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## The Structural Dynamics of Translation

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### Abstract

Accurate protein synthesis (translation) relies on translation factors that rectify ribosome fluctuations into a unidirectional process. Understanding this process requires structural characterization of the ribosome and translation-factor dynamics. In the 2000s, crystallographic studies determined high-resolution structures of ribosomes stalled with translation factors, providing a starting point for visualizing translation. Recent progress in single-particle cryogenic electron microscopy (cryo-EM) has enabled near-atomic resolution of numerous structures sampled in heterogeneous complexes (ensembles). Ensemble and time-resolved cryo-EM have now revealed unprecedented views of ribosome transitions in the three principal stages of translation: initiation, elongation, and termination. This review focuses on how translation factors help achieve high accuracy and efficiency of translation by monitoring distinct ribosome conformations and by differentially shifting the equilibria of ribosome rearrangements for cognate and near-cognate substrates.

### Keywords

cryo-EM; ribosome; RNA dynamics; translation initiation; translation elongation; translation termination

## 1. INTRODUCTION: THE ACCURACY OF TRANSLATION AND GLOBAL RIBOSOME REARRANGEMENTS

The fidelity of protein synthesis (translation) depends on accurate initiation and sequential addition of amino acids to a polypeptide chain until the ribosome reaches the stop codon of the open reading frame of a messenger RNA (mRNA). At each stage of translation—initiation, elongation, or termination—the ribosome must collaborate with the appropriate substrates: transfer RNA (tRNA) or translation factor protein. The correct substrates do not form an orderly queue, awaiting their turn on the ribosome. Rather, the ribosome randomly encounters substrates and quickly decides to accept or reject them while establishing and maintaining the reading frame. How does the ribosome select the correct tRNA or translation factor at each stage of translation, and how does the correct selection of

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substrates drive translation? The process depends in large part on the conformational dynamics of the ribosome.

The ribosome consists of the large and small subunits, each containing three tRNA binding sites (Figure 1a–c). Mobility of the small ribosomal subunit was predicted half a century ago to underlie translation (1). Early structural studies of the bacterial 70S and eukaryotic 80S ribosomes captured four large-scale rearrangements of the small subunit (Figure 1d). The two most extensive rearrangements include a  $\sim 10^\circ$  rotation of the small subunit relative to the large subunit (known as intersubunit rotation or body rotation) and a  $\sim 20^\circ$  rotation of the head of the small subunit relative to its body (known as head swivel) (Figure 1d). Both rearrangements were initially associated with the translocation of tRNAs and mRNA (2–4), consistent with solution fluorescence studies, such as Förster resonance energy transfer (FRET) (5–7). After peptidyl transfer from peptidyl-tRNA in the P site to aminoacyl-tRNA (aa-tRNA) in the A site, forward (also termed counterclockwise) (Figure 1d) rotation of the small subunit is coupled with movement of the tRNA acceptor arms on the large subunit (Figure 1a,b). This results in a rotated ribosome with hybrid tRNA states—e.g., A/P tRNA with the anticodon stem loop (ASL) in the A site of the small subunit and the acceptor arm in the P site of the large subunit (Figure 1b). The first part of the name of hybrid or chimeric tRNA states, such as A/P or ap/P, denotes the position of the ASL on the small subunit, and the last letter denotes the position of the acceptor arm on the large subunit (for more details on this nomenclature, see Table 1). In the presence of translocase elongation factor G (EF-G), reverse (clockwise) rotation propels the ASLs of the A- and P-site tRNAs on the small subunit to the P and E (exit) sites, respectively (Figure 1c) (8). During reverse rotation, the head domain of the small subunit swivels toward the large subunit (forward head swivel), enabling tRNA translocation relative to the body (3). Observed in vacant ribosome crystal structures, head swivel is an inherent ribosomal rearrangement that was proposed to facilitate translocation of deacyl-tRNA by widening a gate between the P and E sites (4). Reverse swivel completes the translocation cycle, restoring the ribosome to a nonrotated conformation, ready to accept the next aa-tRNA. Notably, structural and biophysical studies revealed that the small subunit spontaneously rotates in the absence of EF-G (5) and that rotations coincide with tRNA fluctuations within the ribosome (9), suggesting that they are inherent and thermally driven movements. Spontaneous rotation, however, is futile and does not result in efficient translocation without EF-G.

Two smaller 30S-domain movements identified by early cryogenic electron microscopy (cryo-EM) and X-ray studies were ascribed to translation fidelity. Head tilt, in which the head domain of the small subunit rotates about an axis roughly perpendicular to that of head swivel, brings the head closer to the large 50S subunit (Figure 1d). Head tilt has been observed on the isolated small subunit in the absence (10) and presence of initiator tRNA (11), suggesting that this inherent movement is important for the recognition of initiator tRNA during initiation. Shoulder movement, also termed domain closure, was identified by comparing crystal structures formed with and without tRNA ASLs used to mimic tRNAs; this movement was proposed to underlie the fidelity of mRNA decoding (12).

