interacting with S11, decreased to about 40% in 70 S ribosomes and polysomes, indicating that the population of the mutated 30 S within the 70 S and within the polysomes is lower than in the free subunit pool (see Fig. 4B).

In the results described above, S7 and its mutants had a histidine tag at the C terminus. Since the mutations in S7 are located in its C-terminal region, we also examined the results obtained with plasmids expressing S7, S7/H9004156–178 and S7/H9004148–178 but where the histidine tag was located at the N terminus. In all the assays carried out in this study, whether the histidine tag was located at the N terminus or the C terminus of S7 did not cause any difference (data not shown).

Effect of Disrupting the Interaction between S7 and S11 on Subunit Association—The observation that the overexpression of S7/H9004148–178 and S7subs148–155 into the 30 S subunits causes a relative increase in the free subunit populations prompted us to analyze in vitro the association between the 30 S containing the mutated S7 and the 50 S subunit. Protein S7 or S11 was overexpressed for 90 min and the ribosomal subunits were isolated from the whole cell extract by centrifugation through a sucrose gradient containing 2 mM magnesium. The amount of the plasmid-encoded wild-type or mutated S7 in the isolated 30 S was about 80%, as assessed by Western blot. The 30 S and 50 S subunits were then incubated at a molar ratio of one to one in a buffer containing 10 mM magnesium and their association was monitored by centrifugation through a sucrose gradient (Fig. 5). We found that the 30 S containing either the wild-type S7 (profile 1) or S7/H9004156–178 (profile 2) associated efficiently with the 50 S whereas the 70 S containing S7/H9004148–155 mutation (profile 3) significantly decreased subunit association. Assays with mutant S7/H9004148–178, which was omitted in subsequent experiments, provided a profile that was very similar to that observed with mutant S7subs148–155 (data not shown). The 30 S subunit with mutated S11 was also investigated (profile 4), and, contrasting with the effect of mutations in S7, the presence of the mutated S11 in the 30 S did not affect its capacity to associate with the 50 S. This was expected since no change in the ribosome profiles was observed when overexpressing the S11 mutant.

**Fig. 4.** Incorporation of plasmid-encoded S7, S11, or their mutant derivatives into 30 S, 70 S, or polysomes as determined by Western blot. BLR(DE3)/pLysS cells were transformed with the pET-21a(+) plasmids coding for S7, S11 or their mutant derivatives and overexpression of the ribosomal proteins was induced for 90 min. The 30 S subunits, 70 S ribosomes and polysomes were fractionated as described in the Fig. 3 legend. A, examples of Western blots showing the detection of plasmid-encoded S7 or S7subs148–155 and chromosome-encoded S7 with a goat anti-S7 antibody. Lane 1, cell lysate when wild-type S7 is overexpressed; lane 2, 30 S when wild-type S7 is overexpressed; lane 3, 70 S when wild-type S7 is overexpressed; lane 4, polysomes when wild-type S7 is overexpressed; lane 5, 30 S when S7subs148–155 is overexpressed; lane 6, 70 S when S7subs148–155 is overexpressed; lane 7, polysomes when S7subs148–155 is overexpressed. B, relative incorporation of the plasmid-encoded proteins into 30 S subunits, 70 S ribosomes, and polysomes. The incorporation was assessed by measuring the intensities of the bands detected in the Western blots, as described in the “Experimental Procedures.” The values are means ± S.D. of at least three independent experiments.

