Structure
Review

The Mechanics of Translocation: A Molecular "Spring-and-Ratchet" System

Stephen J. Moran,[1] John F. Flanagan, IV,[1] Olivier Namy,[2] David I. Stuart,[1] Ian Brierley,[3,*] and Robert J.C. Gilbert[1,*]
[1]Division of Structural Biology, Henry Wellcome Building for Genomic Medicine, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, United Kingdom
[2]Institut de Genetique et Microbiologie, Universite Paris-Sud, Batiment 400, 91405 Orsay Cedex, France
[3]Division of Virology, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, United Kingdom
*Correspondence: ib103@mole.bio.cam.ac.uk (I.B.), gilbert@strubi.ox.ac.uk (R.J.C.G.)
DOI 10.1016/j.str.2008.04.001

The translation of genetic information into proteins is a fundamental process of life. Stepwise addition of amino acids to the growing polypeptide chain requires the coordinated movement of mRNA and tRNAs through the ribosome, a process known as translocation. Here, we review current understanding of the kinetics and mechanics of translocation, with particular emphasis on the structure of a functional mammalian ribosome stalled during translocation by an mRNA pseudoknot. In the context of a pseudoknot-stalled complex, the translocase EF-2 is seen to compress a hybrid-state tRNA into a strained conformation. We propose that this strain energy helps overcome the kinetic barrier to translocation and drives tRNA into the P-site, with EF-2 biasing this relaxation in one direction. The tRNA can thus be considered a molecular spring and EF-2 a Brownian ratchet in a "spring-and-ratchet" system within the translocation process.

Introduction

Translocation is the final stage in the elongation cycle and is responsible for moving two tRNAs and mRNA together through the ribosome complex while maintaining the reading frame. During translocation, tRNAs traverse the A, P, and exit (E) sites in a process catalyzed by the translocase EF-2 (EF-G in prokaryotes) (Noller et al., 2002). The starting point for translocation can be defined as the state following the peptidyl-transferase reaction. At this point, the growing polypeptide chain has been transferred to the A-site tRNA from the P-site tRNA, resulting in a change in the structural dynamics of the ribosome. The tRNAs in the A and P sites now fluctuate spontaneously between their "classical" and "hybrid" states, since the acceptor ends of the A- and P-site tRNAs have a greater affinity for the respective P and E sites (Moazed and Noller, 1989; Blanchard et al., 2004; Dorner et al., 2006), while the anticodon ends remain in the A and P sites, generating A/P and P/E configurations. The EF-2 binds to the pretranslocation ribosome while itself in a GTP-bound form, inducing a rotation of the small subunit (SSU) of the ribosome relative to the large subunit (LSU) and activating GTP hydrolysis (Rodnina et al., 1997; Wilden et al., 2006; Pan et al., 2007; Taylor et al., 2007). This hydrolysis leads to a conformational change of the translocase, which in turn stabilizes a conformational change in the ribosome that stimulates translocation, a process known as "unlocking" (Savelsbergh et al., 2003; Taylor et al., 2007). The conformational change involves a disruption of the connection between the mRNA-tRNA moiety and certain nucleotides in the decoding center, plus a rotation of the small subunit head (Taylor et al., 2007). Kinetic analyses have revealed that these conformational changes are followed by a spontaneous movement of tRNA from the A/P configuration into the P site by diffusion, coupled with an independent release of inorganic phosphate from EF-2 (Wilden et al., 2006; Savelsbergh et al., 2003). At the same time, the P/E tRNA enters the E site prior to its exit from the ribosome and the start of a new cycle with the arrival of the next EF-1 (EF-Tu)-GTP-tRNA complex. Within this scheme, the precise role of EF-2 remains unresolved, as does the physical basis of unidirectional translocation—from which derives processive ribosomal activity.

Here, we review the implications of the structure of a functional mammalian ribosome caught in the process of translocation upon stalling at an mRNA pseudoknot derived from a viral frameshifting signal (Namy et al., 2006). When this structure was originally presented, resolving for the first time simultaneous occupancy of the translocase with a connected tRNA, its importance for ribosomal frameshifting was considered paramount (Namy et al., 2006). The additional significance of this stalled complex for understanding translocation has however been sharpened by recent kinetic (Wilden et al., 2006; Pan et al., 2007), cryo-EM (Taylor et al., 2007), and X-ray crystallographic (Selmer et al., 2006) studies. In reviewing all these results, we suggest a mechanism for tRNA displacement during ordinary translocation and provide a physical interpretation for some of the key kinetic steps of the process.

Trapping a Translocating tRNA with the Translocase

Many attempts have been made to trap the ribosome in a translocating state. This has resulted in several structures of ribosomes in "pretranslocation" ("PRE," peptidyl-tRNA in the A site) and "posttranslocation" ("POST," peptidyl-tRNA in the P site) states. Cryo-EM work has focused on the *E. coli* system, using GTP analogs (Agrawal et al., 1999; Frank and Agrawal, 2000) or thiostrepton (Stark et al., 2000) to trap the PRE state and fusidic acid to trap the POST state (Agrawal et al., 1999; Frank and Agrawal, 2000; Valle et al., 2003). In the yeast *S. cerevisiae* system, sordarin has been used to trap EF-2 (Spahn et al., 2004). Unfortunately, simultaneous occupancy of EF-G or EF-2 with tRNA during translocation has not been observed by these means, and the nature of the displacement that is catalyzed by the translocase remains unclear.

There are several reasons for this. Attempts to trap functional ribosomes have met with problems arising from the limitations of the stalling strategies employed, which makes it unsurprising that an interaction between the A-site tRNA and the translocase has not been observed. Translocation is a property inherent to the ribosome, which is capable of translating even in the absence of the translocase, albeit at a rate reduced by several orders of magnitude (Gavrilova et al., 1976; Belitsina et al., 1981). Also, single rounds of translocation can occur even with nonhydrolyzable analogs of GTP (e.g., Inoue-Yokosawa et al., 1974; Sharma et al., 2004; for review, see Wintermeyer et al., 2001), and, consequently, using such analogs to inhibit the translocase cannot be assumed to prevent translocation from occurring. This may explain why attempts to reconstruct functional ribosomes trapped during translocation have revealed fully translocated P-site and E-site tRNAs (Valle et al., 2003). In addition, previously published pretranslocation complexes trapped using GMPPNP have subsequently been revealed as composite reconstructions of heterogeneous states, some with low occupancy and with either tRNA or EF-G present, but not both (Agrawal et al., 1999; Frank and Agrawal, 2000; Penczek et al., 2006). This appears to be because EF-G-GMPPNP cannot form a stable complex with the ribosome if the A site is occupied. Furthermore, although the widely used fusidic acid is assumed to trap EF-G in an EF-G.GDP state after only one round of hydrolysis (and therefore to trap a homogeneous population of POST state ribosomes), it has been recently shown that fusidic acid allows multiple rounds of hydrolysis before inhibition takes effect (Seo et al., 2006). It seems that targeting the translocase alone in a functioning ribosome fails to halt translocation cleanly; a method of stalling the whole ribosome is required such that the intrinsic movement of tRNA and mRNA can be retarded. In a recent study of our own, we discovered a way of naturally pausing the translocating, functional ribosome (Namy et al., 2006) with maintenance of the interaction between the translocase and tRNA engaged with an authentic mRNA. This was the first structure of a translocational intermediate for a mammalian ribosome and has led us to review the existing literature in the field for both prokaryotic and eukaryotic systems.

