Alteration in Location of a Conserved GTPase-associated Center of the Ribosome Induced by Mutagenesis Influences the Structure of Peptidyltransferase Center and Activity of Elongation Factor G*

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Translocation catalyzed by elongation factor G occurs after the peptidyltransferase reaction on the large ribosomal subunit. Deacylated tRNA in the P-site stimulates multiple turnover GTPase activity of EF-G. We suggest that the allosteric signal from the peptidyltransferase center that activates EF-G may involve the alteration in the conformation of elongation factor binding center of the ribosome. The latter consists of the moveable GTPase-associated center and the sarcin-ricin loop that keeps its position on the ribosome during translation elongation. The position of the GTPase-associated center was altered by mutagenesis. An insertion of additional base pair at positions C1030/G1124 was lethal and affected function of EF-G, but not that of EF-Tu. Structure probing revealed a putative allosteric signal pathway connecting the P-site with the binding site of the elongation factors. The results are consistent with the different structural requirements for EF-G and EF-Tu function, where the integrity of the path between the peptidyltransferase center and both GTPase-associated center and sarcin-ricin loop is important for EF-G binding.

The ribosome is a large molecular machine for protein synthesis. During the elongation EF-Tu brings aminoacyl-tRNA to the ribosomal A-site, and its GTPase activity is stimulated by recognition of the mRNA codon by the aminoacyl-tRNA anticodon at the decoding center of the small subunit. EF-G acts after completion of peptide transfer at the peptidyltransferase center of the large subunit.

Before the peptidyltransferase reaction, the growing peptide is attached to the 3′-end of the tRNA bound to the P-site, whereas the aminoacyl-tRNA is located in the A-site. After peptide transfer, the A-site carries the peptidyl-tRNA, whereas the P-site holds the deacylated tRNA. This is the state that should be recognized by EF-G. It has been reported that deacylated tRNA in the P-site stimulates multiple turnover GTPase activity of EF-G, but that peptidyl-tRNA does not (1, 2). This stimulation is attributed to the enhanced binding of EF-G to the ribosome (2).

The P-site is located more than 70 Å away from the binding site of the elongation factors. Thus, an intriguing question is how can information from the P-site be transmitted across half the width of the ribosome? We believe that to answer this question one must seek for elements that are located between the peptidyltransferase center and the elongation factor binding site, the latter consisting of the GTPase-associated center (GAC) and the sarcin-ricin loop (SRL) (3). One can expect that some of the relevant elements should be mobile, as they have to transfer an allosteric signal by its movement relative to the rest of the large subunit. The SRL interacts with the switch regions of the elongation factors close to the GTP binding site (4), and it is likely that it is involved in the activation of GTP hydrolysis. However, in all available atomic structures of large ribosomal subunits from various organisms, as well as in cryoelectron microscopic reconstructions, the SRL always occupies the same position. The GAC, in contrast, is moveable (4, 5); it can move away from the helix 89 and toward the helix 89. These conformations are also referred to as “open” and “closed” in the literature (4). Interestingly, a difference of ~20 Å in the GAC positions can be seen if one compares the large subunit atomic structures from Haloarcula marismortui (6) and Deinococcus radiodurans (7). Furthermore, different locations of the GAC are visible in cryoelectron microscopic reconstructions of various functional complexes of ribosomes; in particular, formation of complexes with EF-G-GMP-PNP is associated with the “closed” conformation of the GAC (5). In the complexes of ribosomes with EF-G-GDP and fusidic acid the GAC is in the open conformation (8). In contrast, the complex of ribosome with EF-Tu-aminoacyl-tRNA-GDP, stabilized by kirromycin, is characterized by a closed conformation of the GAC (4). There are differences in the protection patterns for EF-G and EF-Tu on 23 S rRNA as well (3). EF-G protects A1067 and A1069 residues of the GAC (3), whereas EF-Tu ternary complex weakly protects G1068 and G1071 (9).

