Structural insights into ribosome translocation

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During protein synthesis, tRNA and mRNA are translocated from the A to P to E sites of the ribosome thus enabling the ribosome to translate one codon of mRNA after the other. Ribosome translocation along mRNA is induced by the universally conserved ribosome GTPase, elongation factor G (EF-G) in bacteria and elongation factor 2 (EF-2) in eukaryotes. Recent structural and single-molecule studies revealed that tRNA and mRNA translocation within the ribosome is accompanied by cyclic forward and reverse rotations between the large and small ribosomal subunits parallel to the plane of the intersubunit interface. In addition, during ribosome translocation, the 'head' domain of small ribosomal subunit undergoes forward- and back-swiveling motions relative to the rest of the small ribosomal subunit around the axis that is orthogonal to the axis of intersubunit rotation. tRNA/mRNA translocation is also coupled to the docking of domain IV of EF-G into the A site of the small ribosomal subunit that converts the thermally driven motions of the ribosome and tRNA into the forward translocation of tRNA/mRNA inside the ribosome. Despite recent and enormous progress made in the understanding of the molecular mechanism of ribosome translocation, the sequence of structural rearrangements of the ribosome, EF-G and tRNA during translocation is still not fully established and awaits further investigation. © 2016 The Authors. *WIREs RNA* published by Wiley Periodicals, Inc.

INTRODUCTION

The ribosome translates the sequence of codons in mRNA to synthesize proteins in all living organisms. mRNA codons are decoded by the binding of tRNA molecules charged with amino acids. Both the small and large ribosomal subunits contain three tRNA binding sites: the A (aminoacyl) site, the P (peptidyl) site and the E (exit) site (Figure 1(a)). To extend the polypeptide chain by one amino acid, the ribosome undergoes an elongation cycle that begins with binding of an aminoacyl-tRNA to the A site

followed by the catalysis of peptide transfer from the P- to the A-site tRNA. The elongation cycle is completed when the resulting peptidyl A-site and deacylated P-site tRNAs are translocated to the P and E sites, respectively. tRNA translocation is coupled to the movement of the associated codons of the mRNA through the ribosome and is catalyzed by a universally conserved elongation factor (EF-G in prokaryotes and EF-2 in eukaryotes). Ribosomal translocation is an essential facet of protein synthesis in all organisms. Additionally, studies of the molecular mechanism of ribosomal translocation contribute to the understanding of the general physical and structural principles underlying the mechanics of macromolecules and macromolecular complexes that undergo unidirectional movement in the cell. Because of the fundamental importance of translocation for protein synthesis and the complexity of the translocation mechanism, this problem remains one of the

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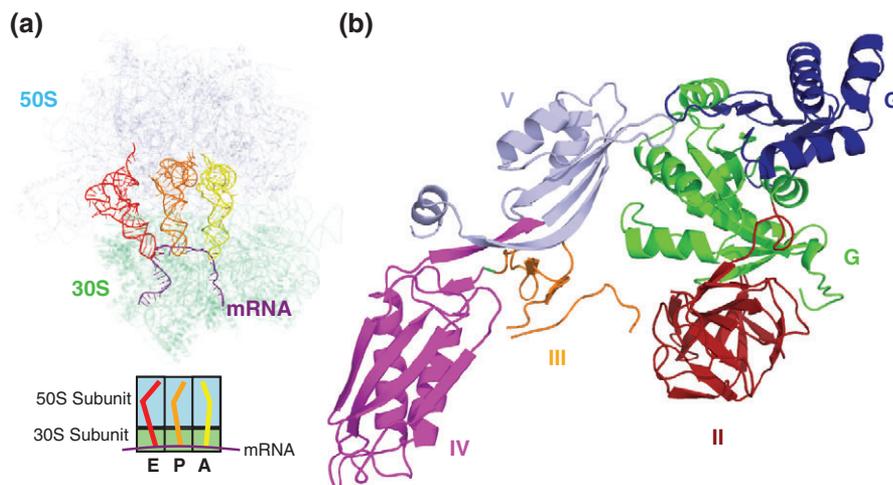
FIGURE 1 | Structural organization of the ribosome and elongation factor G. (a) Crystal structure of the 70S ribosome (Protein Data Bank ID [PDBID] 4V6F[1]). Large, 50S subunit and small, 30S subunit are colored in light blue and light green, respectively. A-site, P-site, and E-site tRNAs are shown in yellow, orange, and red, respectively. mRNA is colored purple. A box diagram of the ribosome showing tRNAs bound in the A, P, and E sites of the 50S and 30S subunits is shown below the crystal structure of the 70S ribosome. (b) Crystal structure of ribosome-free EF-G (PDBID 1DAR[2]) with domains color-coded: G′ domain (dark blue), G domain (green), domain II (dark red), domain III (orange), domain IV (magenta), and domain V (light blue).

most fascinating and popular topics in the field of protein synthesis. The emergence of high-resolution cryo-EM and X-ray crystal structures of the ribosome as well as single-molecule Förster resonance energy transfer (smFRET) and optical tweezers approaches has led to tremendous progress in the understanding of the translocation mechanism in recent years. Nevertheless, a number of important details remain obscure and require further investigation.

Below we summarize recent structural and biochemical studies of the translocation of bacterial 70S ribosomes. The functional core of the ribosome, which includes sites of tRNA and EF-G binding, is conserved throughout all branches of life. Hence, the main features of the translocation mechanism discovered in bacteria are likely similar in archaea and eukaryotes.

FUNDAMENTALS OF RIBOSOMAL TRANSLOCATION

Translocation Is Augmented by the Binding of EF-G · GTP to the Ribosome

EF-G is a five-domain protein[2,3] that accelerates translocation by ~50,000-fold.[4,5] Domain I of EF-G (Figure 1(b)) comprises the G′ and G subdomains; the latter hydrolyses GTP and is structurally similar to the G-domains in other G-proteins.[2,3] Most published reports suggest that EF-G binds to the ribosome with high affinity and induces translocation only in GTP-bound form.[6,7] EF-G · GDP and nucleotide-free EF-G do not show significant translocation activity.[6,8–10] EF-G has low intrinsic GTPase activity, which is dramatically enhanced via interaction of the G domain of EF-G with the universally conserved sarcin-ricin loop (SRL) of the 23S rRNA of the large ribosomal subunit.[11–13] GTP hydrolysis and the subsequent release of inorganic phosphate trigger EF-G dissociation from the ribosome.[7,14]

Although GTP hydrolysis precedes mRNA/tRNA translocation, the release of inorganic phosphate after GTP hydrolysis and mRNA translocation occur at similar rates. Hence, these two events may be coupled through conformational changes in EF-G that are induced by Pi release.[14] However, inhibiting the ability of EF-G to hydrolyze GTP by amino acid substitutions in the G domain of EF-G,[15–18] or replacing GTP with non-hydrolyzable analogues of GTP[4,9,19] severely hamper EF-G turnover but only moderately slow down the rate of a single round of translocation (by 2- to 50-fold, depending on experimental conditions[4,9,19]). Furthermore, a number of antibiotics, such as viomycin or hygromycin B, strongly inhibit translocation by binding to the ribosome without impeding the binding of EF-G, GTP hydrolysis or inorganic phosphate (P$_i$) release[4,20,21] (Table 1). Hence, GTP hydrolysis and translocation do not appear to be directly coupled. Consistent with this model, smFRET experiments show that not every