Structural Details of a Functional Mammalian Ribosome Engaged in Translocation

Studying the structure of a frameshifting ribosome made use of an in vitro translation reaction programmed with an mRNA harboring a coronavirus RNA pseudoknot structure (Namy et al., 2006; Brierley et al., 2007). The pseudoknot promotes −1 ribosomal frameshifting at an adjacent, upstream slippery sequence, and ribosome run-on experiments have demonstrated that this is a fully functional translational system, with the stalled ribosomes resuming translation after encounter with the pseudoknot (Somogyi et al., 1993). Three-dimensional reconstruction revealed a complex ($80S_{PK}$) maintaining simultaneous occupancy of tRNA and EF-2 and the physical contact that EF-2 makes with the translocating tRNA, as well as accompanying ribosomal rearrangements (Figure 1; Namy et al., 2006). One bound tRNA was observed (i.e., there was no E site tRNA), as in other cycloheximide-treated ribosomes (Halic et al., 2004). The ribosomal subunits were in a conformation similar to that observed in previous reconstructions of ribosomes that are interacting with EF-2/EF-G, with a ratchet-like subunit rearrangement (RSR) and a rotation of the small subunit head compared to unbound ribosomes (Frank and Agrawal, 2000; Spahn et al., 2004). As in previous studies, for tRNA-EF-2 bound ribosomes, the RSR is anticlockwise when viewed from the solvent face of the small subunit (Frank and Agrawal, 2000; Spahn et al., 2004); the magnitude of the observed rotation of the head is 5°, with an associated shift of 4 Å, compared to unoccupied ribosomes ($80S_{Apo}$; Figure 1B) (the overall subunit rotation is 2°). This structure therefore supports the idea of a ratcheting subunit rearrangement during translocation. The simultaneous presence of EF-2, with domain IV inserted into the intersubunit space, and displacement of the A-site tRNA indicates that the ribosome is stalled at a point beyond the PRE state. However, although the tRNA is displaced toward the P site, it has clearly not fully entered the P site, and hence the movement has not yet reached the POST state observed in previous reconstructions (Valle et al., 2003; Figure 1E).

When GTP hydrolysis occurs, a rotation of domain III in the translocase extends domain IV such that it is inserted into the A site (Connell et al., 2007). As shown in Figures 1A and 1C, in the frameshifting complex, domain IV of EF-2 overlaps the site occupied by the anticodon arm of A-site tRNAs, meaning they must be displaced by it (Frank and Agrawal, 2000; Agrawal et al., 1998). Related to this, it has long been believed that the translocase is a structural mimic for tRNA (Nissen et al., 1995). Comparison of the P-site tRNA crystal structure (see below, Figure 3; Korostelev et al., 2006) or A-site tRNA (Figure 1C) with the EF-2-interacting tRNA (Figures 1A and 1C) shows that it is significantly deformed. The bending observed appears to result from the opposing pressures of EF-2 insertion and pseudoknot braking of the ribosome's forward movement and is in line with the rotation of the small subunit head. The elbow of the tRNA is displaced into the face of the 60S subunit, rotating round the acceptor arm, and this is accompanied by a pronounced compression of the tRNA toward the large subunit that causes the D-stem to bend (Figure 1E). As emphasized previously (Namy et al., 2006), the pseudoknot is likely to play an important role in tRNA bending, but inspection of the relative positions of the bent tRNA and the pseudoknot reveals that a force directed toward the mRNA entrance in the small subunit, such as would be applied by the pseudoknot, could not result in the compression of the tRNA observed toward the P site of the large subunit. A more plausible explanation is that this aspect of tRNA deformation is brought about by the action of EF-2 and the ribosomal rearrangements associated with translocation. In support of this, the anticodon end of the bent tRNA is positioned some three-quarters of the way up the face of domain IV of EF-2 (Figures 1A and 1C). Given the original position of the anticodon end in the A site as defined by the positioning of tRNAs in previously determined structures (Valle et al., 2002, 2003; Penczek et al., 2006) and the wedge-like shape of domain IV (Savelsbergh et al., 2000), the tRNA seems to have slid up the face of the leading β sheet of EF-2. As observed in this complex, the tip of domain IV contacts the P-site tRNA part way up the anticodon stem, seemingly preventing any bending or movement back toward the A site; the translocase can thus be said to resemble a molecular "catch."

Given the presence of the pseudoknot, it cannot be proved definitively that this complex represents a true intermediate in

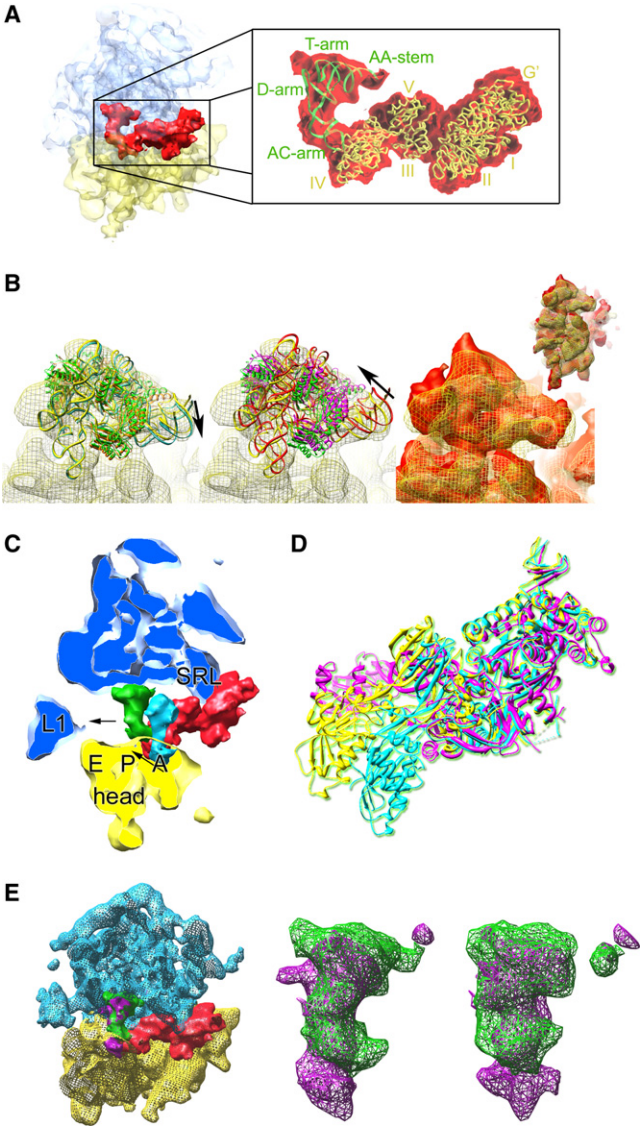

Figure 1. The Interaction of EF-2 and a Translocating tRNA within a Functional Mammalian Ribosome

(A) In the $80S_{PK}$ reconstruction, the translocase EF-2 (yellow ribbon) contacts the anticodon arm of the tRNA (green ribbon) as it extends into the A site. The domains of EF-2 are labeled G′ and I-V and the aminoacyl stem (AA-stem), T-arm, D-arm, and anticodon-arm (AC-arm) are marked on the tRNA. The ribosome large subunit is shown in blue, the small subunit in yellow, and the EF-2 and tRNA complex density in red. Atomic models are as presented in Namy et al., 2006. Images in this display and throughout the manuscript were generated using BOBSCRIPT (Esnouf, 1999) and Raster3D (Merritt and Murphy, 1994) or CHIMERA (Pettersen et al., 2004).

