RNA BIOLOGY
2016, VOL. 13, NO. 12, 1197–1203
http://dx.doi.org/10.1080/15476286.2016.1240140

Taylor & Francis
Taylor & Francis Group

POINT OF VIEW

Translocation as continuous movement through the ribosome

Riccardo Belardinelli, Heena Sharma, Frank Peske, Wolfgang Wintermeyer, and Marina V. Rodnina

Max Planck Institute for Biophysical Chemistry, Department of Physical Biochemistry, Göttingen, Germany

ABSTRACT

In each round of translation elongation, tRNAs and mRNA move within the ribosome by one codon at a time. tRNA–mRNA translocation is promoted by elongation factor G (EF-G) at the cost of GTP hydrolysis. The key questions for understanding translocation are how and when the tRNAs move and how EF-G coordinates motions of the ribosomal subunits with tRNA movement. Here we present 2 recent papers which describe the choreography of movements over the whole trajectory of translocation. We present the view that EF-G accelerates translocation by promoting the steps that lead to GTPase-dependent ribosome unlocking. EF-G facilitates the formation of the rotated state of the ribosome and uncouples the backward motions of the ribosomal subunits, forming an open conformation in which the tRNAs can rapidly move. Ribosome dynamics are important not only in translocation, but also in recoding events, such as frameshifting and bypassing, and mediate sensitivity to antibiotics.

ARTICLE HISTORY
Received 27 July 2016
Revised 14 September 2016
Accepted 16 September 2016

KEYWORDS
EF-G; mRNA; molecular machines; protein synthesis; ribosome; tRNA; translation; translation elongation; translocation

During the elongation phase of translation the ribosome moves along the mRNA while synthesising the nascent polypeptide. The movement takes place during the translocation step of elongation and entails the displacement of 2 tRNAs bound to their respective codons through the ribosome by one codon in each round of elongation. It is now more than 2 decades ago since researchers obtained the first evidence suggesting that the ribosome not only provides the environment but also actively participates in translocation, with the dynamics of the ribosomal subunits (the small and large subunits, SSU and LSU, respectively), and of the tRNAs playing a key role in promoting the movement.[1,2] Structural studies provided high-resolution snapshots of ribosome motions while translocating the tRNAs through the A, P and E sites. They identified the types of motions that play a key role in translocation: the rotation of the SSU relative to the LSU and the swiveling motion of the SSU subdomain, the SSU head, relative to the SSU body.[3-11] In parallel, ensemble and single-molecule kinetics and molecular dynamics simulations provided insights into the link between the movement of the tRNA–mRNA complex and the motions of the subunits.[12-16] The current working model for translocation suggests that each time a peptide bond is formed, the CCA ends of the A-site and P-site tRNA move toward the P and E site on the LSU, respectively, into the A/P and P/E hybrid state.[17-21] This step can proceed spontaneously, driven purely by thermal energy, and is reversible. At the same time, the SSU rotates in counterclockwise (CCW) direction relative to the LSU, and the ribosome alternates between the rotated and the non-rotated state which is loosely coupled to the formation of the hybrid and the classical tRNA state, respectively (Fig. 1A). In the next step, the anticodons of the tRNAs together with the mRNA move relative to the SSU, and the CCA ends complete their movement on the LSU, reaching the classical P/P and E/E state and completing translocation. The SSU moves clockwise (CW) into their initial state relative to the LSU. Movement of the tRNAs on the SSU comprises the main kinetic barrier to translocation,[15,22] which is overcome by elongation factor G (EF-G), a GTPase, at the cost of GTP hydrolysis.[23] However, many fundamental questions remain unanswered, such as: Does EF-G bind to the ribosome in the non-rotated–classical or rotated–hybrid state? How are the movements of the ribosome and the tRNA–mRNA complex coordinated? How do they enable translocation and how is the energy of thermal fluctuations rectified into forward movement? How does EF-G overcome the kinetic barrier of translocation? What does GTP hydrolysis accomplish? Answering these questions is of fundamental interest not only because they address key questions about molecular machines, but also because translation is an important target for antibiotics.[24] Furthermore, movements in molecular machines are interesting from a more broad evolutionary perspective, also given the recent observation that eukaryotic ribosome can employ a peculiar rolling motion.[25]

Several recent studies now provide answers to these pressing questions.[26,27,28] We used a toolbox of fluorescence-labeled translocation components and looked at the moving parts of the ribosome complex in real time, covering multiple angles of translocation, much as a film director would do to better cover its main scene under different perspectives[26,27] (Fig. 1B). Movements, defined as changes in distances between the main players, i.e., tRNAs, mRNA, and different ribosome components, are inferred from the changes in the intensity of fluorescence resonance energy transfer (FRET) between fluorescence reporters attached to different positions. For example, FRET pairs on the SSU and LSU allow us to monitor subunit rotations and SSU swiveling, whereas different combinations of labels on EF-G, tRNA, mRNA, and the ribosome reveal tRNA–mRNA

CONTACT Marina V. Rodnina rodnina@mpibpc.mpg.de
© 2016 Taylor & Francis Group, LLC

