Elongation Factor G Bound to the Ribosome in an Intermediate State of Translocation

David S. Tourigny, Israel S. Fernández, Ann C. Kelley, V. Ramakrishnan*

Introduction: After peptidyl transfer, the movement of messenger RNA (mRNA) and transfer RNAs (tRNAs) with respect to the ribosome places the next mRNA codon in the A site. This process of translocation proceeds via an intermediate state in which the acceptor ends of the tRNAs have moved with respect to the 50 S subunit but not the 30 S subunit, to result in A/P and P/E tRNA hybrid states. The guanosine triphosphatase elongation factor G (EF-G) catalyzes the subsequent movement of mRNA and tRNA with respect to the 30 S subunit. How EF-G binds to the intermediate state of the ribosome and how this results in guanosine 5’-triphosphate (GTP) hydrolysis and translocation are questions that will be greatly facilitated by a high-resolution structure of the complex.

Methods: Thermus thermophilus ribosomes lacking protein L9 were crystallized in an intermediate state with mRNA, a hybrid P/E tRNA, and EF-G with the non-hydrolyzable GTP analog GDPCP. The structure was solved by molecular replacement and refined to 2.9 Å resolution.

Results: The 50 S and 30 S ribosomal subunits are rotated relative to each other, as was expected from previous cryo–electron microscopy studies. The L1 stalk on the 50 S subunit moves inward to stabilize the P/E hybrid-state tRNA, and atomic details of this interaction can now be seen. Domain IV of EF-G takes up an orientation intermediate between that of the isolated form of EF-G and that of EF-G bound to the ribosome in the fully translocated state. The catalytic center of EF-G shows that key switch regions surrounding the γ-phosphate of GDPCP are clearly visible and therefore ordered. Moreover, key conserved residues in EF-G, including a histidine and an aspartate, change conformation relative to both the isolated and fully translocated structure. These activated conformations appear to be stabilized by the highly conserved sarcin-ricin RNA loop (SRL) of the 50 S subunit.

Discussion: Comparison with the post-translocational state suggests that interactions between the tRNA and L1 stalk are preserved throughout translocation and that these are probably an essential feature of translocation required for stabilization of the hybrid P/E state.

In the isolated structure of EF-G, domain IV, because of its orientation, would largely avoid a clash with A-site tRNA, as would be required for formation of a transient initial complex. In our structure, domain IV partly extends into the A site, which is consistent with the observation that EF-G facilitates translocation at a slow rate even without GTP hydrolysis.

The catalytic center of EF-G has essentially the same structure as that previously observed for elongation factor Tu (EF-Tu), with the highly conserved histidine stabilized by the SRL in an orientation that coordinates a water molecule in position for hydrolysis of GTP. This shows that although EF-Tu and EF-G bind to very different states of the ribosome, the mechanism of activation of GTP hydrolysis is probably the same for these two factors, and possibly for other translational GTPases.

Fig. 1. Unbiased difference Fourier maps.
Fig. 2. EF-G bound to the rotated state of the ribosome.
Fig. 3. Dynamics of the L1 stalk during tRNA translocation.
Fig. 4. Interactions of EF-G with L6, L11, and L12.
Fig. 5. Conformational changes in EF-G during translocation.
Fig. 6. The active site of EF-G.
Table 1. Summary of crystallographic data and refinement.

SUPPLEMENTARY MATERIALS
Movies S1 to S3

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A key step of translation by the ribosome is translocation, which involves the movement of messenger RNA (mRNA) and transfer RNA (tRNA) with respect to the ribosome. This allows a new round of protein chain elongation by placing the next mRNA codon in the A site of the 30S subunit. Translocation proceeds through an intermediate state in which the acceptor ends of the tRNAs have moved with respect to the 50S subunit but not the 30S subunit, to form hybrid states.