(B) The first two views of the 40S head from the solvent face show a cryo-EM reconstruction of an unbound ribosome state ($80S_{Apo}$; Namy et al., 2006) as a mesh with fitted coordinates for a yeast homology model (Spahn et al., 2004), the 18S rRNA colored yellow and the small subunit proteins colored green. The left-hand image additionally includes coordinates (cyan rRNA and orange proteins) fitted to the small subunit in a control reconstruction stalled using a stem-loop and possessing an orthodox tRNA in its P site ($80S_{SL}$; Namy et al., 2006), with a small arrow indicating a slight clockwise rotation between the two structures in the view shown. The central image additionally includes coordinates fitted to the pseudoknot-stalled structure with a bent tRNA and EF-2 (red rRNA and magenta proteins), with a larger arrow indicating a more substantial anticlockwise movement of the head in this view. The right-hand image is a superposition of the subunit density for the control ribosome ($80S_{Apo}$; yellow mesh) and the EF-2/bent tRNA complex (red surface), showing the ratchet-like subunit rearrangement which the atomic fits in the other images have allowed us to quantify. An inset thumbnail shows the whole subunit density for this third view. Fitting with atomic models for the ribosomal subunits was accomplished by first fitting atomic models for the 60S to the large subunit density, then fitting the corresponding 40S atomic model to the small subunit density, thus normalizing the 40S fit with respect to the 60S and allowing the calculation of relative angles of rotation. Fitting of the head atomic model alone was then undertaken, giving a figure for the rotation of the head with respect to the body, represented by the fit of the small subunit as a whole. All fits were computed using CCP4 software (CCP4, 1994).

(C) The large and small subunits of the $80S_{PK}$ have been cut away to reveal the environment surrounding the tRNA during translocation. The position of the A-site tRNA is superimposed in semitransparent blue as a reference, for which we used the P-site tRNA from a control reconstruction stalled with a nonframe-shifting stem-loop ($80S_{SL}$; Namy et al., 2006). The displaced tRNA is shown in green. The sarcin-ricin loop (SRL) and L1 stalk of the large subunit and the head of the small subunit are labeled, along with the positions of the A, P, and E sites. Arrows indicate tRNA movements.

(D) The structure of EF-2 bound to the ribosome without additional artificial ligands (yellow ribbon; Namy et al., 2006). Typical structures found for bound (cyan ribbon) and unbound EF-2 (magenta ribbon) that have been determined previously (Jorgensen et al., 2003, Spahn et al., 2004) are overlaid for reference, superimposed using domains G′, I, and II.

(E) Views of the stalled complex with the displaced tRNA colored green and EF-2 colored red superimposed with the position of a P-site tRNA (magenta) to highlight the relative positions of the two tRNAs. On the left, in the context of the whole assembly, in the middle, a close-up of the tRNAs alone in the same orientation, and an orthogonal view (looking toward the E site) on the right.

uninhibited translocation. However, it is clear that the tRNA adopts a position intermediate between the A and P sites, while in contact with the translocase, and it is known that such stalled ribosomal complexes are capable of completing translation (Somogyi et al., 1993). It can also be seen directly from the structure that the forces bending the tRNA arise from an eEF2/ribosome-induced compression and not a pull from the pseudoknot. A model of translocation that includes such a bending of the tRNA by the translocase as part of the process can explain a wealth of structural, thermodynamic, and kinetic data, as discussed below.

Both eukaryotic and prokaryotic translocases have been visualized in many different contexts using both cryo-EM and crystallography. Cryo-EM has been used to observe EF-2 interacting with the ribosome with either GDPNP or sordarin bound (Taylor et al., 2007; Frank and Agrawal, 2000; Spahn et al., 2004) and to observe EF-G with fusidic acid, GMPP(CH_2)P, GDPNP, or GMPPNP bound (Agrawal et al., 1999; Frank and Agrawal, 2000; Valle et al., 2003; Connell et al., 2007). Crystallography has revealed the high-resolution structure of both EF-2 and EF-G in apo forms (Jorgensen et al., 2003; Ævarsson et al., 1994) and of EF-G bound with GDP and GTP (Czworkowski et al., 1994; Laurberg et al., 2000; Hansson et al., 2005). Overlaying all of these structures reveals that the translocase exists in two distinct conformations: an open state found when the molecule is unbound, and a ribosome-bound state where domains III, IV, and V extend into the intersubunit space. The conformation of EF-2 observed in the frameshifting ribosome complex differs significantly from both these conformations in the arrangement of the leading domains and represents the first view of EF-2 bound to the ribosome without an artificial ligand (Namy et al., 2006; Figure 1D). While EF-2 assumes a position on the ribosome very similar to the previous bound conformations, domain IV is extended further and domains III and V are rotated toward the large subunit, accommodating a large displacement of the

sarcin-ricin loop and rpL12 (P1/P2 stalk region; the prokaryotic equivalent is L11 in the L7/L12 stalk base) and producing a conformation intermediate between the previously observed bound and unbound forms. This novel conformation represents the translocase engaged in translocation, a state induced by close interaction with the ribosome and the translocating tRNA.

Normal Mode Analysis of tRNA and EF-2

Rather simple models are now available for estimating the normal modes of molecular assemblies which provide useful information about large-scale motions from a very few low-frequency modes. Ribosome motions have been modeled this way (Tama et al., 2003) and have also been used to fit the crystal structure of EF-2 into the bound density identified in cryo-EM maps (Tama et al., 2004). Normal mode analysis of EF-2 motions reveals a swinging movement of domain IV, pivoting around domains III and V (Figure 2A). Such a motion connects the unbound and bound structural conformations of EF-2 and resembles a lever swinging in and out. Meanwhile, a similar treatment of tRNA reveals a spring-like bending, along the lines predicted previously and as observed in the frameshifting complex (Figure 2A; Namy et al., 2006; Robertus et al., 1974). If the motions of these two molecules are combined, they closely approximate the relative movements and displacement seen in the stalled reconstruction (Figure 2B). Starting from the unbound state of the translocase, normal mode movement of domain IV traces a path to the extended structure seen during frameshifting through the usual position of the A-site tRNA. In order to accommodate this motion, the A-site tRNA adopts a bent and displaced position, following the normal mode motion to end at the position observed (Figure 1A). It appears that the ribosome is constructed to harness the flexibility of the factors that bind to it to orchestrate efficient translocation.

Compression of the Translocating tRNA: A Molecular Spring

An ability to bend was originally proposed for tRNA by Klug and coworkers based solely on the architecture of the molecule (Robertus et al., 1974). They observed that the D-stem and anticodon stem could form a hinge around unpaired base 26 and the opposing bases 44 and 45. A role for this has already been observed during translation (Cochella and Green, 2005)—the tRNA becomes strained as it is delivered into the A site by EF-Tu (EF-1) (Frank et al., 2005). We suggest that a similar strain is imposed as tRNA is moved into the P site by the translocase (Figure 1C). This is in agreement with a recent crystal structure of the ribosome by Noller and coworkers in which significant distortion of the P- and E-site tRNAs was observed (Korostelev et al., 2006), with the P-site tRNA in particular possessing an $\sim 10^{\circ}$ kink toward the large subunit and a rotation toward the A site. Partly on the basis of this deformation, Noller and colleagues present a hypothesis whereby the energy to drive tRNA movement derives in part from the initial strained binding. This deformation is similar to that observed in the frameshifting complex but more modest, leading us to reconsider the significance of the spring-like nature of tRNA. We suggest that the action of EF-2 along with associated movement of the small subunit head builds up strain energy in the A-site tRNA. Relaxation of this strain would then drive spontaneous translocation into the P site. Such a mechanism can explain the action of EF-2 on the tRNA, and the path taken from the A site to P site can be envisioned (Figures 3A–3D). Independent evidence for such a role for the tRNA comes from the observation that mutation in the pivot region of the tRNA reduces translocation rates significantly (Pan et al., 2006).

