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Unraveling the Dynamics of Ribosome Translocation

Jin Chen^{1,2}, Albert Tsai^{1,2}, Seán E. O’Leary², Alexey Petrov², and Joseph D. Puglisi²

¹Department of Applied Physics, Stanford University, Stanford, CA 94305-4090, USA

²Department of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305-5126, USA

Abstract

Translocation is one of the key events in translation, requiring large-scale conformational changes in the ribosome, movements of two transfer RNAs (tRNAs) across a distance of more than 20 Å, and the coupled movement of the messenger RNA (mRNA) by one codon, completing one cycle of peptide-chain elongation. Translocation is catalyzed by elongation factor G (EF-G in bacteria), which hydrolyzes GTP in the process. However, how the conformational rearrangements of the ribosome actually drive the movements of the tRNAs and how EF-G GTP hydrolysis plays a role in this process are still unclear. Fluorescence methods, both single-molecule and bulk, have provided a dynamic view of translocation, allowing us to follow the different conformational changes of the ribosome in real-time. The application of electron microscopy has revealed new conformational intermediates during translocation and important structural rearrangements in the ribosome that drive tRNA movement, while computational approaches have added quantitative views of the translational pathway. These recent advances shed light on the process of translocation, providing insight on how to resolve the different descriptions of translocation in the current literature.

Introduction

Protein synthesis requires the coordination between the ribosome and multiple translation factors to convert the genetic information encoded by the messenger RNA (mRNA) to a polypeptide sequence[1]. The ribosome is a 2.4 MDa RNA-protein enzyme comprised of two subunits (30S and 50S in prokaryotes) [2-4]. After successful initiation, i.e. factor-guided 70S assembly of the two subunits[5, 6], the ribosome commits to the elongation phase. In each cycle of elongation, the ribosome selects the correct aminoacyl transfer RNA (tRNA) specified by the mRNA codon to the aminoacyl-tRNA binding site (A site)[7, 8]. Formation of a peptide bond with the peptidyl-tRNA in the adjacent P site transfers the elongating polypeptide from the P-site tRNA to the A-site tRNA. During the translocation step, catalyzed by elongation factor EF-G[9], the A- and P-site tRNAs must be moved by distances of 20 Å or more to the P site and E site (exit site) respectively[10], accompanied by the movement of the mRNA by precisely one codon[11] with respect to the ribosome to maintain the correct reading frame. The E-site tRNA then dissociates spontaneously[1], leaving the ribosome with a vacant A site and E site, ready for the next round of elongation.

After peptide bond formation ribosome is capable of slowly undergoing spontaneous translocation (at $\sim 3 \text{ h}^{-1}$) [12]. However EF-G greatly accelerates the process. The overall rate of EF-G dependent translocation *in vitro* was measured in bulk to be approximately 20 s^{-1} at $1 \text{ }\mu\text{M}$ EF-G [9, 13]. This matches well with the rate of elongation *in vivo* which is

found to be ~ 20 amino acids per second [14]. After translocation, EF-G dissociates within 50 ms, resetting the ribosome for another round of elongation. These rates frame the timescale for molecular events in translocation.

Determining the molecular mechanism of translocation and open reading-frame maintenance remains one of the key problems in translation. More than four decades ago, a model was proposed by Spirin in which ribosome translocation is controlled by a series of “locking” and “unlocking” events that separates two global conformational states correspondingly termed “unlocked” and “locked” [15]. In this original model, the ribosomal subunits are tightly associated before peptide bond formation to facilitate manipulation of the tRNA and preserve reading frame on the mRNA. After peptide bond formation, the ribosome unlocks. In this unlocked state, the ribosomal subunits and tRNA can move more freely, facilitating translocation of the tRNA and stepping to the next codon of the mRNA [8, 16]. Full translocation returns the ribosome to the locked state, once again restricting the motion of the ribosomal subunits and tRNA.