The guanosine triphosphatase (GTPase) elongation factor G (EF-G) catalyzes the subsequent movement of mRNA and tRNA with respect to the 30S subunit. Here, we present a crystal structure at 3 angstrom resolution of the Thermus thermophilus ribosome with a tRNA in the hybrid P/E state bound to EF-G with a GTP analog. The structure provides insights into structural changes that facilitate translocation and suggests a common GTPase mechanism for EF-G and elongation factor Tu.

It was originally assumed that EF-G simply lowers the free-energy barrier of the spontaneous reaction and that GTP hydrolysis is required to release EF-G from the posttranslocated ribosome. The currently prevailing view, based on kinetic experiments suggesting that GTP hydrolysis precedes and accelerates translocation, is that a rotated-state ribosome plays the role of a GTPase activator for EF-G. Rapid GTP hydrolysis upon ribosome binding is thought to accelerate rate-limiting conformational changes that result in an unlocking of the ribosome leading to translocation.

How GTP hydrolysis is activated by the ribosome remains somewhat controversial. Mutation of the highly conserved His residue of EF-Tu to alanine resulted in a 100-fold reduction in catalytic activity. The structure of the ternary complex bound to the ribosome showed that His is involved in hydrogen-bonding interactions both with A2662 of the in the I1 stalk and main body of the ribosome.

In the second step of translocation, the guanosine triphosphatase (GTPase) elongation factor G (EF-G) catalyzes the movement of mRNA and tRNAs with respect to the 30S subunit, thereby placing the next codon of mRNA in the A site and restoring the ribosome to the canonical, unrotated state. Various experiments suggest that EF-G with GTP stabilizes the rotated state of the ribosome (4) with hybrid tRNAs (5–7). EF-G is structurally similar to the ternary complex of elongation factor Tu (EF-Tu), tRNA, and GTP, with its domain IV mimicking the anticodon stem-loop of tRNA (8–10). The structures of EF-G bound to the ribosome in both the canonical and rotated states have been observed by cryo-electron microscopy (cryo-EM) (11–13). Whereas these studies have greatly advanced our understanding of the changes in the ribosome induced by EF-G binding, a high-resolution structure can provide greater details of the interactions of EF-G with the rotated state of the ribosome and insights into the molecular mechanisms that lead to translocation.

It is not known to what extent EF-G may vary in the posttranslocation canonical state (14, 15). The currently prevailing view, based on kinetic experiments suggesting that GTP hydrolysis precedes and accelerates translocation (16–18), is that the rotated-state ribosome plays the role of a GTPase activator for EF-G. Rapid GTP hydrolysis upon ribosome binding is thought to accelerate rate-limiting conformational changes that result in an unlocking of the ribosome leading to translocation (17).

How GTP hydrolysis is activated by the ribosome remains somewhat controversial. Mutation of the highly conserved His residue of EF-Tu to alanine resulted in a 100-fold reduction in catalytic activity (19). The structure of the ternary complex bound to the ribosome showed that His in the switch II region was involved in hydrogen-bonding interactions both with A2662 of the and a water molecule positioned for hydrolysis of the γ-phosphate of the GTP analog β,γ-methyleneadipate 5′-triphosphate (GDPCP) (20). Suggestions that the histidine might play a role as a catalytic base were later questioned (21–23). Subsequently, a structure of release factor 3 (RF3) bound to a rotated-state ribosome (24) placed the histidine in a very different position, suggesting that it was unlikely to play a direct role in catalysis and that any mechanism of GTP hydrolysis is not general, as was first proposed (20).

A breakthrough in determining high-resolution structures of the ribosome bound to EF-G in various states was made when the crystal structure of guanosine diphosphate (GDP)-bound EF-G stalled on a posttranslocated ribosome was solved (25). Here, we report a crystal structure refined using data to 2.9 Å, of the ribosome bound to EF-G with GDP. In addition, the structure consists of an mRNA with a phenylalanine codon in the P site and a tRNA in the P/E hybrid state. As was done in previous cryo-EM structures (11, 12), the A-site tRNA was left out in order to obtain the intermediate rotated state of the ribosome, because in its presence with wild-type EF-G, the ribosome proceeds within seconds to the posttranslocational canonical state even without GTP hydrolysis (16, 26). This structure with EF-G bound to the rotated state of the ribosome before GTP hydrolysis lacks an A-site tRNA, but otherwise represents a key hitherto missing high-resolution structure in the elongation cycle.