Evidence has accumulated that tRNA hybrid states lie on the translocation pathway, with the translocase moving an A/P state tRNA to a P/P state. Single-molecule studies have shown that after peptidyl transfer, the tRNAs switch between classical and hybrid states spontaneously (Blanchard et al., 2004; Kim et al., 2007), while EF-G stabilizes the hybrid state (Spiegel et al., 2007) to improve the efficiency of translocation (Dorner et al., 2006). Kinetic studies show the energy of translocation catalyzed by EF-G to decrease by 12.5 $kJmol^{-1}$ when tRNA is in the A/P state (Semenkov et al., 2000): the hybrid state tRNAs can thus be considered "poised for movement." If EF-2 enters the A site while the tRNA is in the A/P position, it would displace the tRNA into the configuration observed in pseudoknot-stalled frameshifting ribosomes, where the acceptor arm of the tRNA has entered the P site while the anticodon end remains in contact with EF-2, toward the A site. Such a PRE state that stores potential energy in the tRNA to be translocated would explain the contribution of the PRE state to decreasing the free energy change of the reaction.

The crystal structure of the ribosome in complex with tRNA and mRNA revealed a 45° kink in the mRNA between the A and P sites (Selmer et al., 2006), which delineates the border between the two sites and has been proposed to be important for defining the reading frame and preventing slippage of the mRNA. The tRNA-mRNA complex must move over this kink to progress from the A site to the P site. We suggest that the wedge-like action of EF-2 brings the mRNA codon in the A site into the plane of the P site, simultaneously pulling the mRNA through the entrance tunnel of the ribosome. By compressing the tRNA over this natural catch between the two sites, EF-2 would catalyze the movement to the P site. At the same time the mRNA, bound to the tRNA, would then move freely toward the P site, with EF-2 and the kink acting together to delineate the reading frame. The structure of the tRNA would therefore help to maintain the reading frame, paralleling its role in aiding fidelity during decoding and accommodation (Ogle and Ramakrishnan, 2005; Frank et al., 2005). Together, these actions would retain tension during the mRNA displacement when the rest of the complex is most loose, with the kink between the codon sites presenting part of the kinetic barrier to translocation.

As discussed earlier, the pseudoknot can lead to ribosomal frameshifting in the context of a slippery sequence in the mRNA. We suggest that the pseudoknot, through resistance to the ribosomal helicase, restricts mRNA movement during translocation such that the action of EF-2 stores up sufficient spring energy in the tRNA (tRNAs, being composed of short RNA helices, are relatively stiff) to break the link between codon and anticodon. The tRNA would then relax and repair in an alternative reading frame (Namy et al., 2006). This model explains the sensitivity of frameshifting to the mechanical strength of the stimulatory RNA (Hansen et al., 2007) and to the stability of base-pairing between tRNA and mRNA (Jacks et al., 1988).

In the 2.8 Å 70S crystal structure (Selmer et al., 2006), in addition to the 45° mRNA kink, the C-terminal tail of small ribosomal

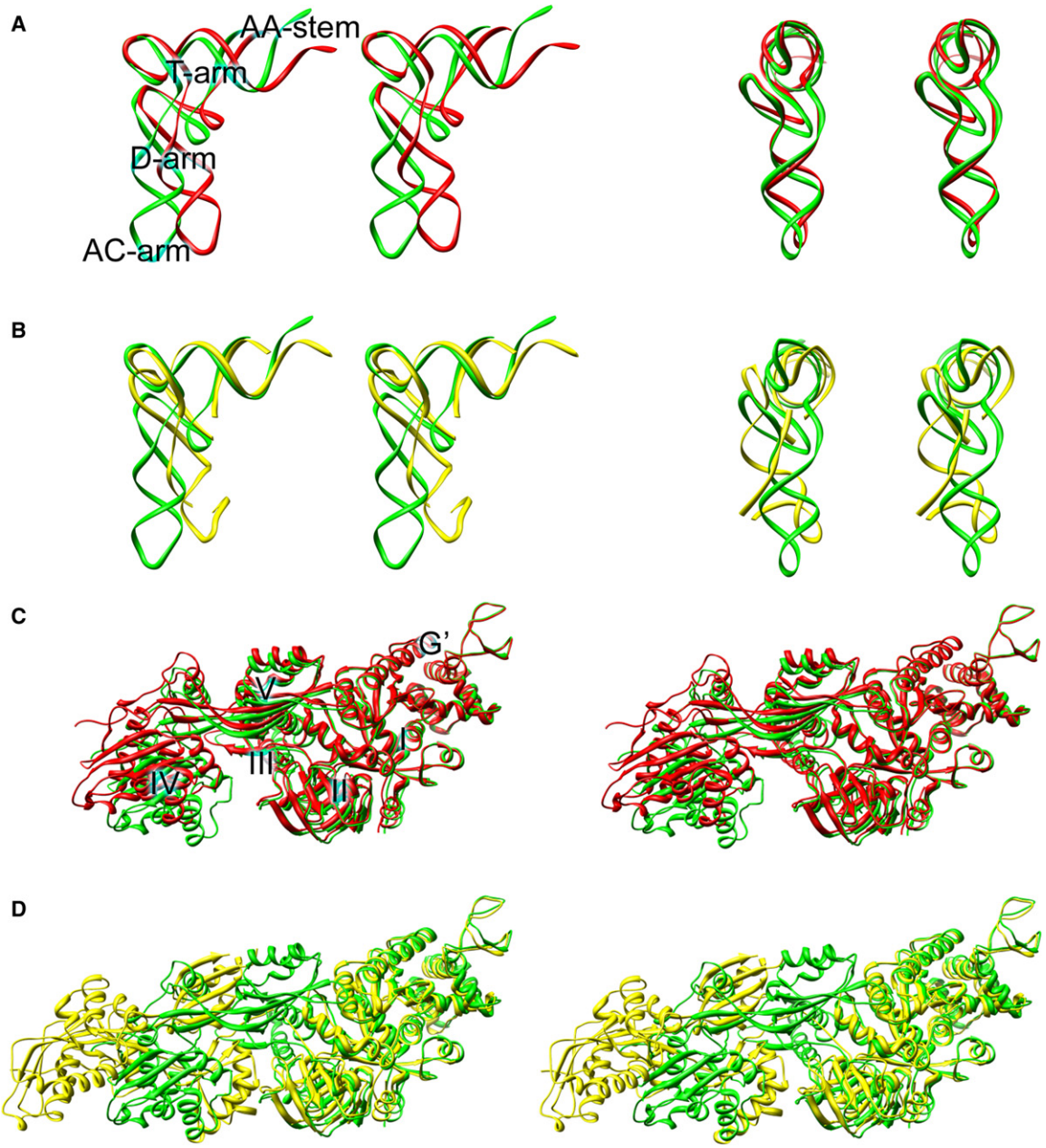

Figure 2. Normal Mode Analysis of tRNA and EF-2

(A) The natural motions of the crystal structure of phenylalanine-tRNA (PDB code: 1EHZ; Shi and Moore, 2000) were determined using normal mode analysis. Structures were submitted to the ElNémo server (Suhre and Sanejouand, 2004), which computes the 100 lowest frequency modes. As it has been shown that the majority of movement can be usually modeled by at most two low-frequency normal modes, only the lowest nontrivial normal mode is included here to demonstrate the dominant natural motion of the molecules (Krebs et al., 2002) (green and red structures show the limits of the displacement, with green unaltered).