The main goal of our study was to model the conformation of the ribosomes with GAC in open or closed position by mutations and to investigate the effects on ribosome function and conformation of 23 S rRNA. The GAC is connected with the rest of the 23 S rRNA by the RNA helix 42. The insertion of a base pair into this RNA helix should cause a displacement of 3.3 Å along the helix axis together with a rotation of 33°. Accordingly, given the specific structure of the GAC connected to helix 42, we expected that deletion or duplication of the helix 42 base

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‡ The abbreviations used are: EF, elongation factor; GAC, GTPase-associated center; SRL, sarcin-ricin loop; GMP-PNP, guanosine 5′-β,γ-imido]triphosphate disodium salt; DMS, dimethyl sulfate.
pair C1030/G1124 would lead to upward or downward movement of the GAC, respectively. The movement, caused by the binding of EF-G-GMP-PNP (5) or Aa-tRNA-EF-Tu-GDP-pkirimycin (4) would resemble the effect of C1030/G1124 deletion, i.e. shortening the distance between the GAC and helix 89 and moving the GAC upwards. For this insertion of the base pair next to C1030/G1124, it could move the GAC downwards, further away from the position, seen in the complexes with EF-G-GMP-PNP (5) or Aa-tRNA-EF-Tu-GDP-pkirimycin (4).

MATERIALS AND METHODS

Mutagenesis and Ribosome Preparation—The mutations InsC1030/G1124 and ΔC1030/G1124 were made by standard site-directed mutagenesis procedures in a HindIII-SphI fragment of the rrnB operon cloned into the plasmid pBR25, a derivative of pRK35 (10) containing a streptavidin-binding aptamer inserted at the position of helix 25 (11). The plasmid contains the rrnB operon of rDNA under control of the phage λ P1 promoter. A similar fragment carrying ΔC1030/G1124 was subcloned to the pLKI25/ vector, a derivative of pLKS (10) without the streptavidin-binding aptamer. Transformation of the AVS9090 strain (12), plasmid substitutions, and checking the purity of mutant tRNA in the cells were carried out as described (13). For expression of the InsC1030/G1124 mutant ribosomal RNA strand POP2136 was used, carrying the temperature-sensitive λ repressor. Translation fidelity was measured by a set of reporter strains (14, 15).

Standard preparations of ribosomes were made according to Blaha et al. (16). Preparations of the InsC1030/G1124 mutant ribosomes and control wild type ribosomes carrying the streptavidin tag by affinity chromatography were made as described in Leonov et al. (11), with the exception that the host strain was POP2136, and expression of the mutant rRNA operon was induced by a temperature shift to 42 °C (17).

Poly(U)-directed poly(rPhe) synthesis was measured according to Bartetzko and Nierhaus (18). All binding experiments were made in a buffer containing 60 mM Hepes-K, pH 7.6, 6 mM MgCl₂, 80 mM NH₄Cl, 8 mM β-mercaptoethanol. The concentration of the ribosomes or the ribosomal complex with poly(U) and tRNA was 0.04 μM. The range of EF-G concentrations was 0.05–1.06 μM. The reactions were stopped after 20 min by addition of equal volume of 20% HCOOH. Time course of the GTP (0.4 μM) hydrolysis by EF-Tu ternary complexes was measured similarly. We used complex of ribosomes (0.1 μM), MFK-mRNA (0.2 μM), and Met-tRNA (0.2 μM), containing the UUC codon in the A-site as the ribosomal substrate.

Chemical Probing and Footprinting—Chemical modification of ribosomes and ribosomal complexes was done as described by Sergiev et al. (13).