**Fig. 5.** Association assays with wild-type 30 S or 30 S containing mutated S7 or S11. The 30 S and 50 S subunits (72 pmol) were incubated in a molar ratio of one to one and the formation of the 70 S was monitored by centrifugation as described in the Fig. 3 legend. The association of the subunits was detected at 260 nm using an ISCO apparatus. The arrows point to the 70 S ribosomes. 1, wild-type 30 S; 2, 30 S with S7/H9004156–178; 3, 30 S with S7subs148–155; 4, 30 S with S11subs55–63.
Effect of Disrupting the S7-S11 Interaction on the Binding of 30 S Subunits to mRNA—A decrease in the capacity of 30 S subunits with mutated S7 to bind mRNA could also account for an increased amount of free subunits containing this mutated protein. To address this possibility, we investigated the interaction between the mutated 30 S and mRNA by toeprinting, using the T4 gene 32 mRNA. A toeprint assay allows the detection of canonical initiation complexes, because an mRNA-bound 30 S, in presence of the initiator tRNA (tRNA^Met), blocks the extension of a primer by the reverse transcriptase. This stop, called the toeprint signal, occurs at position +16 and also, at a lower extent, at +17 on the mRNA (the A of the initiator codon is +1) (45, 52, 53). Examples of typical toeprint experiments are presented in Fig. 6. The 30 S subunits used in these assays were isolated as described for the association assays. With the 30 S subunits that contain S7subs148–155, the toeprint signal was much more intense than that obtained with the wild-type 30 S or with 30 S containing mutant S7Δ156–178 (compare lane 3 with 1 and 2, respectively, in Fig. 6). The relative toeprint, which corresponds to the ratio of the intensity of the toeprint signal to that of the full-length extension signal, was ~10-fold higher for S7subs148–155 than that for wild-type S7. The toeprint assays using wild-type 30 S or 30 S with mutated S11 show that the relative signal with the 30 S with mutated S11 (Fig. 6, lane 4) was also more intense than that seen with wild-type 30 S, and was comparable to the relative signal obtained with the 30 S containing the mutated S7. Similar results were obtained with another mRNA, coding for the chloramphenicol acetyl transferase (data not shown). Thus, the 30 S subunits where the S7-S11 interaction is disrupted have a higher capacity to bind mRNA than wild-type 30 S subunits.

We also directly assessed the binding of the mutated 30 S to mRNA by nitrocellulose filter-binding assays. In these assays, a radiolabeled T4 gene 32 mRNA was mixed with increasing amounts of 30 S subunits and filtered through a nitrocellulose membrane that retains the subunits and the mRNA bound to them. We found that 30 S with mutated S7subs148–155 or mutated S11 retain more mRNA on the filter than wild-type 30 S or those containing mutant S7Δ156–178 (Fig. 7). This fully agrees with the toeprint assays showing that the 30 S subunits in which the S7-S11 interaction is disrupted have an enhanced capacity to bind mRNA.
and misreading, which were measured as described under cell culture. The levels of frameshift efficiency, stop codon readthrough enzyme activity was normalized for the increase in optical density of the ured in the cell lysates after 60 and 120 min of induction and the Luc-R218G, for measuring misreading. Luciferase activity was meas-

Fig. 8. Effect of disrupting the S7-S11 interaction on translation fidelity. E. coli BLR(DE3) cells were co-transformed with a pET-21a (+) plasmid coding for S7, S11 or their mutant derivatives and with plasmid pACYcET-Luc-1, for measuring -1 frameshift, or pACYcET-

Luc-UGA, for measuring misreading. Luciferase activity was measured in the cell lysates after 60 and 120 min of induction and the enzyme activity was normalized for the increase in optical density of the cell culture. The levels of frameshift efficiency, stop codon readthrough and misreading, which were measured as described under “Experimental Procedures,” were, respectively, 0.14 ± 0.04, 3.5 ± 0.8, and 0.05 ± 0.002% when either wild-type S7 or S11 was overexpressed and were arbitrarily ascribed a value of 1. The results are the means of at least four independent experiments with bars representing S.D.

not interfere with the assembly of the mutated proteins into the 30 S, but the mutations made the ribosomes error-prone and increased the capacity of the 30 S to bind mRNA. However, the 30 S with mutated S7 but not those with mutated S11 within the corresponding contact site had a decreased capacity to associate to the 50 S subunits. A simple interpretation of this result could be that, while the mutations in either S7 or S11 that disrupt their interaction make the ribosomes error-prone and enhance the capacity of the 30 S to bind mRNA, the mutations in S7 can additionally perturb the structure of the 30 S subunit in a more dramatic way, affecting its ability to associate to the 50 S subunit. Another interpretation could be that the flexible C-terminal region of S7 must interact with another partner than S11 to promote the association of the 30 S with the 50 S, a likely candidate for this interaction being the 3′-end of 16 S rRNA, which has been UV-cross-linked to S7 (56). The cell growth was identical when overexpressing wild-type or mutated S7. However, overexpression of S7, whether wild-type or mutated, causes a growth defect resulting from the repression of the str mRNA, as explained above. This could mask any effect on growth resulting from the association problem with 30 S having a mutated S7.