TABLE 1 | Inhibitors of Ribosomal Translocation

Inhibitor	Domain of Life	Mechanism
Aminoglycosides (gentamycin, hygromycin B, kanamycin, neomycin, paromomycin)	B, A, E	Aminoglycosides inhibit both EF-G-induced and spontaneous translocation by increasing the affinity of peptidyl-tRNA to the A site.[22]
Spectinomycin	B, A	Spectinomycin is thought to inhibit translocation by interfering with swiveling of the head of the small ribosomal subunit.[22]
Tuberactinomycins (viomycin and capreomycin)	B	Viomycin and capreomycin inhibit both EF-G-induced and spontaneous translocation by increasing the affinity of peptidyl-tRNA to the A site and locking the ribosome in the rotated hybrid state.[20,25,26]
Pactamycin	B, A, E	Pactamycin inhibits translocation by occluding the mRNA-binding channel in the E site of the small ribosomal subunit.[27,28]
Thiostrepton	B, A	Thiostrepton inhibits binding of EF-G to the ribosome.[26,29,30]
Fusidic acid	B, A, E	Fusidic acid inhibits EF-G/EF-2 release after GTP hydrolysis.[31]
Sordarin	E(fungi)	Inhibits EF-2 release from the ribosome after GTP hydrolysis.[32]
EF-2 kinase	E	EF-2 kinase phosphorylates a conserved threonine residue (Thr 56 in human EF-2) in domain I of EF-2, which hampers EF-2 binding to the ribosome.[33]
Diphtheria toxin	E(human)	Diphtheria toxin abrogates EF-2 activity by catalyzing ADP-ribosylation of the diphtamide residue (i.e. posttranslationallly-modified His714 in human EF-2).[34]
α-sarcin and ricin-like ribotoxins	B, A, E	These ribotoxins can cleave (α-sarcin) or depurinate (ricin) the SRL of the rRNA of the large subunit, which activates GTPase activity of EF-G (EF-2) and EF-Tu (EF-1A).[22]

The column 'Domain of life' indicates whether the translocation inhibitors are active in bacteria (B), archaea (A) or eukaryotes (E).

EF-G binding event results in translocation[17,35,36] suggesting that EF-G dissociation triggered by P_i release sometimes occurs before tRNA/mRNA are translocated. Thus, binding of EF-G · GTP to the ribosome makes a major contribution to the catalysis of translocation while GTP hydrolysis mainly serves to stimulate EF-G release.

Translocation of mRNA Is Driven by tRNA Movement

Toeprinting[37] and single-molecule optical tweezers experiments[38] show that mRNA is translocated inside the ribosome three nucleotides at a time in a codon-by-codon manner without detectable intermediates, suggesting that the translocation of mRNA is coupled to the movement of the associated anticodon stem-loops (ASLs) of tRNAs. Indeed, mRNA translocation requires the presence of an ASL bound in the A site of the small subunit and a full-length deacylated tRNA bound in the P site of the ribosome.[37] Binding of EF-G · GTP to ribosomes containing a single tRNA bound in the P site does not induce translocation.[37] By contrast, translocation of tRNAs through the ribosome can be induced by EF-G in the absence of an mRNA template.[39] Mutations in tRNAs that allow the tRNA anticodon loop to form four instead of three basepairs with the nucleotides of mRNA result in the translocation of mRNA by four nucleotides.[40,41] These observations provide further support for the idea that mRNA translocation is passive and is driven by tRNA movement. Nevertheless, structural rearrangements of the small ribosomal subunit may also play a role in directing and facilitating mRNA movement.[42]

Translocation Is an Intrinsic Feature of the Ribosome

Under certain experimental conditions, translocation of mRNA and tRNA inside the ribosome can occur in absence of EF-G, albeit very slowly.[43,44] Spontaneous translocation is stimulated by a decrease in the concentration of magnesium ions,[44] modification of ribosomal proteins with thiol-specific reagents[45,46] or removal of ribosomal proteins S12 and S13.[47]

Furthermore, single-round translocation can be induced by the peptidyl-transferase inhibitors, sparsomycin, linkomycin, and chloramphenicol.[48,49]

A number of antibiotics bind to the ribosome and impede both EF-G-induced and spontaneous translocation[47–49] (Table 1) providing evidence for underlying similarities between the mechanisms of these phenomena and suggesting that tRNA/mRNA translocation is, to a degree, an inherent property of the ribosome. Indeed, structural studies showed that the movement of tRNA/mRNA is accompanied by conformational changes of the ribosome, which will be discussed in detail below.

STRUCTURAL INTERMEDIATES OF RIBOSOMAL TRANSLOCATION

Hybrid-State Intermediate of Translocation

The translocation of tRNAs appears to occur in a stepwise fashion. Early mapping of tRNA binding sites on the ribosomes using chemical probing revealed that the transpeptidation reaction triggers the spontaneous movement of the acceptor stems of the resulting peptidyl-tRNA and deacylated tRNA from A and P to P and E sites of the large subunit, respectively, while tRNA ASLs remain in the A and P sites of the small subunit.[50] Thus, the tRNAs move into the hybrid A/P and P/E sites[50] (Figure 2(a) and (b)). FRET, chemical probing and cryo-EM experiments suggested that, in pretranslocation ribosomes, the spontaneous movement of tRNAs into the hybrid state is coupled to rotation of the platform and body domains of the small ribosomal subunit by 8–10°[25,51–53] (Figure 3(a)). The intersubunit rotation is often described as a ratchet-like movement or simply 'ratcheting'.[57] smFRET experiments show that, in

the absence of EF-G, pretranslocation ribosomes spontaneously fluctuate between nonrotated, classical and rotated, hybrid state conformations.[58–61] smFRET experiments also suggest that the nonrotated, classical and the rotated, hybrid state conformations of pretranslocation ribosomes have similar thermodynamic stabilities.[58,60–62]

Available structures of the ribosome suggest that the movement of deacylated tRNA from the classical P/P to the hybrid P/E state is strictly coupled to intersubunit rotation. By contrast, the peptidyl-tRNA can spontaneously sample both classical A/A and hybrid A/P states in ribosomes that adopt the rotated conformation.[59,63] Furthermore, in rotated bacterial and mammalian ribosomes containing a deacylated tRNA bound in the hybrid P/E state, the peptidyl-tRNA can adopt another conformation that is distinct from canonical A/A and A/P states. This distinct conformation was termed A/P*[55] (A/P in Ref[64] and 'state 5' in Ref[65]). Similar to the canonical A/P-tRNA, the anticodon and CCA ends of the A/P* tRNA are positioned in the A and P sites of the small and large ribosomal subunits, respectively, whereas the tRNA elbow is moved around the A site finger (conserved helix 38 of 23S or 28S rRNA) by 20–25Å toward the P site relative to canonical A/P state[55] (Figure 2(c)).