The following complexes were used for the footprinting of EF-G and EF-Tu: ribosomes (0.3 μM), MFK-mRNA (0.6 μM), fMet-tRNA (0.6 μM), and EF-Tu–fMet-tRNA (0.6 μM); ribosomes (0.1 μM), EF-G (1 μM), and GMP-PNP (0.2 mM); ribosomes (0.3 μM), EF-G (1 μM), GTP (0.2 mM), and fusidic acid (0.2 mM); ribosomes (0.3 μM), MFK-mRNA (0.6 μM), and tRNA (0.6 μM); ribosomes (0.3 μM), MFK-mRNA (0.6 μM), tRNA (0.6 μM), EF-G (1 μM), and GMP-PNP (0.2 mM); ribosomes (0.3 μM), MFK-mRNA (0.6 μM), and tRNA (0.6 μM), EF-G (1 μM), and GTP (0.2 mM), and fusidic acid (0.2 mM); ribosomes (0.3 μM), MFK-mRNA (0.6 μM), fMet-tRNA (0.6 μM), and MFK-mRNA (0.6 μM), fMet-tRNA (0.6 μM), EF-G (1 μM), and GMP-PNP (0.2 mM); ribosomes (0.3 μM), MFK-mRNA (0.6 μM), fMet-tRNA (0.6 μM), and GTP (0.2 mM), and fusidic acid (0.2 mM), and empty ribosomes (0.3 μM) as a control.

RESULTS

In Vivo Effects of the Mutations—Deletion of the C1030/G1124 (ΔC1030/G1124) pair was viable, even if the mutant rRNA was expressed in cells devoid of any source of the wild type rRNA (12); the growth of the strain carrying only ΔC1030/G1124 23 S rRNA was almost not retarded. In contrast, the corresponding insertion of an extra base pair (InsC1030/G1124) was lethal and could only be expressed from a regulated promoter, with the cell life being supported by the expression of the wild type rRNA. The translational fidelity of the mutant ribosomes was measured in vivo with the help of reporter plasmids expressing mutant variants of the lacZ gene (14, 15).

In this system active protein could only be synthesized as a result of various translational errors, such as stop codon readthrough, or +1 and −1 frameshifting. ΔC1030/G1124 mutant ribosomes had almost no effect on translational fidelity. In contrast InsC1030/G1124 caused a significant increase in the translational error level specifically on the stop codon readthrough and −1 frameshifting (Table I). The synthesis of the wild type galactosidase from the control reporter plasmid was also increased in the InsC1030/G1124 mutant. This effect is characteristic for the mutants having severe phenotypes and is explained by increase in the wild type ribosome biosynthesis (22).

Purification of the Mutant Ribosomes—Ribosomes carrying the lethal InsC1030/G1124 mutation were purified from the mixture with wild type ribosomes by means of a streptavidin-binding RNA tag, introduced at a silent site within the 23 S rRNA (11). The wild type ribosomes, carrying the same tag and

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\text{Table I: Effect of InsC1030/G1124 and ΔC1030/G1124 mutations on translation fidelity.}
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| Test plasmid | Type of translation error | Wild type | ΔC1030/G1124 | InsC1030/G1124 |
|--------------|--------------------------|-----------|--------------|---------------|
| pSG 853      | UAA readthrough          | 2.2 ± 0.1 | 3.5 ± 0.1    | 9.3 ± 0.8     |
| pSG 12-6     | UAG readthrough          | 6.8 ± 0.3 | 7.6 ± 0.5    | 18.0 ± 1.8    |
| pSG lac7     | +1 Frameshift            | 45 ± 3    | 48 ± 5       | 92 ± 10       |
| pSG 12DP     | ‒1 Frameshift            | 43 ± 4    | 46 ± 4       | 72 ± 5        |
| pCSH 103     | Glu/Gln substitution     | 4.7 ± 4   | 37 ± 1       | 19 ± 3        |
| pSG25        | No error                 | 3980 ± 700| 4500 ± 400   | 6500 ± 500    |

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\text{GTPase Activities of EF-G and EF-Tu—Multiple turnover [γ-S-32P]GTP (0.5 mM) hydrolysis by EF-G stimulated by various ribosomes.}
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purified similarly, were used as controls in all further experiments. ΔC1030/G1124 mutant ribosomes were isolated according to standard procedures. As was expected for the ribosomes carrying the lethal mutation, the ribosomes carrying the InsC1030/G1124 mutation showed a significant (80%) decrease of activity in poly(U)-directed poly(Phe) synthesis in vitro in contrast to ΔC1030/G1124 ribosomes that demonstrated the same activity as the wild type ones.