(B) The movements observed in the pseudoknot-stalled ribosome structure (yellow model; Namy et al., 2006) relative to the unstrained tRNA structure (green model).

(C) As (A) for apo EF-2 (PDB code: 1NOV; Jorgensen et al., 2003).

(D) As (B) for apo EF-2.

subunit protein S13 (eukaryotic equivalent, S18) extends between the A- and P-site tRNAs (Figure 3E), forming a gate between the sites that must be overcome to achieve translocation. In support of this, ribosomes lacking S13 are able to translate in the absence of EF-G (Cukras et al., 2003), perhaps echoing translocation in ancestral RNA-only ribosomes. S13 is also important for maintaining translational fidelity, and its extended C terminus couples the tRNA binding site to movement of the small subunit head during translation (Cukras and Green, 2005). Furthermore, deletion of either the whole C-terminal tail of S13 or its last five residues reduced growth of engineered *E. coli* strains by half and to a modest extent, respectively, with a particular

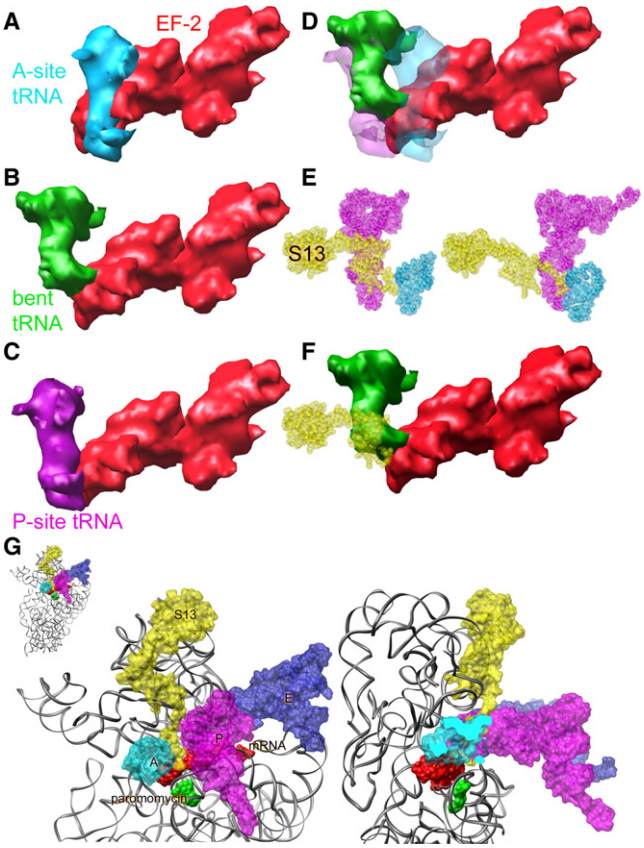

Figure 3. The Displaced tRNA Adopts a Position Intermediate between the A-Site and P-Site tRNAs

(A) The relative positions of an A-site tRNA (blue) and the engaged EF-2 (red). In each case, the density used is from the pseudoknot-stalled ribosome for bent tRNA or from the $80S_{SL}$ reconstruction with an unbent P-site tRNA (Namy et al., 2006).

(B) As (A) for the displaced tRNA observed in our stalled ribosome (green) and EF-2.

(C) As (A) for a P-site tRNA (purple) and the engaged EF-2.

(D) The sequence of tRNA positions from A site to P site superimposed, coloring as in (A)–(C).

(E) Crystal structure of P-site tRNA (purple) and A-site anticodon arm (blue) along with small subunit protein S13 (yellow) (Selmer et al., 2006). On the left, in the same position and orientation as in (D) for our cryo-EM data; on the right, rotated 90° about the D-arm of the tRNA. S13 directly interposes between the two tRNAs.

(F) The arrangement of EF-2 and bent tRNA as observed by (Namy et al., 2006) with respect to S13. Bending of the tRNA would serve to lift it over the gating S13 C terminus.

(G) Two views of the small subunit from *Thermus thermopilus* complexed with A- (cyan), P- (magenta), and E-site (blue) tRNAs. The rRNA is colored gray and shown as a ribbon, while the mRNA is colored red and, like the tRNAs, rendered with a molecular surface. Also shown as a molecular surface is paromomycin, with which the complex was stabilized (green). This antibiotic assists A-site tRNA accommodation by converting bases A1492 and A1493 to a cognate-recognition state, flipped out from helix 44. As shown in this structure (PDB code: 2J00; Selmer et al., 2006), paromomycin binds within helix 44, beneath the mRNA. It enjoys no direct contact with protein S13; hence, while paromomycin inhibits translocation, it does so in a way that does not relate to direct effects on the S13 gate between the A- and P-site tRNAs. The right-hand view is orthogonal to the left-hand one and has been sectioned through the A-site tRNA to assist in visualization, with sectioned surfaces colored cyan. The thumbnail shows the left-hand view in the context of the whole subunit.

effect on the affinity of tRNAs for the P site (Hoang et al., 2004). The PK-frameshifting complex suggests that EF-2 assists in overcoming the S13 (i.e., S18 in eukaryotes) barrier by the spring-like displacement of tRNA, moving the anticodon end neatly over it and into the P site (Figure 3F). In this state, the tRNA appears poised to slide down the β sheet of EF-2 domain IV but remains on the A site side of S13(S18) and, thus, in contact with EF-2. If EF-2 were to leave without successful completion of translocation, the bent tRNA would presumably be drawn back into the A site. While it remains undetermined how the tRNA clears the S13(S18) gate (which could involve RSR-type changes or a further EF-2 conformational change), the role of EF-2-induced anticodon arm bending in setting up the preconditions for successful translocation is clear. Furthermore, the N-terminal globular domain of S13 interacts with protein L5 (to form bridge B1b), and the interaction changes in the different conformational states of the ribosome associated with the RSR of translocation (Valle et al., 2003). Hence, while the C terminus of S13(S18) provides a barrier delimiting the A and P sites, the N terminus is involved in the RSR process. The C terminus of S13 adopts its extended form in the context of an A-site tRNA (Selmer et al., 2006). In another crystal structure containing only P- and E-site tRNAs and no paromomycin (Korostelev et al., 2006), it is instead retracted by being bent back on itself, indicating that this gate closes after A-site tRNA accommodation, precisely in order to then delineate the A and P sites. Paromomycin was included in the three-tRNA complex crystallized by Ramakrishnan and colleagues because it increases the affinity of tRNA for the A site and inhibits translocation (Selmer et al., 2006). As shown in Figure 3G, this inhibition does not derive from effects of paromomycin on the C terminus of S13, since they are not in contact; this indicates that the C terminus of S13 is indeed deployed between the A and P sites on tRNA binding and not as a result of effects of paromomycin. The gating function of S13 may also be important for keeping P-site tRNAs bound (that is, for keeping P-site tRNA in as well as a currently A-site tRNA out), given the reduced affinity of tRNAs for S13 C terminus deletion mutants (Hoang et al., 2004).