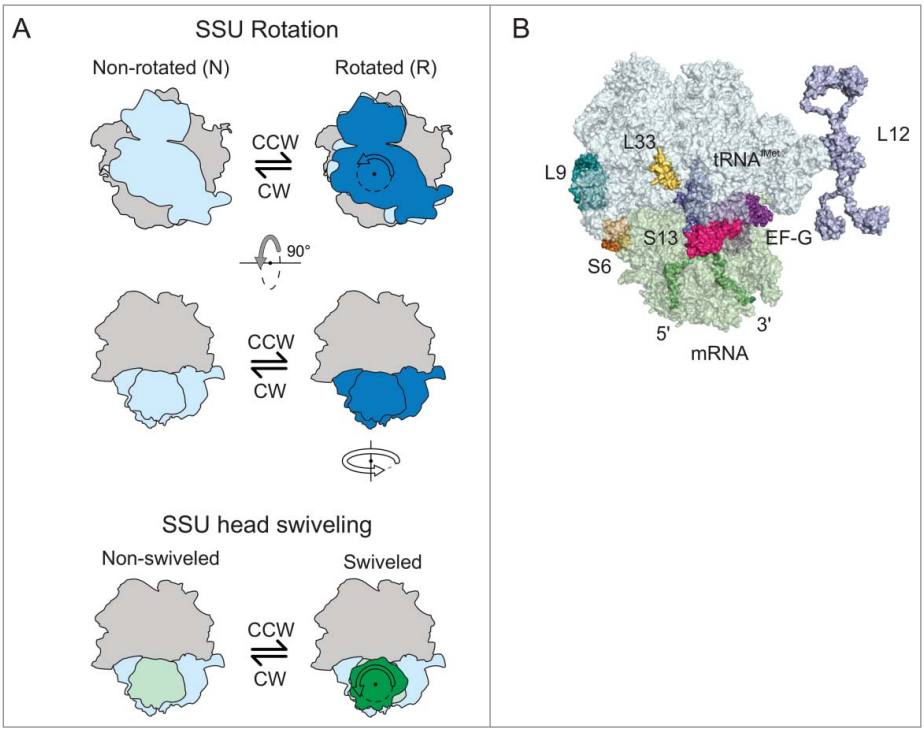

Figure 1. Ribosome dynamics (A) Modes of SSU movements. The rotation states of the SSU relative to the LSU (gray) are indicated by the color intensity of the SSU body (light blue for N, dark blue for R). The swiveling motions of the SSU head are illustrated by a color change from light green (classical non-swiveled SSU head position) to forest green (maximum degree of swiveling relative to the SSU body). (B) The toolbox of fluorescence reporters. Positions of fluorescent reporter groups on ribosomal proteins, tRNA, mRNA, and EF-G are indicated. The fluorescence change of the label on protein S13 reports on EF-G binding. FRET changes monitor the SSU rotation relative to the LSU (S6–L9), SSU head swiveling (S13–L33), or EF-G binding and dissociation (EF-G–L12). FRET changes between the P-site tRNA and SSU or LSU monitor the transit of the deacylated tRNA through the E site and its dissociation from the ribosome. SSU, light green; LSU, light cyan.

displacement and EF-G dissociation. Because the activity of these fluorescence-labeled components is indistinguishable from their non-labeled counterparts, one can integrate the information from all reports into a global model showing the choreography of molecular movements during translocation. In parallel, Blanchard and colleagues[28] used a similar toolbox of fluorescence reporters to study translocation by single-molecule FRET (smFRET) techniques. In the following, we compare the 3 studies and suggest a picture of translocation as seen from these multicolor, multi-angle and multi-perspective studies.

The first questions that we asked were: How rapid is the spontaneous subunit rotation at conditions of rapid translation? And how does EF-G affect the subunit rotation? Using a combination of rapid ensemble kinetics and single-molecule FRET, we estimated the rates of subunit rotations for different tRNA pairs.[27] After the peptide bond is formed, the SSU rotates in CCW direction relative to the LSU and the rate of this spontaneous rotation is 40 s^{-1} (at 37°C) with any tRNA used (Fig. 2). However, the distribution between the rotated and non-rotated states depends on the tRNA,[27,29,30] due to differences in the rate of spontaneous reverse rotation.[27] EF-G can bind to either the rotated or the non-rotated state of the ribosome.[18,31-34] But what happens when EF-G binds to a ribosome that remained in the non-rotated state? It turned out that binding of EF-G dramatically accelerates the rate of CCW rotation to 200 s^{-1} (ref.[27]) and stabilizes the rotated-hybrid state.[3,8,28,31,35,36] At cellular concentrations of EF-G, the life-time of the rotated–hybrid state is very short, because EF-G binds very rapidly and drives the ribosome toward translocation. Nevertheless, the rotated–hybrid state is an authentic translocation intermediate which serves to accelerate tRNA movement through the ribosome.[37-40] EF-G rapidly equalizes a potentially heterogeneous population of ribosome complexes to a uniform state poised for translocation. The high velocity of EF-G-catalyzed CCW rotation may explain why some smFRET studies did not capture the translocation route via the non-rotated state of the ribosome[16,28]: As soon as EF-G binds to the ribosome in the non-rotated state, the complex is rapidly converted into the rotated state, followed by translocation and CW rotation.