In the absence of EF-G, spontaneous fluctuations of the pretranslocation ribosome between nonrotated, classical and rotated, hybrid states do not lead to efficient translocation of tRNA/mRNA on the small subunit, which evidently requires EF-G. Furthermore, peptidyl-tRNAs bound in the A/P state appear to react with the antibiotic puromycin, a 50S A-site aminoacyl-tRNA mimic, with lower efficiency than peptidyl-tRNAs bound in the classical P/P state.[50,66,67] Hence, the movement of the peptidyl-tRNA on the large subunit into a fully puromycin-reactive conformation also necessitates EF-G.[50,66,67]
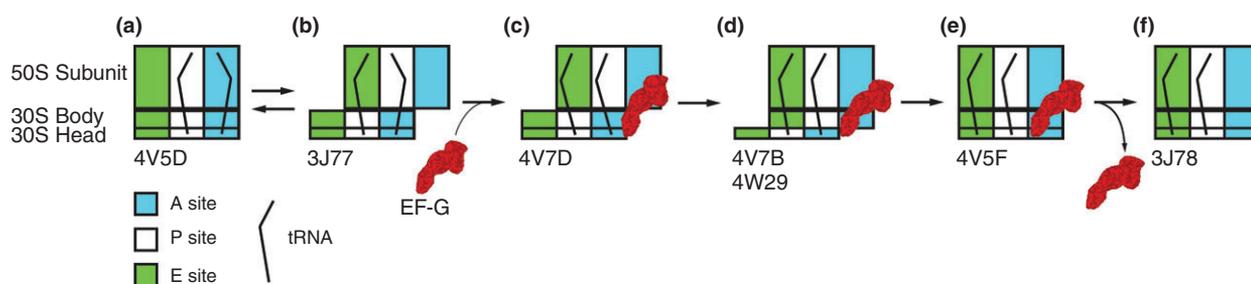

FIGURE 2 | Scheme of tRNA rearrangements during EF-G-catalyzed ribosome translocation. Diagrams show tRNA positions relative to the A, P, and E sites on the 50S subunit and 30S head and body. (a) peptidyl- and deacylated tRNAs are bound in A/A and P/P classical states, (b) A/P and P/E hybrid states, (c) A/P* and P/E states in the presence of ribosome-bound EF-G, (d) ap/P and pe/E chimeric states in the presence of ribosome-bound EF-G, (e) classical P/P and E/E state in the presence of ribosome-bound EF-G, and (f) classical P/P and E/E state after EF-G dissociation. Please see additional details in the text. PDB IDs corresponding to each structural state are indicated under the schematic.
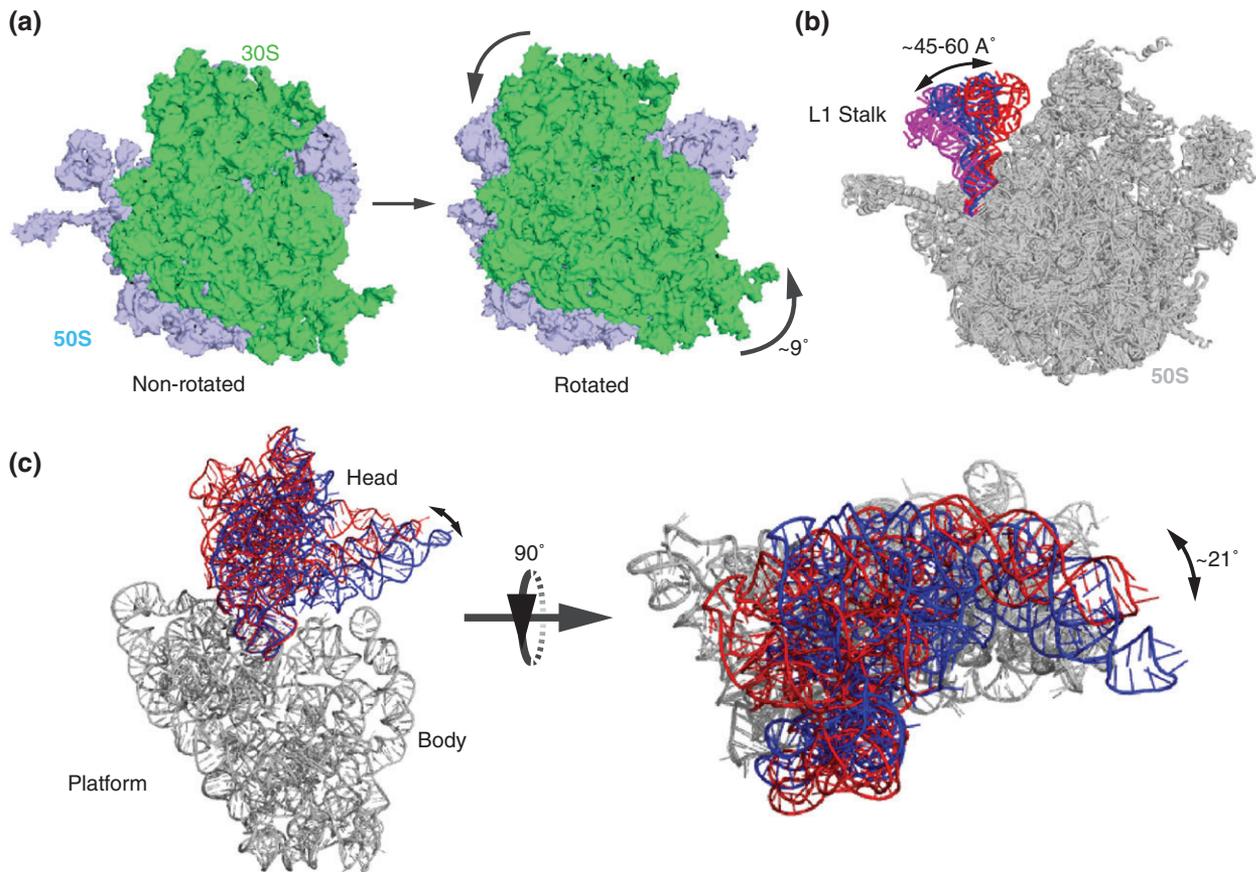
FIGURE 3 | Structural rearrangements of the ribosome that accompany translocation. (a) Structures of the ribosome in the non-rotated, classical state[54] (PDBID 4V9D) and the rotated, hybrid state[55] (PDBID 4V7C). The small subunit is shown in light green and the large subunit is shown light blue. The ribosome is viewed from the solvent side of the small subunit. Curved arrows indicate the counter-clockwise rotation of the small subunit relative to the large subunit. (b) 50S L1 stalk (23S rRNA helices 76, 77, and 78) is shown in the closed[54] (red, PDBID 4V9D), half-closed[1] (blue, PDBID 4V6F), and open[54] (magenta, PDBID 4V9D) positions. The rest of the large subunit is shown in gray. Structures were superimposed by structural alignment of 23S rRNA. (c) The swiveling motion of the head domain of the small subunit is shown by structural alignment of the body and platform domains of 16S rRNA (shown in gray) of crystal structures of nonrotated ribosome containing classical A/A, P/P, and E/E site tRNAs (head domain in blue, PDBID 4V51[56]) and partially rotated ribosome containing a chimeric ap/ap and pe/E tRNAs (head domain in red, PDBID 4W29[42]). The 30S is viewed from its solvent side (left) and the 'top' of the small subunit head (right). Double-headed arrows indicate the direction of head swiveling, which is perpendicular to the long axis of the small subunit.