Our results reveal that disruption of the S7-S11 interaction facilitates the mRNA binding to the 30 S subunits, as shown by toeprinting and filter-binding assays. This can be readily related to the role of the S7-S11 interaction in the formation of the exit channel for the mRNA. Since translation can occur on circular mRNAs (57), one can infer that this exit channel must open to allow mRNA binding to the 30 S subunit, implying a transient disruption of the S7-S11 interaction. A permanent disruption of the S7-S11 interaction would keep the exit channel open, making it easier for the mRNA to bind. In vivo, this does not result in an increase in the portion of the mutated 30 S in the polysomes. However, the 30 S with mutated S7 have an association defect, which prevents a possible increase in the portion of this mutated 30 S in the polysomes. Nevertheless, the 30 S with mutated S11, which do not have an association problem, are not detected in a higher portion in the polysomes than wild-type 30 S. It is possible that, in vivo, the presence of initiation factors, such as IF3, which binds close to the site of the S7-S11 interaction (58, 59), induces the opening of the mRNA channel and thus minimizes the difference observed in vitro in the binding of mRNA between wild-type and mutated 30 S. Previous studies indeed showed that addition of IF3 to the 30 S subunits facilitates mRNA binding (Refs. 60 and 61, reviewed in Ref. 62), an effect similar to that observed when the S7-S11 interaction is disrupted. More recently, cryo-EM studies showed that the regions surrounding the cleft between the head and the platform moves upon binding of IF3 (58). Finally, in the termination process, it was proposed that binding of IF3 enables the 30 S to slide along the mRNA so that it can reach an initiation site (63). Such a sliding obviously requires a loosening of the grip of the 30 S on the mRNA, consistent with the opening of the exit channel.

We also showed that the ribosomes with either S7 or S11 mutated are more error-prone than the wild-type ribosomes. A possible explanation for this result is that disrupting the S7-S11 interaction perturbs the structure of the E site, where the S7-S11 interaction is located. It has been proposed that the E site is allosterically coupled to the A site and that this coupling controls the capacity of the ribosomes to discriminate between cognate and non-cognate tRNAs at the A site (54, 55). One can suggest that a perturbation of the E site impairs the coupling between the E and A sites, which could contribute to decrease the translational fidelity. A communication between the E site and the binding site for the elongation factors EF-G and EF-Tu was also suggested (64) (reviewed in Ref. 6), reinforcing the possibility that perturbations in the E site could affect the binding to the ribosome of the aa-tRNA complexed to EF-Tu. Another explanation for the decreased translational accuracy with the mutated ribosomes could be that the disruption of the S7-S11 interaction facilitates a movement of the head, promoting the so-called closed conformation of the 30 S subunit that is required for the binding and accommodation in the A site of the incoming aa-tRNA (24). The transition to the closed conformation requires a disruption of the S4-S5 interaction at the interface between the shoulder and the platform of the 30 S, and mutations that impair the S4-S5 interaction promote this closed conformation (23, 24), making the ribosomes error-prone (ribosomal ambiguity (ram) mutations). Like the classical S4 and S5 ram mutations, the S7 and S11 mutations that make the ribosomes error-prone also consist in the disruption of a protein-protein interaction. The S7 and S11 ram mutations do not affect cell growth, and this is also the case for the S4 and S5 ram mutants (65, 66). However, one can predict that the simultaneous presence of mutations that break the S4-S5 interaction and those that break the S7-S11 interaction would interfere with cell growth. Interestingly, in line with this prediction, the growth of mutants in which the S7-S11 interaction is broken was more sensitive to sublethal doses of streptomycin, a translational error-inducing agent (data not shown). The increase in frameshifting observed with the ribosomes mutated in S7 or S11 could directly result from the increased misincorporation of the aa-tRNA, as suggested by the dual-error model, a model that proposes that incorporation of a non-cognate tRNA enhances the probability of a frameshift that restores a cognate codon-anticodon interaction (67, 68). Alternatively, the increase in frameshifting could result from an increased probability for the mRNA to slide on the ribosome when the S7-S11 interaction is broken, and the exit channel is open. After tRNA accommodation in the A site and peptide bond formation, the translocation step takes place. It involves a rotation of the head.
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The results of the present study demonstrate that S7 and S11 interact in a functional manner since the disruption of this interaction perturbs the ribosome function. These results also suggest that the S7-S11 interaction participates in the dynamics of the bacterial ribosome. This is a second example, after the S4-S5 interaction, of a protein-protein interaction that affects the dynamic behavior of the ribosome. The S7-S11 interaction is not conserved in eukaryotes. Therefore, it would be interesting to identify the interactions controlling the movement of the head of the small ribosomal subunit in these organisms. A complete mechanistic understanding of the function of the ribosome requires a detailed characterization of its dynamics.

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REFERENCES

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