The next question is how the movements of the ribosome and the tRNA–mRNA complex are coordinated. We addressed this question using rapid ensemble kinetics.[26] The advantage of ensemble kinetics is its high temporal resolution. This allowed us to collect unperturbed translocation trajectories in the time range from milliseconds to seconds, which is the optimal time window for looking at tRNA movements. We designed a novel approach of unbiased fitting to decipher the choreography of movements: instead of trying to understand the nature of each of the FRET and fluorescence changes separately, we performed global data fitting using a minimum model that accounts for all data points (100,000) of the datasets collected with 9 different observables. The minimum model comprises 5 main kinetic steps of translocation; this does not mean that translocation cannot entail further steps, but those steps are unlikely to be rate-limiting, i.e. they should be rapid compared to the 5 steps identified by the global analysis. Another important outcome of the analysis are the values for FRET and fluorescence changes for each reporter and at each step (i.e., intrinsic

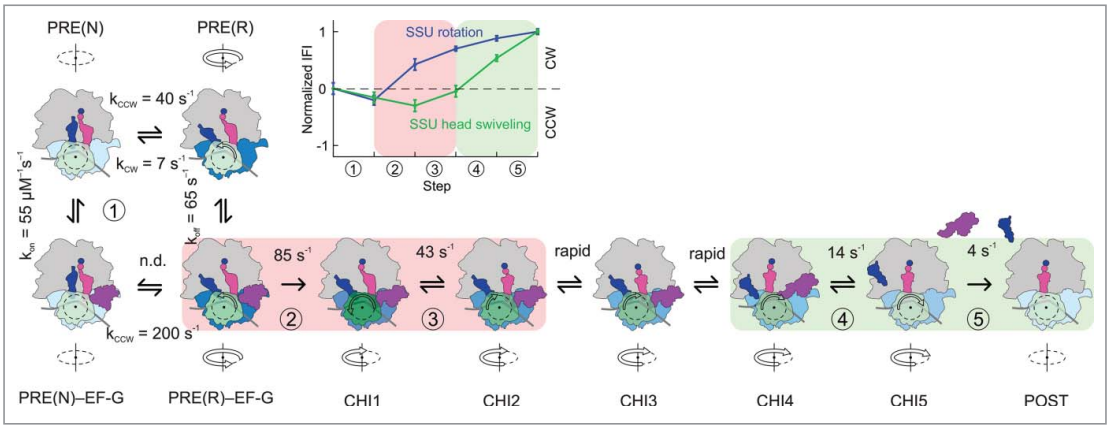

Figure 2. Translocation model The rotation states of the SSU relative to the LSU (gray) are indicated by color intensity of the SSU body (light blue for N, dark blue for R). The swiveling motions of the SSU head are depicted by a color gradient from light green (classical non-swiveled SSU head position) to forest green (maximum degree of swiveling relative to the SSU body).[26] Peptidyl- and deacylated-tRNA in the PRE complex are shown in magenta and blue, respectively. EF-G (purple) is depicted in 2 conformations, a compact[33] and an extended one after engagement with the ribosome.[8,61] The rates of transitions between PRE(N) and PRE(R) and PRE(N)–EF-G and PRE(R)–EF-G are from ref.[27] The rates of EF-G binding and dissociation (step ①) are ensemble rate constants obtained for a mixture of N and R states[26] in which the PRE(R) state is predominant.[27] All other rate constants for the kinetically defined steps ②, ③, ④, and ⑤ are from ensemble kinetics studies with the PRE(fMF) complexes at 37°C.[26] The existence of rapid steps between steps ③ and ④ was demonstrated previously.[22,42] Translocation intermediates (CHI1 to CHI4) are adopted from an smFRET study[31] and are consistent with other smFRET data,[28,35] ensemble kinetics[22,26] and structural studies[3,8,61]. An additional intermediate, CHI5, was identified by ensemble kinetics[26] and smFRET.[28] The POST state may entail further conformational sub-states.[28] Kinetics of GTP hydrolysis and Pi release were described earlier.[22,23] The light red background indicates complexes undergoing unlocking; the light green background shows complexes that move toward relocking. Inset: Distinct timing of CCW and CW movements of the SSU body relative to LSU (blue symbols) and of the SSU head (green symbols) as indicated by normalized intrinsic fluorescence intensities (IFI).

fluorescence intensities, IFIs), which are analogous to FRET values obtained by single-molecule measurements. These values indicate the directions of motions of the ribosomal subunits and the tRNAs and allow us to identify the point in time when unlocking of the ribosome takes place.[22] In the following, we describe these new findings against the background of previous reports identifying translocation intermediates by rapid kinetics[22,41-43] and single-molecule FRET.[31,35] We denote the EF-G-induced translocation intermediates as chimeric states (CHI) in order to distinguish them from the non-rotated–classical (N–C) and the rotated–hybrid (R–H) pre-translocation (PRE) state or the post-translocation (POST) state formed after the dissociation of EF-G.

The first step identified by the kinetic analysis is the initial EF-G binding to the ribosome, which has an association rate constant of 55 $\mu M^{-1}s^{-1}$, and is reversible, with the dissociation constant of 65 s^{-1} (Fig. 2). In the next step, which proceeds at the rate of 85 s^{-1} (at 37°C), EF-G hydrolyzes GTP, engages in the translocation, and uncouples SSU head and body movements. The SSU body starts to move in CW direction, whereas the SSU head continues to swivel in CCW direction. At this step, movements of SSU head and body become uncoupled, which may remove the physical hurdles on the way of the tRNAs and thereby start the unlocking of the ribosome. The resulting CHI1 state converts to CHI2 at a rate of 43 s^{-1}, which corresponds to the rate-limiting step of unlocking and is then followed by rapid tRNA translocation and Pi release from EF-G.[22] The SSU body continues to move into CW direction, but now also the SSU head starts swiveling backward (Fig. 2, inset). Single-molecule FRET studies suggested that the A-site tRNA moves toward the P site, as seen from the decrease in FRET between the tRNA and ribosomal protein L11.[31] This may coincide with the partial movement of the P-site tRNA toward the E site.[41] The exact structural rearrangements leading to unlocking and the following rapid movement of tRNAs and mRNA are not known. Engagement of EF-G domain 4 may promote translocation by altering the conformation of the SSU, e.g. by opening the mRNA-binding cleft, by stabilizing the open conformation of the E-site gate, or by displacing ribosome elements that act as hurdles for 30S translocation, such as intersubunit bridges B1a, B4, B7a, and B8.[4,7,8,33,44-46]