Mutations in rRNA that destabilize tRNA binding in the hybrid states only moderately affect the rate of mRNA and tRNA translocation.[68–70] Therefore, formation of the hybrid state intermediate is not the rate-limiting step of translocation. Additional EF-G-induced rearrangements of the ribosome and tRNAs are likely required to facilitate tRNA/mRNA translocation.

Coupling of Intersubunit Rotation and mRNA Translocation

Upon binding to the pretranslocation ribosome, EF-G was shown to transiently stabilize the rotated,

hybrid state[10,19,57,71] and subsequently induce the transition into the nonrotated, classical state conformation of the ribosome[19,35,51] (Figure 2). In contrast to pretranslocation ribosomes containing a deacylated P-site tRNA that spontaneously fluctuates between nonrotated, classical and rotated hybrid state conformations, posttranslocation ribosomes containing a peptidyl-tRNA in the P/P state are predominately fixed in the nonrotated conformation.[60] The intersubunit rotation and the movement of peptidyl-tRNA from the classical P/P into the hybrid P/E state are likely blocked in posttranslocation ribosomes because the 50S E site binds only deacylated tRNA.[72,73] Therefore, the acylation state of the P-site

tRNA controls the structural dynamics of tRNAs and the ribosome.

Translocation of mRNA and tRNA on the small subunit was shown in kinetic experiments to occur at a rate similar to the rate of reverse rotation of the small subunit from the rotated back into the nonrotated conformation.[19] Inhibition of translocation with the antibiotics spectinomycin or hygromycin B slows down mRNA translocation and the reverse movement of the small subunit to the same extent, further supporting the idea that the translocation of mRNA and ASLs of tRNA are coupled to the reverse rotation of the small subunit.[19] Consistent with these results, the antibiotics viomycin and neomycin, inhibitors of translocation, were shown to perturb intersubunit rotation by trapping the ribosome in conformations in which subunits are rotated relative to each other by ~10°[25,55] and 6°,[74] respectively. Furthermore, a cross-link introduced between proteins S6 of the 30S and L2 of the 50S subunits to block intersubunit rotation was shown to completely abolish ribosomal translocation[75] demonstrating that intersubunit rotation is essential for translocation.

Movement of the L1 Stalk of the Large Ribosomal Subunit

Intersubunit rotation and translocation of tRNAs are also coupled to the movement of a mobile domain of the large ribosomal subunit named the L1 stalk, which comprises ribosomal protein L1 and helices 76, 77 and 78 of 23S rRNA[71] (Figure 3(b)). In the classical, nonrotated state of prestranslation ribosomes containing tRNAs bound in A/A and P/P state the L1 stalk is predominantly positioned in the 'open' conformation, oriented away from the core of the 50S subunit.[54,61,76] In the rotated, hybrid state of pretranslation ribosomes, the L1 stalk is moved inward by 45–60Å (depending on the points chosen for distance measurements) into the 'closed' position and interacts with the elbow of the P/E tRNA[54,61,71,76] (Figure 3(b)). In the absence of EF-G, the L1 stalk spontaneously fluctuates between the open and closed positions in a movement that is coupled to the fluctuations of tRNAs between classical and hybrid states, respectively.[61,76] As a result of EF-G-induced translocation, the L1 stalk adopts an intermediate position between the closed and open positions (i.e., 'half-closed' state) moving outward by 15–20 Å relative to fully closed position[76,77] (Figure 3(b)). In this half-closed conformation, the L1 stalk retains its interactions with the elbow of the deacylated tRNA, which is bound in the E/E classical state.[1,78] The L1 stalk moves into the open position

when the deacylated E-site tRNA disassociates from the ribosome.[76,79] It appears that the movement of the L1 stalk enables remodeling of the 50S E site between the E/E vacant, P/E hybrid and E/E classical states and facilitates the translocation and release of deacylated tRNA (Box 1).

Swiveling Motion of the 30S Head

The small ribosomal subunit comprises three structural domains: head, body and platform (Figure 3 (c)). In a number of structures of both bacterial and eukaryotic ribosomes, a large rotation of the head domain of the small subunit by up to 21° relative to the rest of the small subunit was observed[57,84,85] (Figure 3(c)). The axis of this rotation, termed head swivel, is perpendicular to the axis of intersubunit rotation. The head swivel moves the ASLs of tRNAs and the associated mRNA codons along the direction of translocation through the ribosome. Furthermore, the head swivel is thought to be essential for ribosomal translocation because it opens a wide (over 20 Å) path for tRNA translocation between the P and E sites on the small subunit that is otherwise constricted by the rRNA residues of the head and

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BOX 1

INTERSUBUNIT ROTATION ACCOMPANIES TRANSLATION IN BOTH BACTERIA AND EUKARYOTES.

Nonrotated, classical and rotated, hybrid state conformations of the ribosome appear to be the two predominant conformations that the ribosome adopts during protein synthesis. In addition to sampling the classical and hybrid states during the elongation cycle, the ribosome adopts the nonrotated conformation in complex with release factors 1 and 2 (RF1 and RF2) during translation termination.[23,24,80] The rotated conformation of the ribosome that contains a single deacylated tRNA bound in the hybrid P/E state is sampled during the termination and recycling phases of protein synthesis and is stabilized by bacterial release factor 3 (RF3)[51,80,81] and ribosome recycling factor (RRF).[54,80] Furthermore, the rotated conformation of the ribosome and hybrid states of tRNA binding were also observed in yeast and mammalian ribosomes, suggesting that hybrid state formation is a universal feature of protein synthesis.[64,82,83]

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platform of the small subunit.[85] Kinetic measurements of FRET between fluorophores introduced into the 30S head and body or 30S head and platform demonstrated that the 30S head swiveling indeed accompanies translocation.[86] Moreover, the antibiotic spectinomycin is thought to inhibit translocation in bacteria by binding to one of the hinges connecting the head with the rest of the small subunit and, thus, trapping the head of the small subunit in a partially swiveled state.[87–89] Analysis of available structures suggests that in contrast to intersubunit rotation and L1 stalk movement, the 30S head swivel does not correlate with spontaneous movement of tRNAs into A/P and P/E hybrids states.[88] Consistent with this idea, ensemble kinetic and smFRET experiments suggest that spectinomycin stabilizes an intermediate of translocation that is distinct from the hybrid state intermediate.[9,36]

Kinetic FRET experiments showed that the 30S head swivel is triggered by EF-G binding and may follow the formation of the hybrid, rotated state but precede translocation of tRNAs on the small subunit.[86] The back-swiveling motion of the 30S head into the non-swiveled conformation occurs at a rate that is similar to the rates of mRNA translocation and reverse intersubunit rotation.[86] Although swiveling and back-swiveling motions of the 30S head were resolved in these kinetic translocation experiments, an intermediate of translocation that shows a significant degree (≥8°) of both intersubunit rotation and 30S head swiveling in 2-tRNA · ribosome complexes is yet to be visualized using cryo-EM or X-ray crystallography.