**Results**

**Overall Structure**

Crystallographic data are shown in Table 1. After molecular replacement using the 50S and 30S subunits as search models, the P/E tRNA, mRNA, EF-G, and GDPCP were clearly visible in difference Fourier maps (Fig. 1; mRNA not shown), and the entire structure was built and refined (Fig. 2A). The main body of the 30S subunit is rotated ~7° counterclockwise with respect to the 50S (as viewed from the solvent side (Fig. 2B and movie S1). Although the precise rotation angles differ, the intersubunit interactions and central bridges are similar to those previously seen in the rotated state with ribosome recycling factor (RRF) (27) or RF3 (24, 28), suggesting a ratcheting motion that is conserved across the translational pathway. The head of the 30S is swiveled by ~5° as compared to the canonical (Fig. 2C and movie S2). Two separate ratcheted states that differ in the degree of head swiveling have been identified by cryo-EM of an EF-G–ribosome complex (13). As displayed in Fig. 2C, the 30S head of this structure has a conformation similar to that of the TiPRE state in that cryo-EM study [root mean square deviation (RMSD) of 1.7 Å as opposed to 11.1 Å when compared to TiPOST state]. Recently, it was shown that the TiPRE state also closely resembles cryo-EM reconstructions of ribosomes containing both P/E and A/P hybrid tRNAs after peptidyl transfer (29), which is further evidence that our structure represents a valid model for the main intermediate state of translocation. The head swivel is thought to widen a constriction in the 30S to allow translocation of the P-site tRNA to the E site (13, 30, 31). In the rotated state seen here, this constriction is widened by ~2.7 Å compared to the canonical state, suggesting that further widening must occur at some point to allow translocation of the anticodon stem-loop of tRNA from the P to the E site. It has been proposed that intersubunit ratcheting and 30S head swiveling are sequential events that provide directionality to mRNA and tRNA translocation (32).
Interaction of the L1 Stalk with the P/E Hybrid tRNA

The tRNAPhe in the P/E conformation is distorted, with a twist in the D-stem of the main body enabling the acceptor arm to swing ~35° toward the E site of the 50S subunit, similar to that seen in the hybrid states with RRF (27) or RF3 (28) (RMSD of 0.81 and 1.55 Å, respectively). The elbow of the P/E tRNA is cradled by the L1 stalk of the 50S ribosomal subunit, which has pivoted about the base of helix H76 (Fig. 3A) and swung into the fully closed conformation seen in lower-resolution studies (11, 12). In structures with a canonical E-site tRNA in the posttranslocational state, the L1 stalk is in a “half-closed” conformation (25). Relative to that conformation, the distal part of the L1 stalk has moved inward by ~25 Å to interact with the P/E tRNA (Fig. 3B), resulting in an angle of ~17.4° between these two positions. Moreover, there is a distance of ~37 Å between the closed conformation seen here and the fully open conformation observed in structures of the ribosome with a vacant E site (30). This dynamical nature of the L1 stalk has been studied in two kinds of single-molecule fluorescence resonance energy transfer experiments and demonstrated to have a mechanistic role during translocation (33, 34). In the absence of any factor, the L1 stalk fluctuates between half-closed and closed conformations corresponding to non-ratcheted and ratcheted states of the ribosome; binding of EF-G shifts this equilibrium toward the closed conformation of the ratcheted state. Our structure supports the notion that the L1 stalk-tRNA interaction persists throughout translocation (33). However, a separate study suggests that hybrid state formation and L1 stalk closure are not tightly coupled (35).