The sequence of the 2 following rapid steps is known from earlier ensemble kinetic work.[22,42,43,47] After unlocking, EF-G releases the GTPase product inorganic phosphate, Pi; in parallel, the tRNAs move toward, but not yet quite into, the P site on the LSU (Fig. 2), whereas translocation on the SSU is lagging behind. The resulting CHI3 state is short-lived, because the motions of the tRNA anticodon and CCA ends are normally synchronized with respect to both subunits[42]; the CHI3 state was identified when subsequent steps were blocked by using EF-G mutants, non-hydrolyzable GTP analogs, or antibiotics.[31,42] During unperturbed translocation, the formation of CHI4 signifies the placement of the CCA end of the A-site peptidyl-tRNA in the P site of the LSU where it becomes reactive with puromycin, which is a diagnostic test for the completion of tRNA translocation.[42] Importantly, Pi release and tRNA translocation are 2 parallel, non-coupled reactions independent of one another.[22,47] Blocking tRNA translocation by viomycin, spectinomycin, streptomycin, paromomycin, hygromycin B, or tetracycline does not inhibit Pi release.[22,48] On the other hand, interfering with Pi release by introducing mutations in ribosomal protein L12 or mimicking the Pi-bound state by adding Pi analogs does not block single-round translocation.[22,49] Because the 2 parallel pathways (Pi release first, then translocation, or translocation first, then Pi release) eventually merge, further reactions on the translocation pathway, including EF-G dissociation, are blocked.[49,50]

In the subsequent step, the tRNA, which has been displaced from the P to the E site, moves further through the ribosome via at least one distinct intermediate state (CHI5), and finally

dissociates from the ribosome[26] (Fig. 2). In parallel, the SSU head continues moving backward (the SSU CW rotation is almost complete at CHI4). Concomitantly, EF-G is released from the translocated complex in a stepwise manner – first from the ribosomal interface which then allows a smooth back swiveling of the SSU head and subsequently from the L12 stalk. The ribosome – now in the POST state – presents the SSU head and body in their non-swiveled, non-rotated states, the peptidyl-tRNA in the P site and empty E and A site, with the latter exposing the next mRNA codon which is immediately decoded by the next aminoacyl-tRNA. The dance of the ribosome along the translation axis can then continue with the next round of elongation. In summary, these data provide a picture of a smooth motion along the translocation axis, with a continuum of intermediate states that guide the tRNAs from the PRE to the POST state.

A recent single-molecule FRET study which utilized a similar approach of multi-angle fluorescence detection also suggests that the movements of the SSU head and body are de-coupled by EF-G.[28] The key conclusion, i.e. that the SSU CW body rotation precedes the CW head swiveling, is consistent with the ensemble kinetics study.[26] However, some details of the kinetic models[26,28] are difficult to reconcile due to the use of different observables in the 2 studies. Interestingly, the authors observed exaggerated SSU motions monitored by the S13-L5 FRET pair; it is not certain whether we observe a similar motion upon transition to CHI1 or this is yet a different type of SSU dynamics, as different labeling positions used in the 2 studies may report different motions. The exaggerated head-domain motions may represent the intrinsic motions of the protein L5 following the tRNAs movement,[15] or an as yet unidentified motion of the head outside of the swiveling trajectory (SSU head tilting) or even reflect a rolling motion similar to the one visualized for eukaryotic ribosomes.[25] In fact, subunit rolling was recently observed in SecM-stalled POST complexes from *E. coli*,[51] which would fit to the placement of this intermediate late on the translocation trajectory.[28] It appears that our model of translocation (Fig. 2) is more detailed with respect to the early unlocking steps, whereas ref.[28] identifies additional steps during relocking.

EF-G–mediated GTP hydrolysis plays a fundamental role in translocation. GTP hydrolysis accelerates the unlocking step leading to A to P and P to E tRNA movement (step 3) by 40-fold, as demonstrated by the analysis of the translocation pathway with a slowly hydrolyzable analog (i.e., GTPγS) or a GTPase-deficient EF-G mutant[23,26,42,43] (Fig. 3A). Also, the rate of step 5 (release of EF-G and the E-site tRNA) is dramatically reduced when GTP hydrolysis is inhibited. However, even more strikingly, the comparison of the intrinsic fluorescence intensities (IFI) for the intermediates formed with either GTP or GTPγS suggest that preventing GTP hydrolysis leads to the appearance of a series of intermediates which are structurally different from those on the pathway with GTP hydrolysis (Fig. 3B, C). Thus, our data support the view that uncoupling of the motions of the ribosomal subunits from the movement of the tRNA–mRNA complex is a fundamental consequence of GTP hydrolysis and subsequent ribosome unlocking. EF-G appears to work as a wedge to uncouple the SSU head swiveling