Several cryo-EM and crystallographic studies revealed an EF-G-bound structural state of the ribosome, in which ribosomal subunits are rotated by less than 3.5°, while the 30S head is swiveled by 18–21°.[42,90–92] In this structural state of the ribosome, tRNAs are in unique binding sites called 'chimeric' ap/P (ap/ap) and pe/E states (Figure 2(e)). The acceptor arms of ap/P and pe/E tRNAs are bound in the posttranslocation P and E site on the large subunits. Their ASLs interact with posttranslocation P and E sites of the platform and the body of the 30S while retaining interactions with elements of the 30S A and P site located in the 30S head. Hence, 'chimeric' ap/P (ap/ap) and pe/E tRNAs are trapped midway between canonical hybrid A/P and P/E and posttranslocation classical P/P and E/E states. This structural state of the ribosome may represent a late intermediate of translocation, which is formed as a result of partial reverse rotation of the 30S platform and body from the rotated, hybrid state intermediate of translocation. Further kinetic studies are required

to clarify the possible order and coupling of reverse intersubunit rotation, back-swivel of the head domain of the small subunit and translocation of tRNA/mRNA on the small subunit.

Structural Rearrangements of EF-G during Ribosomal Translocation

Structural and smFRET studies showed that in addition to conformational changes of the ribosome, translocation is also accompanied by rearrangements of ribosome-bound EF-G. Recent cryo-EM and X-ray crystallography studies allowed for visualization of EF-G · ribosome · 2-tRNA complexes in four different conformations: (1) EF-G bound to the nonrotated pretranslocation ribosome containing A/A- and P/P- tRNAs[93] (Figure 4(a)); (2) EF-G bound to the fully rotated pretranslocation ribosome containing A/P*- and P/E- tRNAs[55] (Figure 4(b)); (3) EF-G bound to moderately rotated ribosomes containing tRNAs in chimeric ap/P-(ap/ap) and pe/E- tRNAs[42,94] (Figure 4(c)) and (4) EF-G bound to the nonrotated posttranslocation ribosome containing P/P- and E/E-tRNAs[13] (Figure 4(d)).

The key difference between these structures is the conformation of domain IV of EF-G. The elongated domain IV of EF-G is critical for the catalysis of translocation. Deletion or mutation of domain IV abolishes the translocation activity of EF-G.[4,95,96] Furthermore, diphtheria toxin inhibits protein synthesis in humans by ADP-ribosylation of a conserved post-translationally modified histidine residue located at the tip of domain IV.[34] In the structure of the posttranslocation ribosome, in which the A site of the ribosome is not occupied by tRNA, the elongated domain IV of EF-G is docked in the 30S A site[13,71] (Figures 4(d) and 5). EF-G bound to the ribosome containing tRNAs in the ap/P (ap/ap) and pe/E chimeric states adopts a conformation that is very similar to its posttranslocation conformation[42,94] (Figure 4(c)). However, domain IV of EF-G is not fully docked in the 30S A site because of the large (~21°) swivel of the 30S head. In the pretranslocation EF-G-ribosome complexes captured in the presence of an inhibitor of translocation, the antibiotic viomycin, domain IV of EF-G is positioned approximately 15–20 Å away from the A site relative to the posttranslocation conformation of ribosome-bound EF-G[55] (Figures 4(b) and 5). smFRET data suggest that a similar pretranslocation conformation of EF-G, in which domain IV of EF-G is positioned away from the A site, is sampled during translocation not perturbed by antibiotics.[17] Evidently, tRNA translocation on the small subunit is accompanied by the
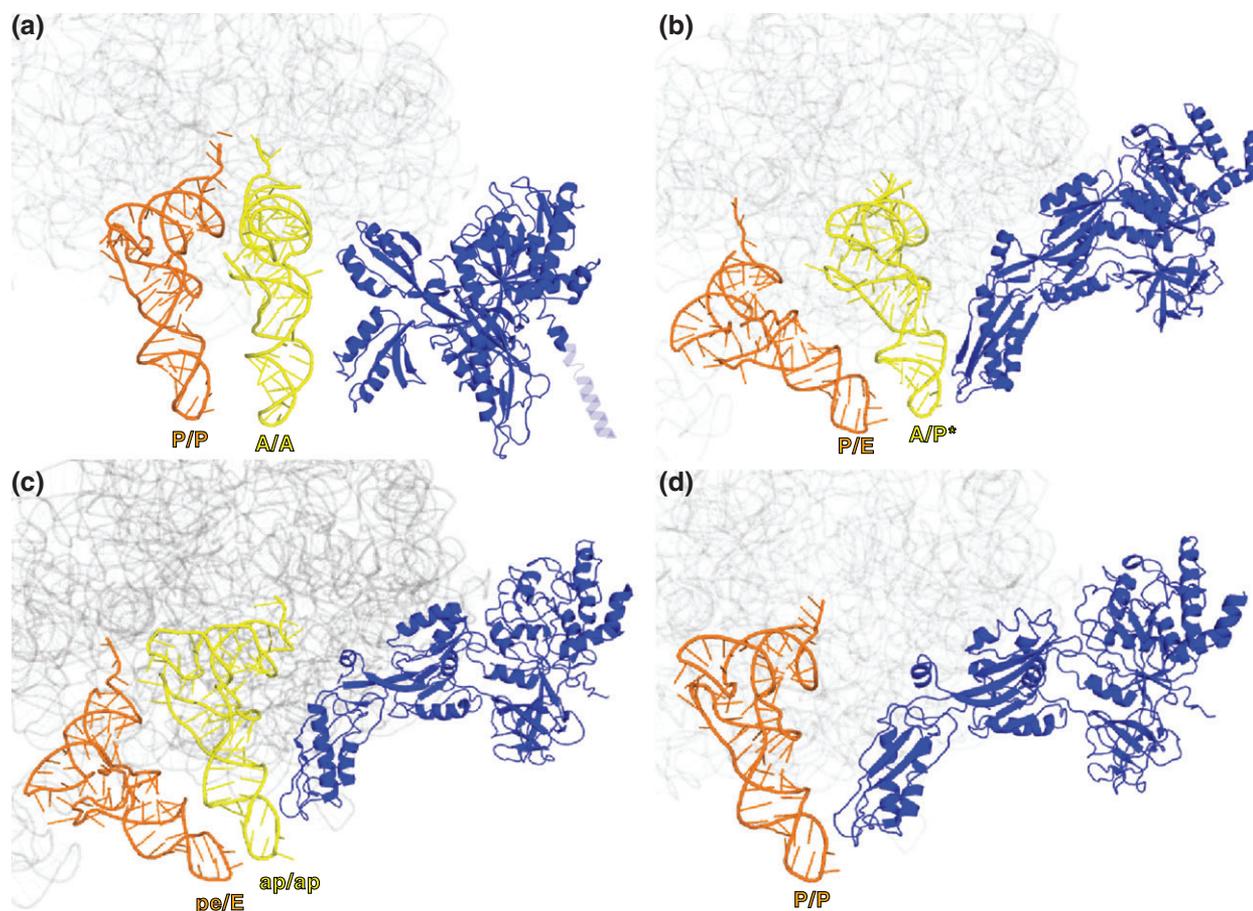
FIGURE 4 | Structural rearrangements of EF-G on the ribosome during translocation. (a) EF-G (blue in all structures) bound to the nonrotated pretranslocation ribosome containing A/A- and P/P- tRNAs[93] (PDBID 4WPO). The N-terminal domain of large subunit protein L9 covalently linked to the N-terminus of EF-G is shown as a transparent blue. (b) EF-G bound to the fully rotated pretranslocation ribosome containing A/P*- and P/E-tRNAs[55] (PDBID 4V7D); (c) EF-G bound to partially rotated ribosomes containing chimeric ap/ap and pe/E- tRNAs[42] (PDBID 4 W29) and (d) EF-G bound to the nonrotated posttranslocation ribosome containing P/P- and E/E- tRNAs[13] (PDBID 4V5F). 23S rRNA in all structures is shown in gray.