Influence of the Mutation on 23 S rRNA Conformation Revealed by Chemical Probing—Movement of the GAC “downward” in InsC1030/G1124 mutant ribosomes should stimulate it to make contact with the tip of rRNA helix 89. Thus we should expect the changes in chemical reactivity of the 23 S rRNA nucleotide bases located at the tip of helix 89 and in helices that have a direct contact with helix 89, namely helices 91 and 95 (Figs. 1 and 2). Indeed, the most prominent difference in chemical reactivity was found for the nucleotide bases 2472–2483, 2529–2532, and 2661–2662 involved in the interactions between the tips of the helices 89, 91, and 95 (SRL) (Figs. 1 and 2). Furthermore, it is important that the conformational change induced by the contact of the GAC with helix 89 is transmitted to the SRL, influencing the structure of the latter.

The second class of 23 S rRNA residues whose reactivity was influenced by the mutation were found in helix 80 (P-loop), helix 39, and helix 89; nucleotides 2250–2256, 956–960, 2454, and 2491–2493 (Figs. 1 and 2). In the ribosome these helices connect the peptidyltransferase center (helix 80, P-loop) through helix 39 and helix 89 with GAC and SRL (Fig. 2).

The third class of nucleotides whose reactivity was changed as a result of the downward movement of the GAC were tertiary contacts of the stem consisting of the helices 96 and 97; nucleotides 2702–2705, 2751, and 2764–2766 (Figs. 1 and 2). One end of this stem is located just below the “elbow” between helix 42 and the GAC. The stem penetrates through almost the entire subunit from “top” to “bottom,” with the SRL being attached at its midpoint.

The structure probing of ribosomes carrying the ΔC1030/ G1124 mutation revealed the changes in protection pattern, consisted with the movement of GAC “upward”, or away from the helix 89 and SRL. Two bases increasing their reactivity as a result of ΔC1030/G1124 mutation (G1115 and G2751) are located in the contact area between the helices 42 and 97. The third base, whose reactivity was increased, is G2529, which forms a contact between helices 89 and 91.

Determination of the Translation Step Affected by the Lethal Mutation in in Vitro Translation System—To check the ability of the mutant ribosomes to fulfill different stages of translation we tested them in the stepwise translation of MFK-encoding mRNA. Progression through the elongation cycle was monitored by toeprinting (Fig. 3). Formation of the 70 S initiation complex by binding of fMet-tRNA^Met to the ribosomes programmed with MFK-mRNA resulted in a clear toeprint signal at position +16 relative to the A in the AUG start codon for both the InsC1030/G1124 and ΔC1030/G1124 mutant ribosomes similar to wild type ones. At the next step the pretranslocation complex was formed by enzymatic binding of PhetRNA^Met to the 70 S initiation complex to the ribosomal A-site (with help of the ternary complex Phet-tRNA^Met·EF-Tu·GTP). That resulted in an arising of the additional toeprint signal at position +17 for both mutant and wild type ribosomes. The translocation was stimulated by EF-G·GTP treatment. In the case of the wild type and ΔC1030/G1124 mutant ribosomes the translocation was complete. A new toeprint signal at positions +19, +20 appeared, and the signal for the pretranslocation complex disappeared. However, in the case of the lethal InsC1030/G1124 mutant the translocation was less efficient and did not proceed to completion (translocation signal at positions +19, +20, and substantial pretranslocation signal at position +17). To test the system in the elongation mode,
Lys-tRNA\textsuperscript{Lys}\textsubscript{EF-Tu}\textsubscript{GTP} was added. Again, the translocation for the InsC1030/G1124 mutant ribosomes was significantly less efficient but not abolished (toeprint signal at position 22).