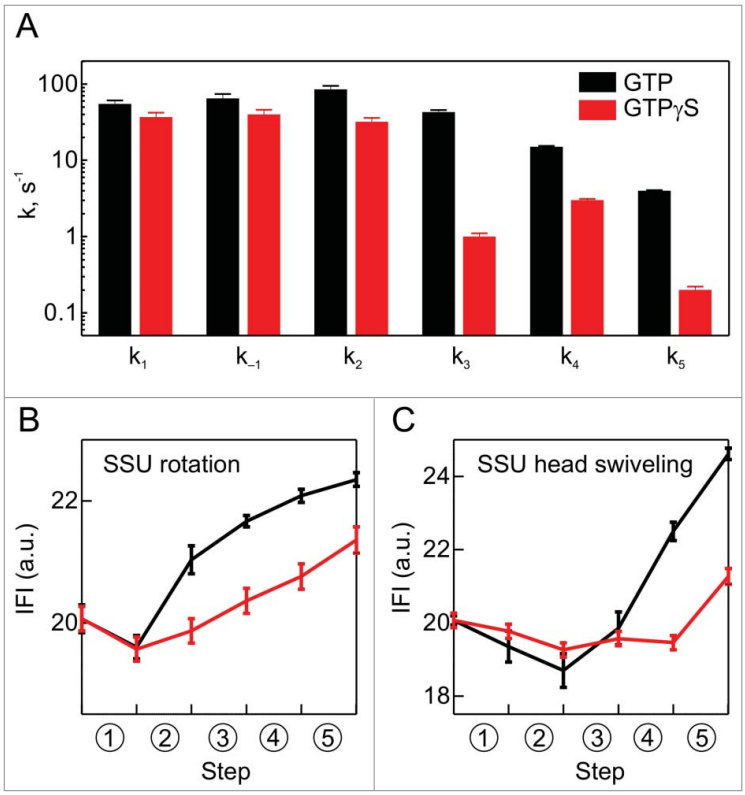

Figure 3. Effect of GTP hydrolysis (A) Rate constants of translocation steps measured with GTP (black bars) and GTPγS (red bars). Note the logarithmic scale used for the rate constants. (B, C) Examples of different trajectories of translocation with GTP (black symbols) and GTPγS (red symbols) for the SSU rotation (B) and SSU head swiveling (C). Intrinsic fluorescence intensities (IFI) reflect structural differences of the intermediates formed in steps ① to ⑤ (Fig. 2).

from the SSU body rotation. EF-G may also actively push the A-site tRNA toward the P site[52] or act as a "door-stop" by following the spontaneous movement of the tRNA out of the A site with its domain 4 and restricting the backward movement of the tRNA.[7,8,53]

While it is clear that a precise balance between synchronization and de-synchronization in SSU head and body motions is fundamental for optimum translocation, it would be of great interest to look at cases when their harmonious dance is disturbed. Antibiotics have already been used to uncouple tRNA–mRNA movements on the SSU and LSU,[42] but how the movements of these ligands correlate with the motions of the SSU body and head in the presence of inhibiting antibiotics is still insufficiently understood. Indeed, we know which ribosome conformations are stabilized by most of the known antibiotics and how ribosome dynamics are affected,[28,29,54,55] but what is still missing are the kinetics and the sequence of motions determining translocation inhibition and consequent translation arrest.

Less dramatic than a translational arrest by antibiotics, but of equally great importance, is the occurrence of translational pauses and their release during programmed ribosome frameshifting. In −1 programmed frameshifting the impaired backward rotation of the SSU head, assisted by a late release of EF-G, drives the slippage of the mRNA into the new reading frame.[56-58] Another exciting example of dynamics is ribosome bypassing, whereupon actively translating ribosomes "hop" over a large distance on the mRNA.[59,60] Understanding the mechanisms of such events from the molecular point of view could help in the design of molecules specifically targeting such motions. In the future, it would be even more challenging to investigate ribosome motions in the context of polysomes to understand whether the neighboring ribosomes in a polysome are synchronized, *de facto* linking ribosome dynamics and regulation of gene expression. Finally, insights into the fundamental principles of translocation on bacterial ribosomes will help to understand the more complex translational dynamics of the eukaryotic ribosome and to test whether a larger ribosome is an equally good dancer.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