Consistent with this model, cryoelectron microscopy (cryo-EM) and x-ray crystallography structures revealed that the two subunits of the ribosome undergo dramatic conformational changes after peptide bond formation, by which the small (30S) subunit is rotated ~3-10° counterclockwise with respect to the large (50S) subunit [17, 18]. This rotational movement is possible because the intersubunit contacts, which consist mainly of RNA-RNA interactions, are relatively labile and can rearrange with little energy cost [18-21]. However, it is still unknown how these intersubunit rotations relate to Spirin’s locking and unlocking mechanism. In addition to the intersubunit rotation, there is also a nearly orthogonal rotation of the head domain of the 30S subunit that seems to play a role in controlling the position of tRNAs within the ribosome [20]. There is thus more than one rotational movement of the ribosome. Recent crystallographic structures of the 70S ribosomal particle have revealed multiple conformational intermediates [22], and here we call the collection of these stepwise conformational changes leading to translocation “ratcheting.” Aside from these global rotational movements of the ribosome, multiple local conformational rearrangements also occur during the various steps of elongation. For example, the L1 stalk is thought to facilitate the movement of tRNA from the P site to the E site [23, 24], fluctuating between three distinct conformational states.

Upon peptide bond formation, not only does the ribosome itself undergo dramatic conformational changes prior to translocation, the tRNAs also fluctuate between multiple states. tRNAs can fluctuate freely between the classical (A/A and P/P) state and the hybrid state (A/P and P/E), facilitating the upcoming translocation step catalyzed by EF-G [8, 25-27]. These conformational changes are possibly driven by one of the many ribosome conformational rearrangements. Chemical probing, subsequent cryo-EM structures, and single-molecule techniques [7, 8] all identified a hybrid tRNA configuration, in which the acceptor stems of the A- and P-site tRNAs interact with the P and E sites of the large subunit, respectively, while the anticodon stem loops of the tRNAs remain in the A and P sites of the small subunit. There have been reports of additional hybrid-state intermediates [28]. This hybrid tRNA configuration can be seen as an intermediate step in translocation. The fluctuation of tRNAs upon peptide bond formation echoes the “unlocking/locking” model proposed by Spirin.

Conformational rearrangements of the ribosome are likely coupled to the tRNA fluctuations and the eventual translocation of the tRNAs and mRNA. Although it is generally assumed that mRNA motion and tRNA movement are coupled mechanically and temporally, this assumption has never been validated directly [22, 25]. However the link between intersubunit rotation and translocation has been established. If two subunits are cross-linked

such that the intersubunit rotational movement is not possible, translocation does not occur[29]. While ribosome can undergo factor free translocation, EF-G and GTP hydrolysis by EF-G greatly accelerates the rate of translocation (see above) possibly by driving one of these conformational changes. Determining molecular mechanisms that link ribosome conformation and ligand dynamics to EF-G-catalyzed translocation remains a key question in translation.

In this review, we address recent advances in understanding the mechanism of translocation through dynamic studies by fluorescence methods, structural results by cryo-electron microscopy and x-ray crystallography, and computation by molecular dynamics. We incorporate these recent results with the current views to formulate a consistent model of translocation.

Real-time dynamics of translocation

Fluorescence approaches, either through single-molecule methods or stopped-flow techniques, probe the dynamics of biological processes with high sensitivity and time resolution. Moreover, fluorescence resonance energy transfer (FRET) provides a method to measure dynamic changes in protein conformation by probing the distance (or potentially the orientation) between a donor dye and acceptor dye (usually separated by 20-80 Å)[30]. With single-molecule fluorescence, dynamics can be observed directly in real time. Despite the nanosecond timescale of fluorescence excitation and emission, the time resolution in single-molecule fluorescence spectroscopy is frequently limited to tens of milliseconds by current camera technologies so that a sufficient number of photons can be integrated. Bulk fluorescence methods, like quench-flow and stopped-flow, provide high time resolutions of kinetic processes (μ s-ms timescales) but suffer from the disadvantage that signals average the behavior of asynchronous ribosomes. Thus bulk fluorescence is limited to investigation of single turnovers, making them unsuitable for observing multiple rounds of translation elongation directly in real-time. Both bulk and single-molecule fluorescence methods have provided a way to track ribosome conformational changes during translocation[14, 30].