movement of domain IV toward the 30S A site from the conformation observed in the viomycin-trapped structure into the conformation observed in the structure of the postranslocation ribosome. This movement mostly results from the rotation of the entire EF-G around the SRL of the 23S rRNA[55] (Figure 5).

In addition to the movement relative to the ribosome, EF-G undergoes interdomain rearrangement that involves the movement of two superdomains, one formed by domains III, IV and V and the other formed by domains I and II, relative to each other (Figure 6). Crystallographic, cryo-EM and FRET studies showed that EF-G adopts fairly similar extended, elongated conformations when bound to (1) hybrid-state pretranslocation,[55] (2) chimeric-state pretranslocation[42,94] or (3) nonroated posttranslocation ribosomes[13] (Figure 6). By contrast, ribosome-free EF-G assumes a more compact conformation where superdomain III-IV-V is positioned closer to

superdomain I-II[2,3] (Figure 6(a)). The rearrangements between ribosome-bound, posttranslocation and ribosome-free conformations result in a movement of the tip of domain IV by approximately 30 Å (Figure 6(a)). An even more dramatic interdomain rearrangement of EF-G is observed in the structure of EF-G bound to a pretranslocation ribosome captured in the nonrotated conformation in the presence of the antibiotic dityromycin that was recently solved using X-ray crystallography.[93] In this structure, the tip of domain IV of EF-G is moved by approximately 100 Å relative to the conformation of EF-G bound to the posttranslocation ribosome (Figure 6(b)).

It has been hypothesized that EF-G promotes translocation by the movement of superdomain III-IV-V relative to superdomain I-II from highly compact to the extended conformation observed in posttranslocation ribosomes.[4,93] Indeed, restricting the conformational dynamics of EF-G by introducing an

intramolecular disulfide crosslink between domains I and V was shown to abolish EF-G translocation activity, suggesting the importance of interdomain rearrangements for translocation.[97] However, recent smFRET experiments showed that neither abrogation of GTP hydrolysis nor inhibition of translocation by the antibiotic viomycin hamper the rearrangement of EF-G from a compact to the extended conformation.[98] Therefore, it is likely that the movement of EF-G from the compact conformation, which EF-G

FIGURE 5 | Movement of EF-G from the pre- to posttranslocation conformation on the ribosome. EF-G (red, PDBID 4V7D[55]) bound to the viomycin-trapped pretranslocation ribosome is superimposed with EF-G bound to the posttranslocation ribosome (blue, PDBID 4V5F[13]). The superimposition is obtained by structural alignment of respective 23S rRNAs. A/P* tRNA is shown in yellow. 23S rRNA of pre and posttranslocation ribosomes are shown in transparent red and blue, respectively.

adopts in the absence of the ribosome, into a more extended conformation is not coupled to but likely precedes both GTP hydrolysis and mRNA/tRNA translocation.[98]

The minimal model for the structural rearrangements of EF-G, the ribosome and tRNAs during translocation includes following steps (Figure 2): (1) Upon binding to the ribosome, EF-G moves from a compact to an extended conformation and transiently stabilizes the rotated conformation of the ribosome with tRNAs bound in the hybrid state (A/P* and P/E states). (2) EF-G binding also leads to the swiveling motion of the head domain of the small subunit. (3) Upon reverse rotation of the small subunit back into the nonrotated conformation and back-swiveling motion of the head of the small subunit (the order or possible coupling of these two events is not fully established) tRNAs are translocated into classical P/P and E/E states. Translocation of tRNAs is accompanied by the movement of mRNA by one codon and docking of domain IV of EF-G into the A site of the small subunit, which is vacated by the peptidyl-tRNA. The movement of domain IV of EF-G toward the A site of the small subunit and the reverse rotation of the small subunit are hampered by translocation inhibitors and, thus, directly coupled to mRNA/tRNA translocation. (4) By contrast, GTP hydrolysis is not directly coupled to any of these rearrangements. Nevertheless, the release of inorganic phosphate on average occurs concurrently with translocation and triggers EF-G disassociation from the ribosome. Presented here, the model includes only those events that were detected in kinetic ensemble and single-molecule biophysical experiments and
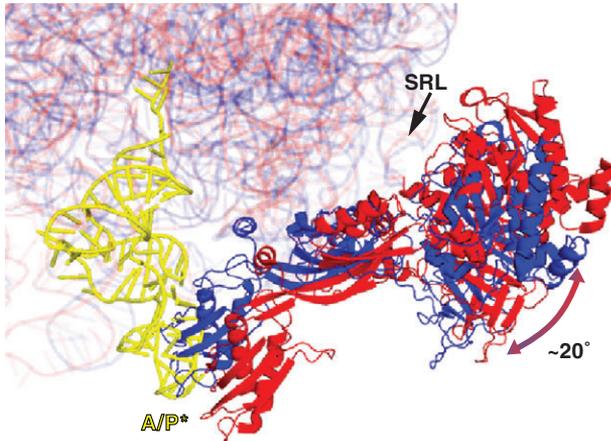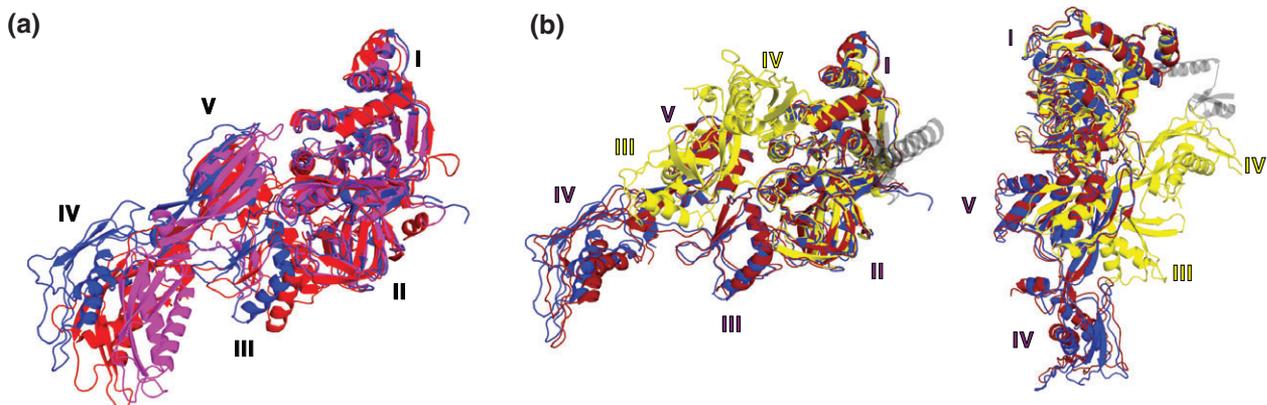