ORCID

Riccardo Belardinelli http://orcid.org/0000-0002-3333-0466

References

1. Frank J, Agrawal RK. A ratchet-like inter-subunit reorganization of the ribosome during translocation. Nature 2000; 406:318-22; PMID:10917535; http://dx.doi.org/10.1038/35018597
2. Moazed D, Noller HF. Intermediate states in the movement of transfer RNA in the ribosome. Nature 1989; 342:142-8; PMID:2682263; http://dx.doi.org/10.1038/342142a0
3. Brilot AF, Korostelev AA, Ermolenko DN, Grigorieff N. Structure of the ribosome with elongation factor G trapped in the pretranslocation state. Proc Natl Acad Sci U S A 2013; 110:20994-9; PMID:24324137; http://dx.doi.org/10.1073/pnas.1311423110
4. Pulk A, Cate JH. Control of ribosomal subunit rotation by elongation factor G. Science 2013; 340:1235970; PMID:23812721; http://dx.doi.org/10.1126/science.1235970
5. Zhou J, Lancaster L, Donohue JP, Noller HF. Crystal structures of EF-G-ribosome complexes trapped in intermediate states of translocation. Science 2013; 340:1236086; PMID:23812722; http://dx.doi.org/10.1126/science.1236086
6. Fischer N, Konevega AL, Wintermeyer W, Rodnina MV, Stark H. Ribosome dynamics and tRNA movement by time-resolved electron cryomicroscopy. Nature 2010; 466:329-33; PMID:20631791; http://dx.doi.org/10.1038/nature09206
7. Ratje AH, Loerke J, Mikolajka A, Brunner M, Hildebrand PW, Starosta AL, Dönhöfer A, Connell SR, Fucini P, Mielke T, et al. Head swivel on the ribosome facilitates translocation by means of intra-subunit tRNA hybrid sites. Nature 2010; 468:713-6; PMID:21124459; http://dx.doi.org/10.1038/nature09547
8. Ramrath DJ, Lancaster L, Sprink T, Mielke T, Loerke J, Noller HF, Spahn CM. Visualization of two transfer RNAs trapped in transit during elongation factor G-mediated translocation. Proc Natl Acad Sci U S A 2013; 110:20964-9; PMID:24324168; http://dx.doi.org/10.1073/pnas.1320387110
9. Dunkle JA, Wang L, Feldman MB, Pulk A, Chen VB, Kapral GJ, Noeske J, Richardson JS, Blanchard SC, Cate JH. Structures of the bacterial ribosome in classical and hybrid states of tRNA binding. Science 2011; 332:981-4; PMID:21596992; http://dx.doi.org/10.1126/science.1202692
10. Agirrezabala X, Lei J, Brunelle JL, Ortiz-Meoz RF, Green R, Frank J. Visualization of the hybrid state of tRNA binding promoted by spontaneous ratcheting of the ribosome. Mol Cell 2008; 32:190-7; PMID:18951087; http://dx.doi.org/10.1016/j.molcel.2008.10.001
11. Julian P, Konevega AL, Scheres SH, Lazaro M, Gil D, Wintermeyer W, Rodnina MV, Valle M. Structure of ratcheted ribosomes with tRNAs in hybrid states. Proc Natl Acad Sci U S A 2008; 105:16924-7; PMID:18971332; http://dx.doi.org/10.1073/pnas.0809587105
12. Guo Z, Noller HF. Rotation of the head of the 30S ribosomal subunit during mRNA translocation. Proc Natl Acad Sci U S A 2012; 109:20391-4; PMID:23188795; http://dx.doi.org/10.1073/pnas.1218999109
13. Ermolenko DN, Noller HF. mRNA translocation occurs during the second step of ribosomal intersubunit rotation. Nat Struct Mol Biol 2011; 18:457-62; PMID:21399643; http://dx.doi.org/10.1038/nsmb.2011
14. Marshall RA, Aitken CE, Dorywalska M, Puglisi JD. Translation at the single-molecule level. Annu Rev Biochem 2008; 77:177-203; PMID:18518820; http://dx.doi.org/10.1146/annurev.biochem.77.070606.101431
15. Bock LV, Blau C, Schroder GF, Davydov, II, Fischer N, Stark H, Rodnina MV, Vaiana AC, Grubmüller H. Energy barriers and driving forces in tRNA translocation through the ribosome. Nat Struct Mol Biol 2013; 20:1390-6; PMID:24186064; http://dx.doi.org/10.1038/nsmb.2690
16. Munro JB, Wasserman MR, Altman RB, Wang L, Blanchard SC. Correlated conformational events in EF-G and the ribosome regulate translocation. Nat Struct Mol Biol 2010; 17:1470-7; PMID:21057527; http://dx.doi.org/10.1038/nsmb.1925
17. Frank J, Jr, Gonzalez RL. Structure and dynamics of a processive Brownian motor: the translating ribosome. Annu Rev Biochem 2010; 79:381-412; PMID:20235828; http://dx.doi.org/10.1146/annurev-biochem-060408-173330
18. Dunkle JA, Cate JH. Ribosome structure and dynamics during translocation and termination. Annu Rev Biophys 2010; 39:227-44; PMID:20192776; http://dx.doi.org/10.1146/annurev.biophys.37.032807.125954
19. Korostelev A, Ermolenko DN, Noller HF. Structural dynamics of the ribosome. Curr Opin Chem Biol 2008; 12:674-83; PMID:18848900; http://dx.doi.org/10.1016/j.cbpa.2008.08.037
20. Munro JB, Sanbonmatsu KY, Spahn CM, Blanchard SC. Navigating the ribosome's metastable energy landscape. Trends Biochem Sci 2009; 34:390-400; PMID:19647434; http://dx.doi.org/10.1016/j.tibs.2009.04.004
21. Holtkamp W, Wintermeyer W, Rodnina MV. Synchronous tRNA movements during translocation on the ribosome are orchestrated by