Aitken and Puglisi (2010) followed the intersubunit rotation of the ribosome in real-time through multiple rounds of elongation by employing single molecule FRET with two dyes on helix 44 of the 30S subunit and helix 101 of the 50S subunit[31] (Figure 1b). The position of the dyes tracks the rotational state of the 30S subunit body domain[32]. The intersubunit FRET signal alternates between a high FRET state and a low FRET state on an elongating ribosome (Figure 1a). The elongation cycle begins with ribosome in non-rotated, high FRET state. The transition from the high to the low FRET state occurs upon peptide-bond formation, and corresponds to the rotation of the 30S body by $\sim 6^\circ$. The subsequent reverse transition (back rotation) from the low FRET state back to the high FRET state is catalyzed by EF-G. Neither FRET transition occurs spontaneously, thus the ribosome needs the free energies of peptidyl transfer and GTP hydrolysis during elongation to facilitate transition between these two global states. The existence of two global conformational states separated by large energy barriers as identified by the intersubunit FRET signal is consistent with Spirin's original model of translation, where in the non-rotated state ribosome is globally locked. Upon peptide bond formation the ribosome rotates and unlocks to permit dynamic tRNA fluctuations between the classic and hybrid states. This puts ribosome in a highly dynamic state in preparation for translocation[33]. The second transition catalyzed by EF-G likely corresponds to the re-locking of the ribosome upon translocation, where the local conformational dynamics of the ribosome are suppressed [31, 34]

In the unlocked state, the dynamic fluctuations go beyond tRNAs and involve other parts of the ribosome. For example, Cornish *et al.*, using FRET between ribosomal proteins S6, S11, and L9, also probed the rotational state of the 30S head domain (though the author did not

explicitly mention, it is likely they are observing the 30S *head* domain rotation based on crystal structures [22]) and showed a dynamic equilibrium between the two rotational states of the ribosome[35] (Figure 1d). This is in contrast with the rotational 30S *body* movement observed by the Puglisi group. Interestingly, rather than observing defined chemical steps controlling the conformational changes of the ribosome, Cornish *et al.* observed spontaneous rotations of the 30S head domain upon peptide bond formation (Figure 1c). The frequency of the head rotation was dependent on tRNA position in the ribosome and the identity of the bound tRNA, suggesting that conformational rearrangements of the 30S head and tRNAs are linked.

Other single molecule studies have shown the L1 stalk in dynamic fluctuation. Fei *et al.* (2008) [24] and Munro *et al.* (2010) [36] used single-molecule FRET between the P-site tRNA and L1 to demonstrate that, prior to peptide bond formation, FRET between L1 and tRNA is stable. Upon peptide bond formation, FRET intensity spontaneously fluctuates. This was interpreted as fluctuations of L1 stalk, however since both L1 and tRNA are mobile these FRET changes do not distinguish between tRNA and L1 mobility. Subsequent studies by Fei *et al.* (2009) [23] using FRET between L1 and L9 also demonstrated L1 fluctuations in pre-translocation complexes, confirming that the original experiments were indeed reporting on L1 dynamics.

These results together with a plethora of structural data demonstrate that upon peptide bond formation, ribosomal subunits rotate relative to each other. In agreement with the original “locking and unlocking” model, the ribosome in the rotated state has greater conformational flexibility as seen in the dynamic fluctuations of the tRNA acceptor stems, the 30S head domain, and the 50S L1 stalk. This ribosomal state is often termed unlocked in the literature. However as indicated by the extremely slow rates of factor free translocation [9, 12], mRNA remains tightly bound to the ribosome. Releasing mRNA to move one codon together with the anticodon loop of the tRNAs requires EF-G binding and/or GTP hydrolysis by EF-G [9, 37]. This event is also called “unlocking” and adds to the confusion in the literature. Therefore, there are two different uses of the terms unlocking and reloading. The first unlocking happens upon peptide bond formation and permits movements of acceptor stems of the tRNAs while keeping mRNA position tightly locked. The second unlocking occurs upon EF-G binding thus allowing the mRNA transition and movement of the anticodon loops of tRNA. In this model, the pre-translocational ribosome, while conformationally flexible, remains tightly associated to the mRNA as it awaits EF-G arrival. A recent study using single-molecule force microscopy tracking the movement ribosomes on the mRNA with highly sensitive optical tweezers reported that the actual movement of the ribosome on the mRNA occurs quickly, accounting only for a very small fraction of the total time spent in an elongation cycle [38]. This observation strongly supports the hypothesis that mRNA movement is allowed only during a short period with EF-G catalyzed translocation to insure reading-frame maintenance.