FIGURE 6 | Interdomain rearrangements of EF-G. (a) EF-G in the posttranslocation conformation (blue, PDBID 4V5F[13]) is superimposed by structural alignment of domains I and II with ribosome-free EF-G•GDP (magenta, PDBID 1DAR[2]) and EF-G bound to the viomycin-trapped pretranslocation ribosome (red, PDBID 4V7D[55]), (b) EF-G in the posttranslocation conformation (blue, PDBID 4V5F[13]) is superimposed by structural alignment of domains I and II with EF-G bound to a ribosome containing a chimeric ap/ap tRNA (dark red, PDBID 4W29[42]) and EF-G-L9 fusion bound to the nonrotated, pretranslocation ribosome (yellow, PDBID 4WPO[93]). The N-terminal domain of L9 covalently linked to EF-G (PDBID 4WPO[93]) is shown in transparent gray. Two differently oriented views of EF-G structures are shown. Domains of EF-G are numbered as indicated.

visualized by cryo-EM or crystallographic reconstruction of 2-tRNA ribosome complexes. However, this minimal model of ribosome translocation is likely incomplete. Indeed, a number of distinct conformations of the ribosome were observed in structures of EF-G bound to ribosomes that either were vacant or contained a single tRNA in the P site.[90,92,99,100] Hence, at least some of these structural states may represent additional intermediates of translocation.

THE MOLECULAR MECHANISM OF TRANSLOCATION

Mechanics of Translocation

Macromolecules and macromolecular complexes move in a unidirectional manner by converting the energy of a chemical reaction into mechanical movement. There are two fundamental mechanisms of such conversion. In the first mechanism, called the power stroke, chemical change occurs either concurrently with the movement or precedes it.[101] In the second mechanism, called the Brownian ratchet, the movement occurs spontaneously and precedes the chemical change.[101] In the Brownian ratchet mechanism, the chemical change traps the macromolecule in the post-movement state thus acting like a pawl that rectifies the movement of the wheel of a mechanical ratchet. These two mechanisms can be distinguished by examination of the load dependence of the translocation rate.[101] Recent optical tweezers measurements of ribosomal translocation against an applied force suggested that the ribosome likely translocates by the Brownian ratchet mechanism.[102] It is noteworthy, however, that the Brownian ratchet and the power stroke mechanisms are idealized models; ribosomal translocation may combine features of both models.[102]

What is the chemical change that biases the thermally driven movements of tRNAs and thus, acts as the 'pawl' of the Brownian ratchet mechanism of translocation? Observation of spontaneous translocation implies that ribosomal translocation is a downhill process driven by the energy of peptide bond formation. Indeed, the maximum mechanical work generated by ribosomal translocation measured in optical tweezers experiments is comparable to the energy of peptide bond formation.[102] Still, it is not clear how the energy of transpeptidation can be conveyed to promote tRNA and mRNA movements. On one hand, an early proposal suggested that peptide bond formation renders the translocation of tRNA thermodynamically favorable because it decreases the affinity of P-site tRNA toward the P site due to

deacylation.[103] However, later measurements revealed that deacylation of tRNA does not significantly change its affinity to the P site, while the affinity of esterified tRNA to the A site is similar to the affinity of deacylated tRNA to the E site, thus, making net free energy change of tRNA translocation from A and P sites to P and E sites close to zero.[72,73,104] Consistent with these data, it was demonstrated that spontaneous forward translocation is unfavorable in some tRNA/mRNA contexts since the reverse spontaneous translocation of tRNAs and mRNA from P and E to A and P sites is instead observed.[105,106] On the other hand, the deacylation of the P-site tRNA during peptide bond formation dramatically alters ribosome and tRNA dynamics[58,60,61] and, thus, may facilitate tRNA translocation. In any case, the reaction of transpeptidation is unlikely to be the only energy source of translocation because it would require an unusually high efficiency of conversion (~80%) of chemical energy into mechanical motion, which is untypical for molecular motors.[102] Therefore, translocation is likely promoted by the energy stored in EF-G · GTP, although, as discussed above, GTP hydrolysis itself may not be directly coupled to translocation. Hence, by binding to the ribosome, EF-G · GTP probably acts as the 'pawl' of the Brownian ratchet of translocation.

The Role of Domain IV of EF-G in Ribosome Translocation

Domain IV of EF-G promotes tRNA translocation, at least in part, by biasing peptidyl-tRNA movement and coupling it to ribosome dynamics. Comparison of the rotated pre- and nonrotated post-translocation states of the EF-G-ribosome complex suggests that domain IV of EF-G acts as a steric hindrance for the return of the peptidyl-tRNA from the hybrid A/P* into the classical A/A state and, thus, promotes the movement of peptidyl-tRNA into the P site of the small subunit upon reverse rotation of the small subunit (Figure 7). Likewise, domain IV of EF-G creates a steric hindrance for the return of peptidyl-tRNA from the chimeric ap/P-(ap/ap) into the classical A/A state upon back-rotation of the 30S head from the swiveled conformation. In addition, domain IV of EF-G docks into the A site of the small ribosomal subunit concurrently or right after the movement of the peptidyl-tRNA into the P site of the small subunit and, thus, prevents its reverse translocation (Figure 5).

The idea that EF-G can promote translocation of tRNAs by biasing tRNA movements inside the ribosome is further supported by the phenomenon of antibiotic-induced translocation. The antibiotics,

sparsomycin, linkomycin and chloramphenicol, that bind to the A site of the large subunit were found to trigger mRNA and tRNA translocation on the small subunit.[48,49] It has been hypothesized that these antibiotics induce translocation by sterically blocking the reverse, nonproductive movement of the peptidyl-tRNA from the hybrid A/P to classical A/A state during spontaneous intersubunit rotation.[49] Therefore, the observation of antibiotic-induced translocation is consistent with the idea that the intrinsic dynamics of the ribosome can be rectified into unidirectional translocation by ligand binding.