elongation factor G and GTP hydrolysis. Bioessays 2014; 36:908-18; PMID:25118068; http://dx.doi.org/10.1002/bies.201400076
22. Savelsbergh A, Katunin VI, Mohr D, Peske F, Rodnina MV, Wintermeyer W. An elongation factor G-induced ribosome rearrangement precedes tRNA-mRNA translocation. Mol Cell 2003; 11:1517-23; PMID:12820965; http://dx.doi.org/10.1016/S1097-2765(03)00230-2
23. Rodnina MV, Savelsbergh A, Katunin VI, Wintermeyer W. Hydrolysis of GTP by elongation factor G drives tRNA movement on the ribosome. Nature 1997; 385:37-41; PMID:8985244; http://dx.doi.org/10.1038/385037a0
24. Wilson DN. Ribosome-targeting antibiotics and mechanisms of bacterial resistance. Nat Rev Microbiol 2014; 12:35-48; PMID:24336183; http://dx.doi.org/10.1038/nrmicro3155
25. Budkevich TV, Giesebrecht J, Behrmann E, Loerke J, Ramrath DJ, Mielke T, Ismer J, Hildebrand PW, Tung CS, Nierhaus KH, et al. Regulation of the mammalian elongation cycle by subunit rolling: a eukaryotic-specific ribosome rearrangement. Cell 2014; 158:121-31; PMID:24995983; http://dx.doi.org/10.1016/j.cell.2014.04.044
26. Belardinelli R, Sharma H, Caliskan N, Cunha CE, Peske F, Wintermeyer W, Rodnina MV. Choreography of molecular movements during ribosome progression along mRNA. Nat Struct Mol Biol 2016; 23:342-8; PMID:26999556; http://dx.doi.org/10.1038/nsmb.3193
27. Sharma H, Adio S, Senyushkina T, Belardinelli R, Peske F, Rodnina MV. Kinetics of spontaneous and EF-G-accelerated rotation of ribosomal subunits. Cell Rep 2016; 16(8):2187-96; PMID:27524615; http://dx.doi.org/10.1016/j.celrep.2016.07.051
28. Wasserman MR, Alejo JL, Altman RB, Blanchard SC. Multiperspective smFRET reveals rate-determining late intermediates of ribosomal translocation. Nat Struct Mol Biol 2016; 23:333-41; PMID:26926435; http://dx.doi.org/10.1038/nsmb.3177
29. Cornish PV, Ermolenko DN, Noller HF, Ha T. Spontaneous intersubunit rotation in single ribosomes. Mol Cell 2008; 30:578-88; PMID:18538656; http://dx.doi.org/10.1016/j.molcel.2008.05.004
30. Fei J, Kosuri P, MacDougall DD, Gonzalez RL, Jr. Coupling of ribosomal L1 stalk and tRNA dynamics during translation elongation. Mol Cell 2008; 30:348-59; PMID:18471980; http://dx.doi.org/10.1016/j.molcel.2008.03.012
31. Adio S, Senyushkina T, Peske F, Fischer N, Wintermeyer W, Rodnina MV. Fluctuations between multiple EF-G-induced chimeric tRNA states during translocation on the ribosome. Nat Commun 2015; 6:7442; PMID:26072700; http://dx.doi.org/10.1038/ncomms8442
32. Li W, Liu Z, Koripella RK, Langlois R, Sanyal S, Frank J. Activation of GTP hydrolysis in mRNA-tRNA translocation by elongation factor G. Sci Adv 2015; 1:e1500169; PMID:26229983; http://dx.doi.org/10.1126/sciadv.1500169
33. Lin J, Gagnon MG, Bulkley D, Steitz TA. Conformational changes of elongation factor G on the ribosome during tRNA translocation. Cell 2015; 160:219-27; PMID:25594181; http://dx.doi.org/10.1016/j.cell.2014.11.049
34. Walker SE, Shoji S, Pan D, Cooperman BS, Fredrick K. Role of hybrid tRNA-binding states in ribosomal translocation. Proc Natl Acad Sci U S A 2008; 105:9192-7; PMID:18591673; http://dx.doi.org/10.1073/pnas.0710146105
35. Chen C, Stevens B, Kaur J, Cabral D, Liu H, Wang Y, Zhang H, Rosenblum G, Smilansky Z, Goldman YE, et al. Single-molecule fluorescence measurements of ribosomal translocation dynamics. Mol Cell 2011; 42:367-77; PMID:21549313; http://dx.doi.org/10.1016/j.molcel.2011.03.024
36. Spiegel PC, Ermolenko DN, Noller HF. Elongation factor G stabilizes the hybrid-state conformation of the 70S ribosome. RNA 2007; 13:1473-82; PMID:17630323; http://dx.doi.org/10.1261/rna.601507
37. Horan LH, Noller HF. Intersubunit movement is required for ribosomal translocation. Proc Natl Acad Sci U S A 2007; 104:4881-5; PMID:17360328; http://dx.doi.org/10.1073/pnas.0700762104
38. Semenkov YP, Rodnina MV, Wintermeyer W. Energetic contribution of tRNA hybrid state formation to translocation catalysis on the ribosome. Nat Struct Biol 2000; 7:1027-31; PMID:11062557; http://dx.doi.org/10.1038/80938
39. Dorner S, Brunelle JL, Sharma D, Green R. The hybrid state of tRNA binding is an authentic translation elongation intermediate. Nat Struct Mol Biol 2006; 13:234-41; PMID:16501572; http://dx.doi.org/10.1038/nsmb1060
40. Lill R, Robertson JM, Wintermeyer W. Binding of the 3′ terminus of tRNA to 23S rRNA in the ribosomal exit site actively promotes translocation. EMBO J 1989; 8(12):3933-8; PMID:2583120
41. Pan D, Kirillov SV, Cooperman BS. Kinetically competent intermediates in the translocation step of protein synthesis. Mol Cell 2007; 25:519-29; PMID:17317625; http://dx.doi.org/10.1016/j.molcel.2007.01.014