Munro *et al.* (2010) examined how EF-G dynamics are correlated with L1 stalk fluctuations[39]. The authors showed that ribosome L1 stalk closure, as probed by the L1 stalk-tRNA FRET, is correlated with EF-G ribosome interactions. The authors described the closure of the L1 stalk as “unlocking,” based on structural modeling that constraints the subunits to ratchet upon L1 stalk closure. In contrast, the “unlocking” as described by the Puglisi group or Spirin would correspond to the first unlocking step while conformational transitions observed by Munro *et al.* likely represent a part of the second unlocking step associated with EF-G. Furthermore, the authors reported an increase of fluorescence as the tRNA is moved from the A site to the P site. The authors found that their measured rate of translocation closely approximates the intrinsic rate at which the ribosome spontaneously closes the L1 stalk, so they argued that EF-G does not measurably accelerate the rate at

which this key intermediate in translocation is achieved. Although GTP hydrolysis does accelerate the overall rate of translocation, they suggested that GTP likely plays a role in enabling EF-G to achieve a high-affinity interaction with the ribosome. Thus, their intermediate state is stabilized by EF-G•GTP, but its rate of formation is unaffected by EF-G•GTP. It remains unclear if L1 stalk closure is an upstream event prior to the EF-G driven unlocking step or itself unlocks the mRNA movement *per se*.

In a bulk FRET experiment, Ermolenko *et al.* (2011) specifically labeled ribosomal proteins S6, S11, and L9 (the same labeling sites as Cornish *et al.*[35]) for bulk fluorescence experiments[40]. These FRET probes were positioned to detect the rotational movements of the ribosome subunits, proposed to produce an increase in S11-L9 FRET and an anti-correlated decrease in S6-L9 FRET resulting from a counterclockwise rotation of the 30S subunit (possibly rotation of the head domain) (Figure 1d). Through the use of antibiotics that slow translocation (spectinomycin and hygromycin), Ermolenko *et al.* were able to resolve the fast counterclockwise rotation of the two subunits, followed by a slow clockwise reverse rotation. By measuring the quenching of pyrene-labeled mRNA fluorescence, the authors showed that mRNA translocation occurs on the same timescale as the clockwise rotation back from the rotated state to the original state, thereby concluding that the two processes are coupled. Thus, the first counterclockwise rotation would correspond to the mRNA unlocking step in our model. The authors further used EF-G•GDPNP (a nonhydrolyzable GTP analog) or EF-G•GTP with fusidic acid (an antibiotic that inhibits EF-G release after GTP hydrolysis) to demonstrate that the subsequent clockwise rotation does not require EF-G release or GTP hydrolysis. The translocation rate in the presence of GDPNP is slowed down by a factor of ~2.5. However, this clearly is in contrast with the 50-fold inhibition of translocation rate reported by Rodnina *et al.*[9] and Munro *et al.*[39].

These dynamic studies revealed that there are multiple locking and unlocking events occurring during an elongation cycle, and they are associated with distinct conformational changes. These conformational transitions are differentially affected by EF-G binding, dissociation, and GTP hydrolysis. Unfortunately, the ambiguous use of the terms “unlocking” and “unlocked state” by the different groups creates unnecessary confusion. However this could be resolved by carefully identifying what conformational movements are tracked and by explicitly specifying what molecular transitions become unlocked or locked.