Single-state ribosome complexes from early X-ray crystallography and cryo-EM studies provided important insights into different ribosome conformations. Nevertheless, key

structural intermediates have been missing that could explain how the ribosomes and translation factors regulate translation fidelity. For example, crystal structures of ribosomes with cognate tRNA (complementary to the paired mRNA codon) or near-cognate tRNA (anticodon with one noncomplementary nucleotide) revealed markedly similar interactions (13, 14), even though near-cognate tRNAs are efficiently rejected by the ribosome in cellular and biochemical studies. Furthermore, structural studies have relied on the use of inhibitors, mutations, or GTP analogs to capture complexes with translation factors, such as the essential translational GTPases. These complexes might capture off-pathway intermediates and/or fail to visualize the conformations essential for function.

Recent advances in cryo-EM have helped to bridge these gaps by enabling near-atomic structure determination of complexes formed under conditions that mimic authentic translation. Improved data quality and computational approaches now allow high-resolution differences to be resolved within an ensemble of structures from a single sample. Moreover, time-resolved cryo-EM approaches allow temporal separation and capture of transient structural states, including those involved in GTP hydrolysis. Arranging these structures along a reaction trajectory, informed by biochemical and biophysical studies, offers a high-resolution movie-like view of translation steps that had long evaded structural characterization. Recent time-resolved and ensemble cryo-EM studies have revealed that the inherent large-scale inter- and intrasubunit rearrangements are essential at all three stages of translation; these studies also demonstrated how ribosome dynamics collaborate with translation factors to ensure accurate and efficient translation.

This review presents a concise account of recent cryo-EM studies, which allow a more complete structural reconstruction of translation than previously available. The review focuses on the structural mechanisms that explain translation accuracy at all three steps of bacterial translation and draws parallels with eukaryotic translation. The discussion outlines how the inherent ribosome dynamics define the high fidelity of each step. Some local structural elements of the ribosome, which rearrange to assist translation [e.g., the tRNA-binding L1 stalk and factor-binding L11 stalk (P-stalk in eukaryotes) (Figure 1)] and eukaryote-specific subunit rolling, are not discussed in detail, as their roles have been discussed elsewhere (e.g., 15–18).

## 2. INITIATION

To initiate translation, the ribosome must correctly position the mRNA open reading frame. The correct positioning is achieved when the AUG start codon in the P site pairs with the initiator tRNA: formyl-methionyl-tRNA in bacteria (fMet-tRNA<sup>fMet</sup>, where the prefix denotes the aminoacylation status, and the superscript identifies the tRNA) or methionyl-tRNA in eukaryotes. A wobble pairing in the first nucleotide position of the codon allows infrequent initiation from GUG, UUG, and CUG, whereas other codons are strictly discriminated against (19, 20). The molecular players of initiation have been characterized by decades of research (reviewed in 21, 22). In the bacterial ribosome, the AUG codon is held in the P site by the upstream Shine–Dalgarno sequence (23, 24), which base pairs with the 3' end of 16S rRNA (25, 26). Dynamic RNA-binding protein S1, upstream from the E site, also contributes to positioning the mRNAs, in particular those with weak or absent

Shine–Dalgarno sequences (27). Formation of the 70S initiation complex with initiator tRNA in the P site is aided by initiation factors IF1, IF2, and IF3. Although a bacterial 70S initiation complex can be formed without initiation factors in vitro, the three factors are required for fast and accurate initiation (22, 28).

IF1 binds the 30S subunit A site and bridges IF2 and IF3 (Figure 2a) (29–31). The GTPase IF2 recognizes the formyl-methionyl moiety of initiator tRNA (32) and coordinates the binding of the 50S subunit to form the 70S initiation complex (Figure 2a). GTP hydrolysis by the sarcin-ricin loop (SRL) of the large subunit allows dissociation of IF2 and formation of the elongation-competent 70S complex (70S-E), in which the fMet moiety is bound in the peptidyl transferase center (PTC), and the mRNA exposes the first elongation codon in the A site. IF3 plays a central role in initiation fidelity by discriminating against elongator tRNAs (33, 34). The dynamic IF3 (35) has evaded high-resolution structural characterization on the ribosome until recently (11). The N-terminal and C-terminal halves of IF3 align with the E and P sites, respectively, along the mRNA path. This destabilizes tRNA binding to the small subunit and, together with IF1, precludes premature subunit joining (36, 37).

