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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. Gilbert1,*

1Division of Structural Biology, Henry Wellcome Building for Genomic Medicine, University of Oxford, Oxford OX3 7BN, United Kingdom
2Institut de Genetique et Microbiologie, Universite Paris-Sud, Batiment 400, 91405 Orsay Cedex, France
3Division of Virology, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QH, United Kingdom
*Correspondence: ib103@mole.bio.cam.ac.uk (I.B.), gilbert@strubi.ox.ac.uk (R.J.C.G.)

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” (Savelberg 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; Savelberg 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 stalled 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 Wintemeyer 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<sub>PK</sub>) 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/EFG, 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<sub>Apo</sub>; 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, Figures 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
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 translocation (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(CH2)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; Lauberberg 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 face, 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).

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

(A) In the 80Sap 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 (80Sap; 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 tRNA 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 (80SSSL; 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 tRNA 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 (80Sap, yellow mesh) and the EF-2/bent tRNA complex (red sur-
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state of the translocase, normal mode movement of domain IV
53x281] the prokaryotic equivalent is L11 in the L7/L12 stalk base) and producing a con-
formation 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 nor-
mal modes of molecular assemblies which provide useful infor-
mation 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 motion of domain IV, pivoting around do-
main 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 approx-
imate 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 accom-
modate 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 orches-
trate 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 (Rob-
ertus et al., 1974). They observed that the D-stem and anticodon
stem could form a hinge around unpaired base 26 and the oppos-
ing 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
(Figure 1C). This is in agreement with a recent crystal structure
of the ribosome by Noller and coworkers in which significant dis-
tortion of the P- and E-site tRNAs was observed (Korostelev et al.,
2006), with the P-site tRNA in particular possessing an ~10° kink
among the large subunit and a rotation toward the A site. Partly
on the basis of this deformation, Noller and colleagues present a hy-
thesis 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 spontane-
ous 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 translo-
cation 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 translo-
cation 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
place the tRNA into the configuration observed in pseudo-
knot-stalled frameshifting ribosomes, where the acceptor arm
of the tRNA has entered the P site while the anticodon end re-
 mains 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 be-
tween 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 cata-
lyze 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 de-
coding 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 trans-
location 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
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 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 (Suhr 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; Narny 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.
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$_{340}$ 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 thermophilus 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 $	heta$-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; Savelbergh 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$_{	ext{i}}$-bound state that drives the reaction by
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 (k₄), 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 (k₃, 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 k₃ and k₄ 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 dissipaged 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.

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REFERENCES

ÀEVarsen, 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.

Beltinsa, 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, 12893–12899.

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 m7G-cap of mRNA. Cell 12, 321–328.

Czworkowski, J., Wang, J., Steltz, 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.

Domer, 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.

Einsouf, 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., Hao, 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., Kostashkina, O.E., Kotellansky, V.E., Rutkevich, N.M., and Spirin, A.S. (1978). Factor-free (“non-enzymic”) and factor-dependent systems of translation of polyuridylic acid by Escherichia coli ribosomes. J. Mol. Biol. 107, 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., Rehiani, 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 all-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., Masfiaz, 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., Lauberg, M., and Noller, H.F. (2006). Crystal structure of a 70S ribosome–RNA 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 49, 682–695.

Lauberg, 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 mechanistic explanation of RNA pseudoknot function in programmed ribosomal frameshifting. Nature 441, 244–247.

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

Noller, H.F., Yusupov, M.M., Yusupova, G.Z., Baicorn, 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.

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.

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 miRNA and tRNA. Science 313, 1935–1942.

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., 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., Emolchenko, 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 Saneljouand, 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., Ill. (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., Ill. (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 77, 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.