Structural Insights into Translocation

Despite the power of fluorescence methods to illuminate dynamics, they provide sparse structural information. Cryo-electron microscopy has been used extensively in structural studies of the ribosome, and acts as a potent complement to FRET. Time-resolved and multiparticle cryo-electron microscopy (cryoEM) have revealed of how structural rearrangements on different length scales act concertedly to facilitate mechanical processes central to translation, such as translocation. Despite its relatively lower resolution, cryo-EM has advantages over crystallography because conformationally-diverse substates can be identified within an ensemble of molecules through a variety of existing and emerging particle-classification algorithms. The relative populations of these substates allow assessment of the energetics of their interconversion, provided that they are of sufficiently low energy to appear as detectable populations in the EM data. Thus cryoEM can probe the overall energy landscape of translocation.

Fischer *et al.* (2010) employed a time-resolved approach to study thermally-driven ribosomal back-translocation, and identified a large number of ribosomal pre- and post-translocation configurational substates for the *E. coli* 70S ribosome in the absence of EF-G[41]. Analysis of the time-dependence of the relative substate populations indicated that

substates on each side of the translocation event are in rapid equilibrium, and that the movement of tRNAs from the A/P, P/E hybrid state to the P/P, E/E state itself fully limits the observed rate of translocation. Movement of tRNAs through the ribosome was linked to the formation of tRNA-ribosome contacts, including with the L1 stalk, helix 69 of the 23S rRNA, and the L5 protein. In general, the tRNA motion was shown to be mediated by the sequential formation and dissolution of these tRNA-ribosome contacts, in the context of ongoing 30S body rotation. For each substate of tRNA movement, the authors observed a nearly continuous 30S body rotation and 30S head movements, suggesting a coupling of tRNA movement and global changes in ribosome conformation (Figure 2). The transition from Pre1 to Pre2 (likely peptide bond formation) results in a large 30S body rotation counterclockwise, consistent with single-molecule FRET measurements discussed above. Up until translocation, the 30S head also rotates. After translocation (from Pre5 to Post1), both the 30S head and 30S body rotates back, resetting the ribosome for the next round of elongation.

A similar paradigm for ribosome dynamics was also uncovered in the cryo-EM study of Ratje *et al.* (2010) on *Thermus thermophilus* 70S ribosomes bound to EF-G in the presence of fusidic acid, which results in EF-G catalyzed GTP hydrolysis, but not the subsequent translocationally-relevant conformational changes in, or dissociation of, EF-G•GDP[42]. Results from this study also revealed a number of configurations of the pre-translocation ribosome that were intermediates on the translocation pathway. In particular this study identified an “intra-subunit” hybrid state, termed a pe/E state, where the tRNA made contacts with both the P-site components of the 30S head and its E-site platform components at the same time. The data led to the proposal that EF-G binds to the pre-translocation ribosome, stabilizing the fully “ratcheted” state, before translocation occurs in a manner that is facilitated by GTP hydrolysis. GTP hydrolysis was proposed to lead to reverse-ratcheting of the 30S head and body, which drives tRNA motion. As the tRNAs then move through the hybrid ap/P and pe/E states into the classical P/P and E/E states, interaction of Domain IV of EF-G is proposed to decouple back-ratcheting from reverse tRNA motion, providing the net directionality. Molecular dynamics and other studies also implicated Domain IV as playing a central role in translocation (see below). This cryo-EM study also implicated a swiveling motion of the 30S head as being instrumental in guiding tRNA transit.

These combined results highlight the importance of multiple ribosome conformational dynamics in guiding tRNA and mRNA motion, and show that contacts between the ribosome and tRNAs facilitate an energetically inexpensive progression through a large number of intermediates states during translocation. The two studies are consistent with the model proposed by an earlier crystallography study by Zhang *et al.*[22], showing that there are multiple conformational intermediates during elongation. In that study, Zhang observed at least four intermediates of ratcheting: ratcheting likely begins with the 30S subunit body rotation, continuing with the 30S platform and head domains, and completes with rearrangement of the central bridges. It is clear that in addition to the rotational movement of the 30S subunit, other large-scale conformational changes, like the 30S head movement, are essential for translocation. High-resolution structures of the ribosome in different rotated states with intact tRNAs will be required to complete our molecular understanding of the large-scale conformational rearrangements in the ribosome that allow ratcheting and translocation.