42. Holtkamp W, Cunha CE, Peske F, Konevega AL, Wintermeyer W, Rodnina MV. GTP hydrolysis by EF-G synchronizes tRNA movement on small and large ribosomal subunits. EMBO J 2014; 33:1073-85; PMID:24614227; http://dx.doi.org/10.1002/embj.201387465
43. Cunha CE, Belardinelli R, Peske F, Holtkamp W, Wintermeyer W, Rodnina MV. Dual use of GTP hydrolysis by elongation factor G on the ribosome. Translation (Austin) 2013; 1:e24315; PMID:26824016; http://dx.doi.org/10.4161/trla.24315
44. Borovinskaya MA, Shoji S, Fredrick K, Cate JH. Structural basis for hygromycin B inhibition of protein biosynthesis. RNA 2008; 14:1590-9; PMID:18567815; http://dx.doi.org/10.1261/rna.1076908
45. Tourigny DS, Fernandez IS, Kelley AC, Ramakrishnan V. Elongation factor G bound to the ribosome in an intermediate state of translocation. Science 2013; 340:1235490; PMID:23812720; http://dx.doi.org/10.1126/science.1235490
46. Schuwirth BS, Borovinskaya MA, Hau CW, Zhang W, Vila-Sanjurjo A, Holton JM, Cate JH. Structures of the bacterial ribosome at 3.5 A resolution. Science 2005; 310:827-34; PMID:16272117; http://dx.doi.org/10.1126/science.1117230
47. Savelsbergh A, Mohr D, Kothe U, Wintermeyer W, Rodnina MV. Control of phosphate release from elongation factor G by ribosomal protein L7/12. EMBO J 2005; 24:4316-23; PMID:16292341; http://dx.doi.org/10.1038/sj.emboj.7600884
48. Peske F, Savelsbergh A, Katunin VI, Rodnina MV, Wintermeyer W. Conformational changes of the small ribosomal subunit during elongation factor G-dependent tRNA-mRNA translocation. J Mol Biol 2004; 343:1183-94; PMID:15491605; http://dx.doi.org/10.1016/j.jmb.2004.08.097
49. Salsi E, Farah E, Ermolenko DN. EF-G Activation by Phosphate Analogs. J Mol Biol 2016; 428:2248-58; PMID:27063503; http://dx.doi.org/10.1016/j.jmb.2016.03.032
50. Savelsbergh A, Rodnina MV, Wintermeyer W. Distinct functions of elongation factor G in ribosome recycling and translocation. RNA 2009; 15:772-80; PMID:19324963; http://dx.doi.org/10.1261/rna.1592509
51. Zhang J, Pan X, Yan K, Sun S, Gao N, Sui SF. Mechanisms of ribosome stalling by SecM at multiple elongation steps. eLife 2015; 4:e09684; PMID:26670735; http://dx.doi.org/10.7554/eLife.09684
52. Chen C, Cui X, Beausang JF, Zhang H, Farrell I, Cooperman BS, Goldman YE. Elongation factor G initiates translocation through a power stroke. Proc Natl Acad Sci U S A 2016; 113:7515-20; PMID:27313204; http://dx.doi.org/10.1073/pnas.1602668113
53. Wilson KS, Noller HF. Mapping the position of translational elongation factor EF-G in the ribosome by directed hydroxyl radical probing. Cell 1998; 92:131-9; PMID:9489706; http://dx.doi.org/10.1016/S0092-8674(00)80905-8
54. Wang L, Pulk A, Wasserman MR, Feldman MB, Altman RB, Cate JH, Blanchard SC. Allosteric control of the ribosome by small-molecule antibiotics. Nat Struct Mol Biol 2012; 19:957-63; PMID:22902368; http://dx.doi.org/10.1038/nsmb.2360
55. Ermolenko DN, Spiegel PC, Majumdar ZK, Hickerson RP, Clegg RM, Noller HF. The antibiotic viomycin traps the ribosome in an intermediate state of translocation. Nat Struct Mol Biol 2007; 14:493-7; PMID:17515906; http://dx.doi.org/10.1038/nsmb1243
56. Caliskan N, Katunin VI, Belardinelli R, Peske F, Rodnina MV. Programmed -1 frameshifting by kinetic partitioning during impeded translocation. Cell 2014; 157:1619-31; PMID:24949973; http://dx.doi.org/10.1016/j.cell.2014.04.041
57. Qin P, Yu D, Zuo X, Cornish PV. Structured mRNA induces the ribosome into a hyper-rotated state. EMBO Rep 2014; 15:185-90; PMID:24401932; http://dx.doi.org/10.1002/embr.201337762

58. Kim HK, Liu F, Fei J, Bustamante C, Gonzalez RL, Jr, Tinoco I, Jr. A frameshifting stimulatory stem loop destabilizes the hybrid state and impedes ribosomal translocation. Proc Natl Acad Sci U S A 2014; 111:5538-43; PMID:24706807; http://dx.doi.org/10.1073/pnas.1403457111
59. Samatova E, Konevega AL, Wills NM, Atkins JF, Rodnina MV. High-efficiency translational bypassing of non-coding nucleotides specified by mRNA structure and nascent peptide. Nat Commun 2014; 5:4459; PMID:25041899; http://dx.doi.org/10.1038/ncomms5459
60. Chen J, Coakley A, O'Connor M, Petrov A, O'Leary SE, Atkins JF, Puglisi JD. Coupling of mRNA structure rearrangement to ribosome movement during bypassing of non-coding regions. Cell 2015; 163:1267-80; PMID:26590426; http://dx.doi.org/10.1016/j.cell.2015.10.064
61. Zhou J, Lancaster L, Donohue JP, Noller HF. How the ribosome hands the A-site tRNA to the P site during EF-G-catalyzed translocation. Science 2014; 345:1188-91; PMID:25190797; http://dx.doi.org/10.1126/science.1255030