Some part of the peptidyl-tRNA underwent translocation, when it appeared in the A-site of the wild type ribosomes but not of the InsC1030/G1124 mutant (Fig. 3B) either spontaneously or because of the admixture of EF-G traces (in EF-Tu preparation). We repeated the stepwise translation experiments using the AcPhe-tRNAPhe as an A-site ligand (Fig. 3C) bound to the ribosomes non-enzymatically. It is known that spontaneous translocation is almost absent for the AcPhe-tRNA analogue (23). The P-site of ribosomes was prefilled with deacylated tRNAPhe. The binding of the deacylated tRNA to the P-site produced the toeprint signal, indistinguishable between the wild type and the InsC1030/G1124 mutant (Fig. 3C). As expected (23), the A-site bound AcPhe-tRNAPhe did not undergo spontaneous translocation even if bound to the wild type ribosomes.

Upon the addition of EF-G-GTP, wild type ribosomes translocated AcPhe-tRNAPhe to the P-site, whereas the translocation...
Selection of Translation Elongation Factors by the Ribosome

Mutation of EF-Tu binding by comparison of characteristic bands intensities (Fig. 4C). We did not observe essential differences for the mutant and wild type ribosomes. This indicates that EF-Tu binding properties are not affected by the mutation. ΔC1030/G1124 mutant ribosomes were the same as wild type ones in these tests. The fact that InsC1030/G1124 mutation does not affect EF-Tu function but significantly reduces EF-G binding indicates that this mutation is likely to fix the position of GAC favorable for interaction with EF-Tu but not EF-G.

Stimulation of EF-Tu GTPase by Correct Codon-Anticodon Interaction in the Ribosomes—GTPase activity of EF-Tu upon its binding to the ribosome at the posttranslocation complex is stimulated by correct codon-anticodon interactions at the small subunit decoding center. We checked the influence of InsC1030/G1124 mutation on the elongation factor Tu GTPase activity using the model posttranslocation complex (Met-tRNA<sub>Met</sub>-ribosome-MFK-mRNA) to which cognate ternary complex Phe-tRNA<sub>Phe</sub>-EF-Tu<sub>γ-32P</sub>GTP was then bound. This binding was accompanied by GTP hydrolysis for both the wild type and InsC1030/G1124 mutant ribosomes (Fig. 5A). In contrast, if the non-cognate Lys-tRNA<sub>Glu</sub>-EF-Tu<sub>γ-32P</sub>GTP ternary complex was added, no stimulation of GTP hydrolysis was observed for both wild type and InsC1030/G1124 ribosomes (Fig. 5A). Thus, the movement of the GAC has no influence on the GTPase stimulation of EF-Tu.

Stimulation of EF-G GTPase by Decacylated tRNA in the Ribosomes—Whereas EF-Tu binding and its GTPase activity are regulated by codon-anticodon interactions at the decoding center of the small subunit, both the binding and GTPase activity of EF-G depend on the state of the tRNA bound to the P-site. Decacylated tRNA bound to the P-site mimics the situation after peptide transfer and stimulates the binding and multiple turnover GTP hydrolysis of elongation factor G (1, 2). The question was whether ribosomes carrying the InsC1030/G1124 mutation would retain this type of regulation. First, we measured the multiple turnover GTP hydrolysis of EF-G activated by empty ribosomes (Fig. 5B). The activation of the GTP hydrolysis of EF-G by the ribosomes, carrying the InsC1030/G1124 mutation was decreased in comparison with the wild type ribosomes. These data are in agreement with decreased binding of EF-G to the mutant ribosomes, although other steps of the EF-G cycle could also be affected by mutation. Second, poly(U)-programmed ribosomal complexes with decacylated tRNA<sub>Glu</sub> were used to test the stimulation of EF-G GTPase activity by decacylated tRNA. As expected, decacylated tRNA in the P-site of the wild type ribosomes stimulated the GTPase activity of EF-G (Fig. 5B). At the same time, the GTPase activity of EF-G at the presence of decacylated tRNA bound to the P-site of the InsC1030/G1124 mutant ribosomes remained at the same level as in empty ribosomes (Fig. 5B). These data are in agreement with the EF-G binding tests described above. Although we currently could not say definitely whether diminished GTPase activity is explained solely by decreased EF-G binding. Other stages of EF-G cycle could also be affected. ΔC1030/G1124 mutant ribosomes revealed the same properties as the wild type ribosomes in these tests (data not shown). Thus, the mutation that models downward GAC movement not only significantly affects EF-G binding to the ribosomes but almost completely abolishes the EF-G GTPase stimulation by decacylated tRNA.