Linking structural and dynamic information

Structural snapshots from X-ray crystallography and cryo-EM suggest that many conformational rearrangements of the ribosome, tRNAs, and EF-G must occur during translocation. Nevertheless, high-resolution structures of the transition states in between those snapshots are not available, due to the intrinsic high energy and short lifetimes of these

states. Cryo-EM imaging techniques have been successful in capturing some of the intermediate states, albeit at lower resolution and sometimes with missing components. Moreover, the short time-scales (microseconds or shorter) involved in some highly energetic states along the reaction coordinate for translation are simply inaccessible to most methods. Computational approaches provide a link between structure and dynamics. While the size of the ribosome (~3.2 million atoms) and the long time scales (up to 100 ms) involved in translocation [43] render all-atom molecular dynamics (MD) simulations of the complete process intractable, shorter simulations on parts of the elongation cycle starting from high-resolution structures have probed important structural and dynamic features of the transition states.

Simulations of the whole ribosome remain challenging, considering the large number of atoms and need for robust potential energy functions; however, recent advances are exciting. Li *et al.* [44] simulated structural dynamics of the large conformational changes of EF-G on a ribosome to drive translocation. Their results suggest that EF-G has a large degree of conformational flexibility, especially of Domain IV (the elongated domain that reaches into the A site of the ribosome) with respect to the rest of the molecule, and this flexibility likely lies at the center of the function of EF-G during translocation (Figure 3). The conformational variability of EF-G when bound to the ribosome could be driven by interactions of EF-G with the GTPase-associated center of the large subunit, specifically the L11 lobe, as the ribosome changes its ratcheting state.

Recent achievements in all-atom MD simulations are promising for dissecting the structural and dynamic features of short-lived transition states of the ribosome complex with various elongation factors during translocation. MD provides a great complement to cryo-EM studies with low spatial and time resolution and the dynamic fluorescence studies with higher time resolution but little spatial resolution. While improvements in computational techniques will make MD an essential tool for understanding ribosomal function, observations originating from MD simulations must be experimentally tested. The integration of computation and experiment is a challenge for upcoming years.

Discussion

Recent results in fluorescence assays, structural studies, and molecular dynamics simulations have shed additional light onto the mechanisms of translocation. However, the current literature in translocation is still marred with confusing and seemingly contradictory models. This problem mostly stems from the misuse of terminology. The terms “ratcheting”, “unlocking and locking,” and “rotated and non-rotated” have been used repeatedly in literature (including by us), although each of the studies were probing different parts of the ribosome conformation. The confusing picture presented by these results can be reconciled by looking at translocation as a process with multiple conformational motions, as revealed by the various structural and dynamic studies.

Generally, the term “unlocking and locking” are not confined to just a single intersubunit transition. In Spirin’s “unlocking locking” model, translation is separated into two major conformational states: one that is waiting for EF-Tu tRNA arrival (the locked state), and the other waiting for EF-G and translocation (the unlocked state). This model is consistent with the single-molecule FRET data by the Puglisi group [31, 33, 34]. The FRET labeling positions are helix 44 of 30S subunit (helix 44 is known to be the axis of rotation of the intersubunit rotational movement) and helix 101 of 50S subunit, away from the head domain of the 30S subunit, suggesting that this FRET signal reports on the rotational movement of the 30S body [32]. Thus, the 30S body rotation may be consistent with this definition of “unlocking and locking.” This is echoed by the cryo-EM result by Fischer *et al.*, which

showed a large clockwise rotation of the 30S subunit upon translocation and a more gradual counterclockwise rotation upon peptide bond formation[41]. In this “unlocked” state a large number of conformations become accessible, including tRNA fluctuations, 30S head rotations and so on, while mRNA movement remains restricted. The second “unlocking” event, catalyzed by EF-G is required to facilitate mRNA movement. Other groups have used this second molecular event to define of “unlocking” of the ribosomal particle [45]. Both events are required for rapid and efficient translocation, but the lack of clear distinctions between two mars the literature and impedes the field.