**Fig. 1. Unbiased difference Fourier maps.** Unbiased difference Fourier maps obtained after initial refinement with an empty ribosome as a starting model, showing (A) P/E tRNA, (B) switch I and GDPCP in the active site, and (C to E) key conserved residues in the active site with water molecules.

A detailed description of the interactions between the L1 protein and tRNA is made possible by the stabilization of the stalk in the closed conformation, resulting in excellent maps that show side-chain conformations (Fig. 3C). Most of these interactions are electrostatic, such as

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### Table 1. Summary of crystallographic data and refinement.

<table>
<thead>
<tr>
<th>Data collection</th>
<th>Data set 1</th>
<th>Data set 2</th>
<th>70S-tRNA-EF-G-GDPCP (merged)</th>
</tr>
</thead>
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<tr>
<td>Beamline</td>
<td>ID14-4 (ESRF)</td>
<td>IO4 (DLS)</td>
<td>P2₁</td>
</tr>
<tr>
<td>Space group</td>
<td>P2₁</td>
<td>P2₁</td>
<td>P2₁</td>
</tr>
<tr>
<td>Cell dimensions</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>a, b, c (Å)</td>
<td>203.42, 243.05, 309.97</td>
<td>201.58, 241.65, 305.80</td>
<td>201.58, 241.65, 305.80</td>
</tr>
<tr>
<td>α, β, γ (°)</td>
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<td>90.00, 99.48, 90.00</td>
<td>90.00, 99.48, 90.00</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>39.6–3.1 (3.2–3.1)</td>
<td>39.6–2.9 (3.0–2.9)</td>
<td>39.6–2.9 (3.0–2.9)†</td>
</tr>
<tr>
<td>Rsym (%)</td>
<td>17.8 (58.7)</td>
<td>24.1 (138.4)</td>
<td>22.4 (137.8)</td>
</tr>
<tr>
<td>I/σ(I)</td>
<td>8.15 (2.42)</td>
<td>4.93 (1.08)</td>
<td>7.56 (1.02)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>99.6 (97.1)</td>
<td>97.8 (98.9)</td>
<td>99.8 (98.9)</td>
</tr>
</tbody>
</table>

Refinement

| Resolution (Å) | 39.6–2.9 |
| No. unique reflections | 635,092 |
| Rwork/Rfree | 19.6/24.5 |
| No. atoms | 150,122 |
| B values | RNA 37.8 Protein 51.4 |
| Bond length RMSD (Å) | 0.004 |
| Bond angles RMSD (°) | 1.488 |

* I/σ(I) = 2.15 at 3.1 Å (using a bin from 3.2 to 3.1 Å resolution).
† I/σ(I) = 3.31 at 3.1 Å (using a bin from 3.2 to 3.1 Å resolution).
Changes in the Catalytic Site

The catalytic site of EF-G shows distinct differences from the posttranslocated GDP form (25) or the isolated EF-G with GDPNP (36) that yield insights into activation of GTP hydrolysis. The switch I region was unresolvable in previous crystal structures of both posttranslocated and isolated EF-G, but is ordered in this structure from Met55 onward. The switch I region adopts a single turn of a 3_10 helix that contacts helix B1 of domain III, as in the isolated structure of the EF-G homolog EF-G-2 in the GTP form and as also seen at lower resolution by cryo-EM studies of a ribosomal complex similar to the structure described here (12). The γ-phosphate of GDPCP is surrounded by several highly conserved residues, notably His87 of switch II and Asp22, and Lys25 in the P loop (Fig. 6, A and B). His87 and Asp22 point away from bound nucleotide in the isolated and posttranslocated states of EF-G, but have moved by ~6.4 and ~3.3 Å, respectively, toward the γ-phosphate of GDPCP upon ribosome binding (Fig. 6C and movie S3) to assume a conformation very similar to that seen in EF-Tu (Fig. 6D) (20). As with EF-Tu, the conformation of the activated His87 is stabilized by hydrogen bonding interactions with both A2662 of the SRL and the catalytic water molecule poised for hydrolysis of the phosphate ester (Fig. 6B). Two Mg^{2+} ions uniquely positioned by the GAGA tetrad of the SRL stabilize the inward conformation of Asp22 where it coordinates a second water molecule above the γ-phosphate of GDPCP (Fig. 6B). This second water could play a further role in catalysis by donating a hydrogen bond to the γ-phosphate O2. The structure strongly suggests that the change in orientation of Asp22 and His87 upon EF-G binding is part of GTPase activation by the ribosome.
and that the mechanism of GTP hydrolysis is essentially the same for both EF-Tu and EF-G.