The Mechanical Basis of Translocation

Based on kinetic and structural data reviewed above, we suggest a mechanical model for translocation in which tRNA behaves as a molecular spring and EF-2 as a Brownian ratchet (Figure 4). Extensive pre-steady-state analysis of translocation has revealed the kinetic steps of the reaction in some detail (Rodnina et al., 1997; Wilden et al., 2006; Pan et al., 2007; Savelsbergh et al., 2003). First, EF-2.GTP (EF-G.GTP) binds to give the PRE state (k_1) leading to the ribosome inducing a structural change in EF-2 that initiates GTP hydrolysis (k_2) (a merely GDP-bound state is not capable of driving translocation; Pan et al., 2007). The free energy of hydrolysis is not dissipated but brings about a further conformational change in EF-2, which drives or stabilizes conformational changes in the ribosomal subunits (k_3). Here, EF-2 is thought to have an active chemomechanical function, transducing the energy of GTP hydrolysis into work. This work can be identified with a movement of domain IV into the A site, which disrupts the decoding center interaction with the codon-anticodon helix (Taylor et al., 2007). It is EF-2/EF-G in the GDP-P_i-bound state that drives the reaction by

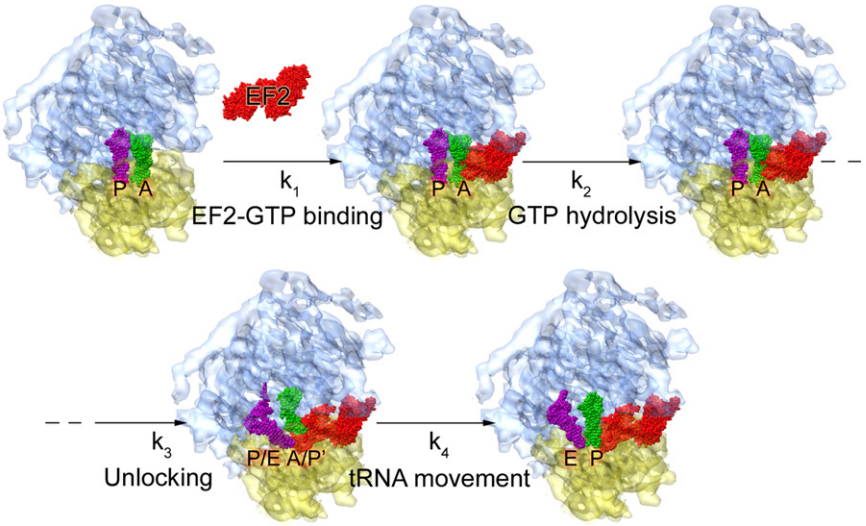

Figure 4. A Physical Model for Some of the Key Kinetic Steps in Translocation: A "Spring-and-Ratchet" System

With peptidyl tRNA in the A-site, EF-2.GTP binds to the pretranslocation ribosome (k_1). Binding induces conformational changes between the translocase and the ribosome that activates GTP hydrolysis (k_2). An unlocking event is then triggered that sees a rotation of the small subunit head and release of the codon-anticodon helix from the decoding center (k_3). These movements and insertion of EF-2 into the A-site induce a spring-like deformation of the tRNA. As it relaxes, the tRNA is passively guided into the P site by EF-2 acting as a Brownian ratchet (k_4). Different positions for the tRNAs are labeled A, P, E, A/P′, and P/E.

stabilizing conformational states of the ribosome that facilitate tRNA movement (Wilden et al., 2006). The next significant kinetic step has been identified as an intrinsically rapid and spontaneous movement of tRNA that takes place by diffusion (k_4), with the independent release of phosphate leading to EF-2 dissociation and completion of translocation to the POST state. The details of how this occurs are not known.

The extended conformation of EF-2 in the frameshifting pseudoknot-stalled complex (Namy et al., 2006), stretched into the A site, implies that GTP hydrolysis has already occurred. However, since the tRNA has not fully entered the P site, translocation has not completed, and this complex lies on the translocation pathway. We suggest that a snapshot of the stable 80S/EF-2 (70S/EF-G) interaction just after GTP hydrolysis has been captured, where EF-2.GDP.Pi stabilizes a state of the ribosome that facilitates translocation (k_3, above). In the absence of other fixing agents (such as GTP analogs or sordarin) and given the intimate physical contact between the ribosome and EF-2, the ribosome appears to be holding EF-2 in place, preventing it from leaving until the inorganic phosphate product of GTP hydrolysis is released. The novel EF-2 conformation observed is likely to represent an activated form, with the domain arrangement driven by the ribosome placing the frameshifting complex at a point between the k_3 and k_4 events of the kinetic pathway. The existence of such a kinetic intermediate (termed the INT complex) has come from a recent kinetic study of tRNA movement during translocation (Pan et al., 2007). The INT complex forms after GTP hydrolysis but before completion of translocation and phosphate release. In the INT complex, there is clear evidence of a movement of the 3′ terminus of the peptidyl tRNA to a hybrid state denoted A/P′ to distinguish it from the original A/P hybrid state. The position of the bent tRNA in the PK-frameshifting complex would agree with that inferred for the A/P′ intermediate.

Unlocking and tRNA movement are kinetically separate events. This can be explained if one considers the strain induced in the tRNA in the stalled complex where it is compressed within the intersubunit space. We suggest that the spontaneous movement of the tRNA corresponds to a release of its strain energy, with EF-2 and S13(S18) directing the movement into the P site. EF-2 thereby takes the role of a Brownian ratchet, as suggested previously by others (Savelsbergh et al., 2003), demonstrating how an energy-driven translocase activity can also play a passive role, with the relaxing tRNA being helped into the P site as it slides down the β sheet face of EF-2. A recent study of translocation by single ribosomes that measured the magnitude of forward strokes of translating complexes along mRNA (Wen et al., 2008) is entirely in line with our argument. There, the rate-determining step of forward ribosomal movement did not involve translocation itself, which was rapid, but the establishment of preconditions necessary for translocation. In the context of this review, we would argue that the physical basis of the rate-determining step thus detected is the establishment of an intermediate complex, such as we observe, in which a bent tRNA can surmount the physical barrier represented by S13(S18). Interestingly, the distributions of lengths for the rapid actual translocational event following the rate-determining establishment of a transition (i.e., hybrid) state could best be fit with three rate constants, suggesting the existence of a series of specific hybrid states for tRNAs in their trajectory from A to P sites, as inferred by us from the kinetic and structural data reviewed here.

Conclusion

We conclude that tRNA plays a dynamic role in the translocation process. In combination with EF-2 and a pseudoknot in the mRNA jamming the ribosomal helicase, it has been shown to assume a bent conformation in moving from the A site to the P site. The insertion of EF-2-GTP by GTP hydrolysis into the A site exerts a force on the tRNA such that it follows a trajectory set by the A/P hybrid state with its elbow pressed into the large subunit and its anticodon end sliding up the face of EF-2 domain IV. The energy released by GTP hydrolysis is transduced to the tRNA, which acts as a molecular spring. Subsequent release of this energy drives movement of the tRNA into the P site by relaxation, guided by the face of EF-2 domain IV. EF-2 thereby acts passively to bias the directionality of the tRNA movement, preventing slippage back into the A site.