**DISCUSSION**

We selected the sites for mutagenesis of 23 S rRNA on the basis of previous observations showing that the GAC behaves as a mobile element in the functioning ribosome (4, 5). It could be found in either open or closed conformations, shifted away or
toward the helix 89. A closed conformation is characteristic for the ribosomal complex with EF-G/H18528GMP-PNP (8) and EF-Tu/H18528Aa-tRNA/H18528GDP/H18528kirromycin (4), whereas the open one is characteristic for the complex with EF-G/H18528GDP/H18528fusidic acid (8). The A1067 residue is not protected by EF-Tu from DMS modification, in contrast to EF-G, which causes this protection (3). However, the Aa-tRNA/EF-Tu/H18528GDP/H18528kirromycin complex protects G1068 and G1071 from kethoxal attack (9).

The binding of thiostrepton, an antibiotic that inhibits the multiple turnover GTP hydrolysis by EF-G (24) also protects A1067 from DMS modification by EF-G (25). In contrast, a similar antibiotic, micrococcin, enhances the reactivity of A1067 (25) and stimulates the multiple turnover GTPase activity of EF-G (24). Perhaps these opposite effects could also be attributed to stabilization of open and closed positions of the GAC.

We made two mutations that simulate movements of the GAC irrespective of the functional state of the ribosome. In the absence of appropriate x-ray data for the mutant ribosomes, one can model the structural changes using the basic knowledge of RNA structure and results of chemical probing analyses as well as elongation factor activity tests we have performed.

The insertion and the deletion of a base pair in the RNA stem should cause the elongation or shortening of the stem by 3.5 Å. The mutations should promote oppositely directed rotational movements. Because helix 42 and GAC connected to it are nearly L-shaped, the rotational component must be more pronounced. The deletion of the base pair C1030/G1124 was silent in all assays that could indicate the ability of the ribosome to overcome this artificial upward movement and still keep the ability to move down. The only differences in the structures of the rRNA are putative disruptions of the contacts between helices 42 and 97 as well as 89 and 91. On the other hand the movement downward expected for the InsC1030/G1124 mutation was lethal. Movement of the GAC downward in the InsC1030/G1124 mutant ribosomes according to x-ray ribosome structure should create a contact with the tip of rRNA helix 89 (Fig. 5).

Indeed, the most prominent difference in chemical reactivity was found for the nucleotide bases in the helix 89 itself and for nucleotide bases involved in the interactions of the helix 89 with the helices 91 and 95.

EF-G-related functions were the only ones compromised by the InsC1030/G1124 mutation. Other translation steps were not affected. The ribosomes carrying the InsC1030/G1124 mutation were able to bind tRNA molecules to the P- and A-sites equally to that of the wild-type ribosomes. Binding of EF-Tu ternary complex to the InsC1030/G1124 mutant ribosomes was the same as to the wild type ribosomes. The activation of GTP hydrolysis by EF-Tu was also the same and depended on a correct codon-anticodon recognition. Thus, it is likely that the InsC1030/G1124 mutation perturbed the ribosome structure in a specific way, affecting the EF-G-related activity. The increase in –1 frameshifting in vivo observed for the cells expressing.
EF-G the downward GAC movement, caused by mutation, switches the GAC into the conformation restrictive to EF-G binding. At the same time this conformation could either be favorable or neutral for EF-Tu ternary complex binding. Despite the observation that the mutant ribosome was still able to bind EF-G, it almost completely lost the ability to stimulate EF-G GTPase at deacylated tRNA binding. Such an effect is expected if the signal transition system that stimulates EF-G GTPase upon deacylated tRNA binding is affected. The allosteric signal carrying the information that the peptidyltransferase reaction has been completed is likely to originate from the P-loop. This loop interacts with the CCA end of the tRNA bound to the P-site, but not with that of the deacylated tRNA bound to the hybrid P/E-site (27). Formation of the P/E hybrid state, but not the classical P-state, is likely to determine the enhancement of the multiple turnover GTPase activity of EF-G (2). The conformation of the P-loop is influenced by mutations in helix 39 (13). Mutations in the P-loop affect the contacts between helices 89, 91, and the SRL (17). This very set of contacts is also affected in part by the binding of the antibiotic evernimicin (28).