It is also important to distinguish the multiple types of intersubunit rotation. Upon peptide bond formation the ribosome undergoes counterclockwise rotation of the 30S *body* to the “unlocked” state. In this state, spontaneous fluctuations are facilitated: tRNAs fluctuate between the classical and hybrid state, 30S *head* spontaneously rotates as reported by Ha and Noller[35], and the L1 stalk fluctuates between the open and closed states. Noller and Ha labeled FRET fluorophores at S6, S11, and L9, which are close to the head domain of the 30S subunit [35, 46]. Thus, it is possible that their FRET probes are measuring 30S head domain rotation. They reported that spontaneous rotations occur upon peptide bond formation, consistent with the “unlocking” theory. Though here we only distinguish between head and body rotation, there are likely many more subtle rotational movements.

When EF-G binds in complex with GTP, it undergoes dramatic conformational changes, possibly to accommodate into the A-site by rearranging its domain IV [17]. As a result, the 30S head was stabilized in the swiveled and rotated conformation, as shown in the cryo-EM result by Ratje *et al*[42]. Single-molecule studies by Noller and Ha also showed a stabilization of the “rotated” conformation upon binding of EF-G•GDPNP, suggesting the possibility of head domain rotation[35]. Binding of EF-G also stabilizes the tRNA in the hybrid state[47]. GTP hydrolysis then results in another conformational change of EF-G and the ribosome, which is the second mRNA “unlocking” step suggested by Noller and Rodnina[40, 45]. The back rotation of the 30S body and the back rotation of the head domain “relock” the ribosome, acting together with the L1 stalk to translocate tRNA and mRNA to the next codon. The molecular details of each of these events will require additional high-resolution structures[20].

The role of GTP hydrolysis in translocation remains still unclear, though it is generally accepted that GTP hydrolysis is not necessary for translocation, as seen in experiments with GDPNP. EF-G driven translocation with GDPNP may appear to contradict intuition; however, from the perspective of an energy landscape for translation[48], the energy barrier for translocation may still be crossed with finite probability under factor-free situations, as evident from experiments showing spontaneous translocation. The binding of EF-G in GTP form stabilizes certain conformations of the ribosome, possibly lowering the energy barrier, resulting in a higher rate of translocation. That said, GTP hydrolysis is clearly required for rapid translocation catalyzed by EF-G.

The pathway for translocation along the energy landscape is likely to be very heterogeneous. The ribosome probably samples multiple sub-states of conformation along the pathway towards translocation. Translocation may be viewed as a series of multiple conformational changes, rather than just as a simple rotated/non-rotated two-state model. Although a complete mapping of the energy landscape of translocation remains challenging, further structural and dynamic studies of conformational intermediates, combined with molecular dynamics, will provide better understanding of certain pathways for translocation.

Conclusions

Moore once described the current literature on translocation as a “proverbial group of blind men trying to describe the proverbial elephant on the basis of what each learns about the elephant by groping it at random”[49]. This description is not far from the truth. Every group uses different methods to probe translocation, be it cryo-EM, single-molecule FRET, bulk fluorescence methods, or molecular dynamics modeling, they each shed light on different aspects of the process. Thus, further understanding the molecular mechanism of translocation will require the humble discussion of the various views. The detailed mechanism of translocation will be elucidated through the combined application of structural, single-molecule and computational methods

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Highlights

- Translocation requires large-scale ribosome conformational changes, tRNA movement into adjacent sites, and mRNA movement by one codon
- How the conformational rearrangements of the ribosome are linked to translocation is still unclear.
- Multiple intersubunit rotations and conformational sub-states drive the movement of tRNA and mRNA.
- Additional experiments using cryo-electron microscopy, single-molecule fluorescence, and molecular dynamics will provide further insights into the mechanism of translocation.