Thus, translocation uses chemical energy to store potential energy, which is then dissipated against the framework of the ribosome, acting to orient the players so that their motions are steered along their normal vibrational modes and leading to

productive movement of the mRNA-tRNA complex. This simple spring-and-ratchet model provides a useful interpretative mechanism for kinetic and other observations of the progress of translocation.

ACKNOWLEDGMENTS

This work was supported by grants from the Biotechnology and Biological Sciences Research Council UK and the Medical Research Council, UK. S.J.M. held a Wellcome Trust Structural Biology Studentship, O.N. is supported by a grant from ANR (ANR-06-BLAN-0391-01), R.J.C.G. is a Royal Society University Research Fellow and D.I.S. is an MRC (UK) Research Professor.

REFERENCES

Ævarsson, A., Brazhnikov, E., Garber, M., Zheltonosova, J., Chirgadze, Yu., Al-Karadaghi, S., Svensson, L.A., and Liljas, A. (1994). Three-dimensional structure of the ribosomal translocase: elongation factor G from *Thermus thermophilus*. EMBO J. *13*, 3669–3677.

Agrawal, R.K., Penczek, P., Grassucci, R.A., and Frank, J. (1998). Visualization of elongation factor G on the *Escherichia coli* 70S ribosome: The mechanism of translocation. Proc. Natl. Acad. Sci. USA *95*, 6134–6138.

Agrawal, R.K., Heagle, A.B., Penczek, P., Grassucci, R.A., and Frank, J. (1999). EF-G-dependent GTP hydrolysis induces translocation accompanied by large conformational changes in the 70S ribosome. Nat. Struct. Biol. *6*, 643–647.

Belitsina, N.V., Tnalina, G.Z., and Spirin, A.S. (1981). Template-free ribosomal synthesis of polylysine from lysyl-tRNA. FEBS Lett. *131*, 289–292.

Blanchard, S.C., Kim, H.D., Gonzalez, R.L., Jr., Puglisi, J.D., and Chu, S. (2004). tRNA dynamics on the ribosome during translation. Proc. Natl. Acad. Sci. USA *101*, 12893–12898.

Brierley, I., Pennell, S., and Gilbert, R.J.C. (2007). Viral RNA pseudoknots: versatile motifs in gene expression and replication. Nat. Rev. Microbiol. *5*, 598–610.

CCP4 (1994). The CCP4 suite: Programs for protein crystallography. Acta Crystallogr. D Biol. Crystallogr. *50*, 760–763.

Cochella, L., and Green, R. (2005). An active role for tRNA in decoding beyond codon:anticodon pairing. Science *308*, 1178–1180.

Connell, S.R., Takemoto, C., Wilson, D.N., Wang, H., Murayama, K., Terada, T., Shirouzu, M., Rost, M., Schuler, M., Giesebrecht, J., et al. (2007). Structural basis for interaction of the ribosome with the switch regions of GTP-bound elongation factors. Mol. Cell *25*, 751–764.

Cukras, A.R., and Green, R. (2005). Multiple effects of S13 in modulating the strength of intersubunit interactions in the ribosome during translation. J. Mol. Biol. *349*, 47–59.

Cukras, A.R., Southworth, D.R., Brunelle, J.L., Culver, G.M., and Green, R. (2003). Ribosomal proteins S12 and S13 function as control elements for translocation of the mRNA:tRNA complex. Mol. Cell *12*, 321–328.

Czworkowski, J., Wang, J., Steitz, T.A., and Moore, P.B. (1994). The crystal structure of elongation factor G complexed with GDP, at 2.7 Å resolution. EMBO J. *13*, 3661–3668.

Dorner, S., Brunelle, J.L., Sharma, D., and Green, R. (2006). The hybrid state of tRNA binding is an authentic translation elongation intermediate. Nat. Struct. Mol. Biol. *13*, 234–241.

Esnouf, R.M. (1999). Further additions to MolScript version 1.4, including reading and contouring of electron-density maps. Acta Crystallogr. D Biol. Crystallogr. *55*, 938–940.

Frank, J., and Agrawal, R.K. (2000). A ratchet-like inter-subunit reorganization of the ribosome during translocation. Nature *406*, 318–322.

Frank, J., Sengupta, J., Gao, H., Li, W., Valle, M., Zavialov, A., and Ehrenberg, M. (2005). The role of tRNA as a molecular spring in decoding, accommodation, and peptidyl transfer. FEBS Lett. *579*, 959–962.

Gavrilova, L.P., Kostiashkina, O.E., Koteliansky, V.E., Rutkevitch, N.M., and Spirin, A.S. (1976). Factor-free ("non-enzymic") and factor-dependent systems of translation of polyuridylic acid by *Escherichia coli* ribosomes. J. Mol. Biol. *101*, 537–552.

Halic, M., Becker, T., Pool, M.R., Spahn, C.M.T., Grassucci, R.A., Frank, J., and Beckman, R. (2004). Structure of the signal recognition particle interacting with the elongation-arrested ribosome. Nature *427*, 808–814.

Hansen, T.M., Reihani, S.N., Oddershede, L.B., and Sorensen, M.A. (2007). Correlation between mechanical strength of messenger RNA pseudoknots and ribosomal frameshifting. Proc. Natl. Acad. Sci. USA *104*, 5830–5835.

Hansson, S., Singh, R., Gudkov, A.T., Liljas, A., and Logan, D.T. (2005). Crystal structure of a mutant elongation factor G trapped with a GTP analogue. FEBS Lett. *579*, 4492–4497.

Hoang, L., Fredrick, K., and Noller, N.F. (2004). Creating ribosomes with an all-RNA 30S subunit P site. Proc. Natl. Acad. Sci. USA *101*, 12439–12443.

Inoue-Yokosawa, N., Ishikawa, C., and Kaziro, Y. (1974). The role of guanosine triphosphate in translocation reaction catalyzed by elongation factor G. J. Biol. Chem. *249*, 4321–4323.

Jacks, T., Madhani, H.D., Masiarz, F.R., and Varmus, H.E. (1988). Signals for ribosomal frameshifting in the Rous sarcoma virus *gag-pol* region. Cell *55*, 447–458.

Jorgensen, R., Ortiz, P.A., Carr-Schmid, A., Nissen, P., Kinzy, T.G., and Andersen, G.R. (2003). Two crystal structures demonstrate large conformational changes in the eukaryotic ribosomal translocase. Nat. Struct. Biol. *10*, 379–385.

Kim, H.D., Puglisi, J.D., and Chu, S. (2007). Fluctuations of tRNAs between classical and hybrid states. Biophys. J. *93*, 3575–3582.

Korostelev, A., Trakhanov, S., Laurberg, M., and Noller, H.F. (2006). Crystal structure of a 70S ribosome-tRNA complex reveals functional interactions and rearrangements. Cell *126*, 1065–1077.

Krebs, W.G., Alexandrov, V., Wilson, C.A., Echols, N., Yu, H., and Gerstein, M. (2002). Normal mode analysis of macromolecular motions in a database framework: Developing mode concentration as a useful classifying statistic. Proteins *48*, 682–695.

Laurberg, M., Kristensen, O., Martemyanov, K., Gudkov, A.T., Nagaev, I., Hughes, D., and Liljas, A. (2000). Structure of a mutant EF-G reveals domain III and possibly the fusidic acid binding site. J. Mol. Biol. *303*, 593–603.

Merritt, E.A., and Murphy, M.E. (1994). Raster3D Version 2.0. A program for photorealistic molecular graphics. Acta Crystallogr. D Biol. Crystallogr. *50*, 869–873.