Structural probing experiments of InsC1030/G1124 mutant ribosomes revealed a defined chain of nucleotides connecting the P-loop of 23 S rRNA via helices 39, 89, 91, and 42 with the elongation factor binding site, consisting of the GAC and the SRL (Figs. 4 and 5). Consideration of these data together favors the suggestion that P-loop and EF-G binding sites are connected by the chain of interactions of nucleotide bases located in helices mentioned above.

Structural probing of the InsC1030/G1124 mutant ribosomes allowed us to suggest a new mobile 23 S rRNA element interacting with helix 42 that could be involved in the signal transition chain and be essential for determining upward or downward position of GAC. This element is a long stem consisting of the helices 96 and 97 that penetrates the large subunit from the top to the bottom on the L7/L12 side. This stem moves downwards within the subunit, as a result of the pressure from the GAC making nucleotides in the loop-end of helix 96/97 accessible for chemical modification. Restoration of the original position of this stem causes the protection of these bases (in the wild type ribosomes). The formation and disruption of the contact between the loop of the helix 96 and helix 42 could be seen upon comparison of the large ribosomal subunit structures of *H. marismortui* (6) and *D. radiodurans* (7).

Thus, we can conclude that movement of GAC in a way to shorten the distance between GAC and SRL has no effect on EF-Tu function but compromises EF-G binding and its GTPase activity stimulation by deacylated tRNA. The conformational signal can be transferred between peptidyltransferase and elongation factor binding centers of the ribosome via helices 39, 89, 42, and 91 with the help of the mobile element (helix 96/97). Most likely, the ribosome utilizes the GAC movement to favor or restrict EF-G binding in dependence of the peptidyl transferase reaction has been completed is likely to originate from the P-loop. This loop interacts with the CCA end of the tRNA bound to the P-site, but not with that of the deacylated tRNA bound to the hybrid P/E-site (27). Formation of the P/E hybrid state, but not the classical P-state, is likely to determine the enhancement of the multiple turnover GTPase activity of EF-G (2). The conformation of the P-loop is influenced by mutations in helix 39 (13). Mutations in the P-loop affect the contacts between helices 89, 91, and the SRL (17). This very set of contacts is also affected in part by the binding of the antibiotic evernimicin (28).

**FIG. 5. Measurement of the ribosome dependent GTP hydrolysis by EF-G and EF-Tu.** A, time course of the GTP hydrolysis by EF-Tu ternary complexes, stimulated by the complex of ribosomes, MFK-mRNA and fMet-tRNA<sup>fMet</sup>. Squares correspond to the non-cognate ternary complex Lys-tRNA<sup>Cys</sup>-EF-Tu<sup>Δ-32P</sup>GTP, circles correspond to the cognate Phe-tRNA<sup>Cys</sup>-EF-Tu<sup>Δ-32P</sup>GTP ternary complex. Black symbols and lines of trend correspond to wild type ribosomes, gray symbols and lines of trend correspond to ribosomes carrying the InsC1030/G1124 mutation. B, the rate of the multiple turnover [γ<sup>32P</sup>]GTP hydrolysis by EF-G, stimulated by 40 nM empty ribosomes (squares) and 40 nM ribosomal complex with poly(U) and deacylated tRNA<sup>ΔMS</sup> (circles), plotted against EF-G concentration. Black symbols and lines of trend correspond to wild type ribosomes, gray symbols and lines of trend correspond to ribosomes carrying the InsC1030/G1124 mutation.

the InsC1030/G1124 mutant rRNA correlates well with the model of frameshifting (26), according to which the −1 frameshifting event is associated with the pretranslation state; thus, a delay in translocation would increase the probability of −1 frameshifting.