Figure 1.**Single-molecule translation assays**

(a) Cy5-labeled 50S subunits, tRNA ternary complex, and EF-G are delivered to surface-immobilized single pre-initiation complexes containing Cy3-labeled 30S subunits and illuminated at 532 nm; both Cy3 and Cy5 fluorescence are simultaneously detected. Immobilization with an mRNA coding for six phenylalanines (6F) permits the observation of ribosome conformation during multiple rounds of elongation via the intersubunit FRET signal. The arrival of FRET corresponds to 50S subunit joining during initiation and is followed by multiple cycles of high-low-high FRET, each reporting on ribosome unlocking and locking during one round of elongation, according to Spirin's model. The unlocked state is rotated, while the locked state is non-rotated.

(b) FRET between helix 44 (30S) and helix 101 (50S) reports on the relative rotational states of the 30S body with respect to the 50S subunit. Figure obtained from [50]

(c) Single-molecule time traces of S6-Cy5/L9-Cy3 ribosome complexes showing intersubunit rotation of the 30S head domain induced by peptide bond formation (puromycin), EF-G binding, and translocation. The ribosome starts in the high FRET state. Peptide bond formation results in a transition to the low FRET state but also induces spontaneous intersubunit head rotational movements, as indicated by the fluctuations between the low FRET state and high FRET state. EF-G binding stabilizes the low FRET state. Translocation by EF-G is observed as the transition back to the high FRET state. This also echoes Spirin's unlocking/locking model.

(d) Ribosomes labeled at L9 and S6 or S11. FRET reveals spontaneous conformational fluctuations similar to those observed for the L1 stalk and tRNA. Reproduced with modifications and with permission from Aitken *et al.* and Cornish *et al.* [31, 35].

Figure 2. Cryo-EM reveals sub-states of translation

(a) Distribution of 30S substates for different tRNA positions in pre and post states. The heat map indicates the fraction of particles relative to the total number of particles in the respective state. The transition from Pre1 to Pre2 (likely peptide bond formation) results in a large 30S body rotation counterclockwise. Up until translocation, the 30S head also rotates. After translocation (from Pre5 to Post1), both the 30S head and 30S body rotates back, resetting the ribosome for the next round of elongation.

(b) Schematic of 30S body rotation. The 30S body rotates around a pivot point at helix 27 of 16S rRNA independent of the 30S head. Schematic of 30S head movement. 30S head movement comprises a rotation and swiveling motion around the neck region (h28). This indicates that multiple ribosome conformational changes coordinate translocation.

(c) Free energy landscape of global ribosome conformation of the different tRNA substates. Reproduced with modifications and with permission from Fischer *et al.* [41].

Figure 3. EF-G Dynamics

The conformations of EF-G in various bound states, as observed by both X-ray crystallography and cryo-EM: EF-G•GTP in unbound form (red); EF-G•GTP bound to the ribosome (yellow); EF-G•GDP bound to the ribosome (blue); and EF-G•GDP in unbound form (pink). EF-G conformations changes throughout the elongation cycle. These conformations may be significant in determining binding dynamics of EF-G to the ribosome as well as catalyzing ribosome conformational changes. Reproduced with permission from Li *et al.* [44].

Figure 4. Schematic of elongation

The ribosome is separated into two global conformations, the “unlocked” state and the “locked” state. Upon peptide bond formation, the ribosome “unlocks” and the 30S subunit rotates with respect to the 50S subunit, permitting fluctuations of the L1 stalk between open and closed states, fluctuations of tRNAs between the classical and hybrid states, and spontaneous rotations in the 30S head domain. EF-G•GTP binding then stabilizes the L1 stalk in the closed state and tRNA in the hybrid state, as well as causing the head of the 30S subunit to rotate. GTP hydrolysis by EF-G unlocks movement of the mRNA for translocation, followed by relocking of the ribosome (back-rotation of the 30S body and head domains) and translocation. Relocking of the mRNA arrests its movement to preserve reading frame. The E-site tRNA and EF-G•GDP departs rapidly, returning the ribosome to the original state.