Diffusion of tRNAs inside the ribosome is hindered by their tight binding to the A and P sites of the small subunit as evident from the very slow rate of antibiotic-induced translocation. Hence, in addition to biasing tRNA movement, EF-G likely also induces conformational changes of the ribosome that facilitate the diffusion of tRNA/mRNA through the ribosome. This rate-limiting step of translocation is often referred to as 'unlocking.' However, after almost five decades of intense studies of ribosomal translocation since the term 'unlocking' was first introduced,[108] the identity of the conformational change induced by EF-G that 'unlocks' the ribosome and, thus, facilitates tRNA diffusion remains unclear.

EF-G may enable diffusion of tRNAs inside the ribosome by inducing conformational changes in the 30S decoding center and, thus, destabilizing the binding of peptidyl-tRNA to the A site of the small ribosomal subunit.[96,109] In addition, EF-G may also facilitate tRNA translation by inducing the head swivel of the small subunit, which opens the otherwise constricted path for the movement of tRNA from the P to the E site on the small subunit.[85,86] Additional studies are required to precisely identify the rearrangement of the ribosome that is rate-limiting for translocation.

CONCLUSION

Concluding Remarks

Emerging over the last 15 years, X-ray crystal and cryo-EM structures of the ribosome revolutionized our understanding of translation and revealed dramatic conformational rearrangements of the ribosome, tRNA and EF-G that accompany ribosome translocation. Ensemble kinetic measurements together with single-molecule FRET and optical tweezers experiments provided important insights into the structural dynamics, kinetics and mechanics of translocation. However, structural and biophysical studies of translocation also raised new unanswered questions.

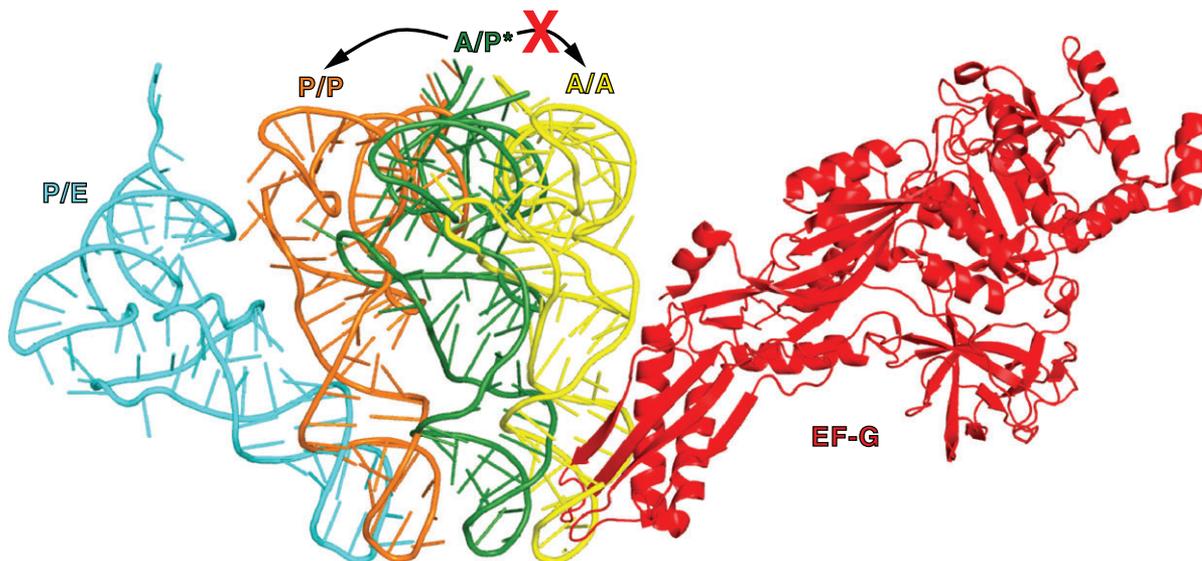Instead of converging onto a few defined structural states that likely resemble intermediates of

FIGURE 7 | EF-G may promote tRNA translocation by clashing with A-site tRNA. A/A (yellow, PDBID 4V5D[107]), A/P* (dark green, PDBID 4V7D[55]), P/P (orange, PDBID 4V5D[107]), and P/E (cyan, PDBID 4V7C[55]) tRNAs are superimposed by structural alignment of 23S rRNA in the respective structures. EF-G bound to the viomycin-trapped pretranslocation ribosome is shown in red (PDBID 4V7D[55]). In the absence of EF-G, spontaneous intersubunit rotation is coupled to the fluctuation of tRNAs between classical (A/A and P/P) and hybrid (A/P [A/P*] and P/E) states. In the presence of ribosome-bound EF-G, upon the reverse intersubunit rotation from the rotated to nonrotated conformations of the ribosome, the movement of peptidyl-tRNA from A/P* to A/A state is disallowed because of the clash with domain IV of EF-G and, thus, the peptidyl-tRNA translocates into the P/P state.

translocation, the dozens of cryo-EM and crystal structures obtained to date reveal great structural heterogeneity of elongation-like complexes of the ribosome. In particular, these structures show large variability in the degree of intersubunit rotation and the 30S head swivel.[88] In equilibrium structural studies, transient intermediates of translocation are trapped by use of antibiotics inhibiting translocation (Table 1) and non-hydrolysable GTP analogues impeding EF-G release. Other factors such as crystal lattice in X-ray crystallography and interactions of the ribosome with the water–air interface and the supporting film in cryo-EM microscopy[110] may also alter the relative stability of various structural states of the ribosome. Using the terminology of energy landscape theory, crystal and cryo-EM structures likely represent energy minima (i.e., mesoscopic states) in an energy landscape[111] that are perturbed by antibiotics and other experimental conditions. Here lies of one of the major challenges as at least some structures of elongation-like ribosome complexes may represent off-pathway states that are not sampled during translocation. In addition, translocation from the pre- to postranslocation state of the ribosome may occur through several alternative pathways.[112,113] Finally, the unperturbed energy landscape of translocation may be smooth and, thus, structural states of the ribosome captured by antibiotics and/or GTP analogues and visualized using X-ray crystallography and cryo-EM microscopy may represent artificially stabilized snapshots of the trajectory of structural changes associated with translocation rather than discrete meta-stable intermediates.

Further kinetic ensemble and single-molecule experiments are required to complement existing structural data in order to reconstruct the trajectory (or alternative trajectories) of the structural transitions of the ribosome, tRNA, mRNA and EF-G during ribosomal translocation and identify authentic intermediates of translocation. However, kinetic biophysical studies do not reveal high-resolution structural details. Hence, developing time-resolved cryo-EM[114,115] and molecular dynamic simulation approaches[113] will likely be instrumental in the investigation of ribosome translocation in the future. Finally, the studies of the translocation mechanism in bacteria need to be further extended to the examination of ribosomal translocation in eukaryotes, which likely has a number of unique features.

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