According to different *in vitro* tests with the InsC1030/G1124 mutant ribosomes, the translocation was affected in agreement with reduced binding of EF-G to mutant ribosomes. Although the EF-G binding to mutant ribosomes was certainly affected by the InsC1030/G1124 mutation, we do not exclude the possibility that other steps of EF-G related function were also compromised. The explanation of the observed specific effects of the Ins1030/1124 mutation on the EF-G binding could be that for
Bartels, H., Franceschi, F., and Yonath, A. (2001) Cell 107, 679–688
8. Agrawal, R. K., Linde, J., Sengupta, J., Nierhaus, K. H., and Frank, J. (2001) J. Mol. Biol. 311, 777–787
9. Moazed, D., and Noller, H. F. (1989) Cell 57, 585–597
10. Powers, T., and Noller, H. F. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 1042–1046
11. Lesnov, A. A., Sergiev, P. V., Bogdanov, A. A., Brimacombe, R., and Dontsova, O. A. (2003) J. Biol. Chem. 278, 25664–25670
12. Asai, T., Zaporojets, D., Squires, C., and Squires, C. L. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1971–1976
13. Sergiev, P. V., Bogdanov, A. A., Dahlberg, A. E., and Dontsova, O. (2000) J. Mol. Biol. 299, 379–389
14. O’Connor, M., and Dahlberg, A. E. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9214–9218
15. Cupples, C. G., and Miller, J. H. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5345–5349
16. Blaha, G., Stelzl, U., Spahn, C. M., Agrawal, R. K., Frank, J., and Nierhaus, K. H. (2000) Methods Enzymol. 317, 292–309
17. Gregory, S. T., and Dahlberg, A. E. (1999) J. Mol. Biol. 285, 1475–1483
18. Bartetzko, A., and Nierhaus, K. H. (1988) Methods Enzymol. 164, 650–658
19. Shimizu, Y., Inoue, A., Tomari, Y., Suzuki, T., Yokogawa, T., Nishikawa, K., and Ueda, T. (2003) Nat. Biotechnol. 19, 751–755
20. Hartz, D., McPheeters, D. S., Traut, R., and Gold, L. (1988) Methods Enzymol. 164, 419–425
21. Ehrenberg, M., Bilgin, N., and Kurland, C. G. (1990) in The Ribosomes and Protein Synthesis A Practical Approach (Spedding, G., ed), pp. 101–129, Oxford University Press, Oxford, UK
22. Moine, H., and Dahlberg, A. E. (1994) J. Mol. Biol. 243, 402–412
23. Bergemann, K., and Nierhaus, K. H. (1983) J. Biol. Chem. 258, 15105–15113
24. Cundliffe, E., and Thompson, J. (1981) Eur. J. Biochem. 118, 47–52
25. Egelsee, J., Douthwaite, S., and Garrett, R. A. (1989) EMBO J. 8, 607–611
26. Harper, J. W., Kedroskias, A., and Dinman, D. J. (2002) Trends Biochem. Sci. 27, 448–454
27. Moazed, D., and Dahlberg, H. F. (1989) Nature 342, 142–144
28. Belova, L., Tenson, T., Xiong, L., McNicholas, P. M., and Mankin, A. S. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 3726–3731
29. Guex, N., and Peitsch, M. C. (1997) Electrophoresis 18, 2714–2723
Alteration in Location of a Conserved GTPase-associated Center of the Ribosome Induced by Mutagenesis Influences the Structure of Peptidyltransferase Center and Activity of Elongation Factor G

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