Although the final activated state of EF-Tu and that of EF-G GTP are highly similar, in EF-Tu the equivalent Asp\(^{21}\) has its activated conformation even in the isolated ternary complex. Thus, different steps may be required to reach the same activated state. Interestingly, the toxin ricin depurinates A2660 of the GAGA tetrad. It is likely that depurination of A2660 prevents the surrounding region from adopting the conformation required to bind the metal ions necessary to stabilize Asp\(^{22}\) and neighboring regions of EF-G in the activated form. EF-Tu does not make these interactions, explaining why ricin only affects EF-G function (37).

Although a proposal was made that His\(^{87}\) might be acting as a general base in EF-Tu (20), the structure is consistent with an alternate mechanism that was proposed subsequently (27). In this mechanism, the environment of the SRL may result in an increase in the acid dissociation constant (pK\(_a\)) of His\(^{87}\) and stabilize the protonated state of its N\(_6\), thus enabling His\(^{87}\) to donate a hydrogen bond to the hydrolytic water. The water can in turn donate a hydrogen bond to the carbonyl oxygen of Thr\(^{64}\) and to one of the three oxygen atoms on the γ-phosphate. Under these circumstances, the occurrence of a substrate-promoted catalytic mechanism, whereby the γ-phosphate abstracts a proton from the water molecule to generate a hydroxide ion that in turn cleaves the phosphate ester, appears feasible. It has also been suggested that the role of the histidine is not to behave as a donor or acceptor of protons at all, but to contribute to an allosteric effect that results in stabilization of the transition state by the general electrostatic effect of the P loop (23).

This scenario is compatible with the observation that in EF-G-2, a ribosome-activated GTPase that can substitute for EF-G in polyU-directed protein synthesis in vitro (12), the histidine and aspartate have been replaced by tyrosine and glycine, respectively (Fig. 6A).

**Discussion**

The structure sheds light on the GTPase mechanism of EF-G and on its role in translocation. Globally, a distinctive feature is that the interactions of the L1 stalk with the P/E tRNA appear to be the same as those with the posttranslational E-site tRNA (25), implying that the interactions are preserved throughout translocation, as previously suggested (33). This also suggests that the stabilization of the closed conformation of the L1 stalk through its interaction with the P/E tRNA is an essential feature of translocation through the stabilization of hybrid states.

Another large-scale movement is the swiveling of the head, which is required to open a constriction that allows passage of the P-site tRNA to the E site in the 30S subunit (13, 38). It has previously been suggested that spectinomycin, an antibiotic that inhibits translocation, may act by inhibiting the movement of the head by binding to a crucial hinge point (39, 40). Our structure shows that in the rotated state, the swivel angle of the head would cause a steric clash with spectinomycin, thus supporting this idea.

Notably, key residues in EF-Tu and EF-G change conformation in different ways upon binding to very different states of the ribosome to form a nearly identical catalytic site (Fig. 6D), suggesting a common mechanism for activation of translational GTPases by the ribosome. This mechanism also implies that the SRL plays a crucial role in stabilizing key residues of the catalytic site in their activated conformations, which would be in keeping with their very high degree of conservation.