Moazed, D., and Noller, H.F. (1989). Intermediate states in the movement of transfer RNA in the ribosome. Nature *342*, 142–148.

Namy, O., Moran, S.J., Stuart, D.I., Gilbert, R.J.C., and Brierley, I. (2006). A mechanical explanation of RNA pseudoknot function in programmed ribosomal frameshifting. Nature *441*, 244–247.

Nissen, P., Kjeldgaard, M., Thirup, S., Polekhina, G., Reshetnikova, L., Clark, B.F., and Nyborg, J. (1995). Crystal structure of the ternary complex of Phe-tRNAPhe, EF-Tu, and a GTP analog. Science *270*, 1464–1472.

Noller, H.F., Yusupov, M.M., Yusupova, G.Z., Baucom, A., and Cate, J.H.D. (2002). Translocation of tRNA during protein synthesis. FEBS Lett. *514*, 11–16.

Ogle, J.M., and Ramakrishnan, V. (2005). Structural insights into ribosomal fidelity. Annu. Rev. Biochem. *74*, 129–177.

Pan, D., Kirillov, S., Zhang, C.-M., Hou, Y.-M., and Cooperman, B.S. (2006). Rapid ribosomal translocation depends on the conserved 18–55 base pair in P-site transfer RNA. Nat. Struct. Mol. Biol. *13*, 354–359.

Pan, D., Kirillov, S.V., and Cooperman, B.S. (2007). Kinetically competent intermediates in the translocation step of protein synthesis. Mol. Cell *25*, 519–529.

Penczek, P.A., Frank, J., and Spahn, C.M.T. (2006). A method of focused classification, based on the bootstrap 3D variance analysis, and its application to EF-G-dependent translocation. J. Struct. Biol. *154*, 184–194.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. (2004). UCSF Chimera – a visualization system for exploratory research and analysis. J. Comput. Chem. *25*, 1605–1616.

Robertus, J.D., Ladner, J.E., Finch, J.T., Rhodes, D., Brown, R.S., Clark, B.F.C., and Klug, A. (1974). Structure of yeast phenylalanine tRNA at 3Å resolution. Nature *250*, 546–551.

Rodnina, M.V., Savelsbergh, A., Katunin, V.I., and Wintermeyer, W. (1997). Hydrolysis of GTP by elongation factor G drives tRNA movement on the ribosome. Nature *385*, 37–41.

Savelsbergh, A., Matassova, N.R., Rodnina, M.V., and Wintermeyer, W. (2000). Role of domains 4 and 5 in elongation factor G functions on the ribosome. J. Mol. Biol. *300*, 951–961.

Savelsbergh, A., Katunin, V.I., Mohr, D., Peske, F., Rodnina, M.V., and Wintermeyer, W. (2003). An elongation factor G-induced ribosome rearrangement precedes tRNA-mRNA translocation. Mol. Cell *11*, 1517–1523.

Selmer, M., Dunham, C.M., Murphy, F.V., IV, Weixlbaumer, A., Petry, S., Kelley, A.C., Weir, J.R., and Ramakrishnan, V. (2006). Structure of the 70S ribosome complexed with mRNA and tRNA. Science *313*, 1935–1942.

Semenkov, Y.P., Rodnina, M.V., and Wintermeyer, W. (2000). Energetic contribution of tRNA hybrid state formation to translocation catalysis on the ribosome. Nat. Struct. Biol. *7*, 1027–1031.

Seo, H.S., Abedin, S., Kamp, D., Wilson, D.N., Nierhaus, K.H., and Cooperman, B.S. (2006). EF-G-dependent GTPase on the ribosome. Conformational change and fusidic acid inhibition. Biochemistry *45*, 2504–2514.

Sharma, D., Southworth, D.R., and Green, R. (2004). EF-G-independent reactivity of a pre-translocation-state ribosome complex with the aminoacyl tRNA substrate puromycin support an intermediate (hybrid) state of tRNA binding. RNA *10*, 102–113.

Shi, H., and Moore, P.B. (2000). The crystal structure of yeast phenylalanine tRNA at 1.93Å resolution: a classic structure revisited. RNA *6*, 1091–1105.

Somogyi, P., Jenner, A.J., Brierley, I., and Inglis, S.C. (1993). Ribosomal pausing during translation of an RNA pseudoknot. Mol. Cell. Biol. *13*, 6931–6940.

Spahn, C.M.T., Gomez-Lorenzo, M.G., Grassucci, R.A., Jorgensen, R., Andersen, G.R., Beckmann, R., Penczek, P.A., Ballesta, J.P., and Frank, J. (2004). Domain movements of elongation factor eEF-2 and the eukaryotic 80S ribosome facilitate tRNA translocation. EMBO J. *23*, 1008–1019.

Spiegel, P.C., Ermolenko, D.N., and Noller, H.F. (2007). Elongation factor G stabilizes the hybrid-state conformation of the 70S ribosome. RNA *13*, 1473–1482.

Stark, H., Rodnina, M.V., Wieden, H.-J., van Heel, M., and Wintermeyer, W. (2000). Large-scale movement of elongation factor G and extensive conformational change of the ribosome during translocation. Cell *100*, 301–309.

Suhre, K., and Sanejouand, Y.-H. (2004). ElNemo: A normal mode web server for protein movement analysis and the generation of templates for molecular replacement. Nucleic Acids Res. *32*, W610–W614.

Tama, F., Valle, M., Frank, J., and Brooks, C.L., III. (2003). Dynamic reorganization of the functionally active ribosome explored by normal mode analysis and cryo-electron microscopy. Proc. Natl. Acad. Sci. USA *100*, 9319–9323.

Tama, F., Miyashita, O., and Brooks, C.L., III. (2004). Normal mode based flexible fitting of high-resolution structure into low-resolution experimental data from cryo-EM. J. Struct. Biol. *147*, 315–326.

Taylor, D.J., Nilsson, J., Merrill, A.R., Andersen, G.R., Nissen, P., and Frank, J. (2007). Structures of modified eEF-2.80S ribosome complexes reveal the role of GTP hydrolysis in translocation. EMBO J. *26*, 2421–2431.

Valle, M., Sengupta, J., Swami, N.K., Grassucci, R.A., Burkhardt, N., Nierhaus, K.H., Agrawal, R.K., and Frank, J. (2002). Cryo-EM reveals an active role for aminoacyl-tRNA in the accommodation process. EMBO J. *21*, 3557–3567.

Valle, M., Zavialov, A., Sengupta, J., Rawat, U., Ehrenberg, M., and Frank, J. (2003). Locking and unlocking of ribosomal motions. Cell *114*, 123–134.

Wen, J.-D., Lancaster, L., Hodges, C., Zeri, A.-C., Yoshimura, S.H., Noller, H.F., Bustamante, C., and Tinoco, I., Jr. (2008). Following translation by single ribosomes one codon at a time. Nature *452*, 598–603.

Wilden, B., Savelsbergh, A., Rodnina, M.V., and Wintermeyer, W. (2006). Role and timing of GTP binding and hydrolysis during EF-G-dependent tRNA translocation on the ribosome. Proc. Natl. Acad. Sci. USA *103*, 13670–13675.

Wintermeyer, W., Savelsbergh, A., Semenkov, Y.P., Katunin, V.I., and Rodnina, M.V. (2001). Mechanism of elongation factor B function in tRNA translocation on the ribosome. Cold Spring Harb. Symp. Quant. Biol. *66*, 449–458.