Recently, lethal mutations in the SRL were found not to affect GTP hydrolysis (41), suggesting that the SRL does not play a direct role in stabilizing the transition state for GTP hydrolysis. However, the interactions with the SRL occur via phosphate backbone interactions...
rather than specific bases, so it is possible that in these mutant ribosomes, other nucleotides of the mutated SRL play the role of key residues in the wild-type ribosome.

In contrast to EF-Tu and EF-G, the catalytic site of RF3 on the ribosome appears different; the histidine is far from the $\gamma$-phosphate of GTP and makes different interactions with the SRL (24). It is therefore possible that the GTPase mechanism for RF3 is different or that the structure, which lacks the expected P/E tRNA, does not represent the GTPase-activated state of RF3.

The structure reported here offers some clues into how conformational changes associated with GTP hydrolysis could facilitate translocation. GTP hydrolysis results in changes in switch I, switch II, and P-loop regions that form an interface between the ribosome, domain III, and GTP. These changes in switch I and II may be communicated to domain III and cause the large movements of the helices that serve to bridge the I-II and III-V superdomains (Fig. 5). This would account for the relative change in the orientation of these superdomains upon GTP hydrolysis (Fig. 5). Deletion of domain III decreases EF-G activity 103-fold on the ribosome (42), supporting the notion that this region may couple GTP hydrolysis to the interdomain movements that allow domain IV to adopt the favored conformation of the posttranslocational state. Such a conformation may be adopted after tRNA translocation has occurred transiently, allowing domain IV to enter the A site and prevent a reversal of translocation. Details of the mechanism of action of EF-G will require concerted studies by many complementary techniques.

In conclusion, this work provides an atomic model of EF-G bound to the ribosome in a rotated state before GTP hydrolysis. It has enabled a complete description of the inward movement of the L1 stalk, stabilization of the P/E tRNA, and conformational changes in EF-G that are the key steps in facilitating translocation. GTP hydrolysis leads to a series of changes in the switch I, switch II, and P-loop regions of EF-G, which result in an interdomain reorientation about domain III that is expected to promote translocation.
were incubated at 55°C for 6 min before addition of volumes used for crystallization experiments did not reflect the final values in the sample. Typical total sample volumes used for crystallization experiments did not exceed 500 μl.

Crystals were grown by streak seeding and vapor diffusion in sitting-drop trays by mixing 3 μl of sample with 3 μl of reservoir solution [100 mM MES (pH 6.3), 75 mM KCl, 6.0 to 6.5% (w/v) polyethylene glycol (PEG) molecular weight 20,000 (20K)]. Crystals of plate morpholoogy grew to full size (~200 μm by 100 μm by 50 μm) over a period of 3 weeks and were cryoprotected in a stepwise fashion by sequentially increasing the concentrations of PEG 20K and PEG 400 in the crystallization buffer to 6.8 and 30%, respectively, while maintaining the concentration of other components. Crystals were plunged into liquid nitrogen and stored until data collection.

Two independently complete sets of data were collected from single crystals on beam line ID 14-4 at the European Synchrotron Radiation Facility (ESRF) and on beam line IO4 at the Diamond Light Source, Harwell, United Kingdom, respectively. Data were integrated, merged, and scaled using XDS (44) and found to be consistent with space group P21 with unit cell dimensions a = 201.58 Å, b = 241.65 Å, c = 305.80 Å and β = 99.48°.

Molecular replacement was performed with MOLREP (45) in two stages, first with the 50S subunit of the 70S T. thermophilus structure (31) as a search model, followed by inclusion of the 30S. The solution showed a single ribosome in the asymmetric unit in the fully rotated conformation. Refinement was carried out in alternating cycles of automated refinements with either PHENIX (46) or REFMAC5 (47), with manual refinement and model building in COOT (48). A summary of refinement and data collection statistics is displayed in Table 1. All figures were generated with PyMOL (49) or Jalview for sequence alignments (50).

### References and Notes


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**Supplementary Materials**
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10.1126/science.1235490