How does the ribosome cooperate with initiation factors to select the initiator tRNA and to effectively discriminate against elongator tRNAs? Complementarity of the initiator tRNA anticodon to the start codon must be a key determinant of this discrimination. However, the differences in the thermodynamic stabilities of the cognate and mismatched base pairs are too small (38) to account for stringent discrimination of  $>10^3$  fold (28). Rather, the ribosome and initiation factors rely on thermal fluctuations and meta-stability of the three-base-pair codon–anticodon helix to shift the equilibrium toward dissociation of mismatched tRNA or stabilization of cognate initiator tRNA (39). Cryo-EM ensembles of initiation structures suggest how the ribosome might accomplish this (11).

In the absence of fMet-RNA<sup>fMet</sup>, the C-terminal domain (CTD) of IF3 shields the AUG codon in the P site and interacts with IF1, which blocks the A site (see the left side of Figure 2c). The 30S subunit samples open and closed states, whereby the head tilts away from or toward the body, stochastically widening the mRNA tunnel by more than 10 Å (Figure 2a). Binding of tRNA next to the AUG codon on the open 30S initiation complex (30S-I) requires a small shift of IF3 (see the middle of Figure 2c). Three G–C pairs in the anticodon stem of tRNA<sup>fMet</sup> are important for selecting and stabilizing the tRNA via interactions with G1338 and A1339 at the head of the 30S subunit (11, 40, 41). Further stabilization of tRNA coincides with the 30S subunit head tilt, which moves the head closer to the body than in the open state, thus latching the subunit. Latching of 30S-I (closed) requires dissociation of the IF3 CTD from the AUG codon and from IF1 (see the right side of Figure 2c). Thus, the IF3 CTD works as a switch that prevents stable binding of tRNA unless a cognate Watson–Crick codon–anticodon helix is formed in the closed 30S state (Figure 2a).

In the latched 30S-I (closed) complex, the  $\sim 10$ -Å head tilt (at G1338) shifts the tRNA, moving the CCA-fMet end by  $\sim 25$  Å toward the body (Figure 2a). In this position, tRNA<sup>fMet</sup> interacts with IF2 bound at the 30S body (Figure 2a), which triggers subunit joining. Formation of the 70S complex involves intersubunit dynamics that resemble those of translocation. Single-molecule (sm)FRET (42) and time-resolved cryo-EM (43) reveal that

subunit joining with IF2 (to form 70S-I) involves partial head swivel and partial subunit rotation ( $\sim 4^\circ$ ). Upon IF2 dissociation, the ribosome adopts a nonrotated 70S-E conformation ready to bind the first elongator tRNA (Figure 2a). Curiously, an earlier cryo-EM study captured a small population of fully rotated 70S•fMet-tRNA<sup>fMet</sup> ribosomes with IF2 and the nonhydrolyzable GTP analog, GDPNP (44). This structure was proposed as an on-pathway intermediate of subunit joining. However, this fully rotated structure was not identified in the solution smFRET or time-resolved cryo-EM studies of GTP-catalyzed subunit joining, so the possible role of this structural state remains to be shown. Nevertheless, the specificity of IF2 for the initiator tRNA further contributes to initiation fidelity (32, 45), so the combination of all three initiation factors achieves the highest initiation efficiency and accuracy (28, 46, 47). In summary, recent studies visualized how initiation factors rectify the inherent 30S and 70S dynamics to bias initiation toward formation of the 70S complex with initiator tRNA. Restricting the conformational dynamics of the 30S subunit (e.g., with the antibiotic streptomycin) decreases initiation fidelity (46), in keeping with the essential role of ribosome rearrangements.

Several questions concerning the mechanism of initiation remain to be addressed. First, to delineate the roles of 30S rearrangements and IF3 in tRNA discrimination, structures of cognate initiation complexes need to be compared with those formed with near-cognate tRNAs. Biochemical studies showed that IF3 uniformly destabilizes binding of tRNAs to the 30S subunit, including initiator tRNA. Comparison of initiation complexes with different tRNAs is needed to visualize detailed structural differences that enable the ribosome to discriminate against near-cognate tRNAs. Second, current structural data do not distinguish whether initiation follows one or more pathways (Figure 2a). For example, the capture of a closed 30S•IF3•fMet-tRNA<sup>fMet</sup> complex without IF1 (Figure 2a) suggests at least two scenarios: (a) IF1 can bind or dissociate at initial stages of tRNA recognition and thus contribute to tRNA discrimination or (b) IF1 binds 30S-I at a later stage to stabilize IF2 (48). Alternatively, parallel pathways of initiation that allow different orders of initiation factor binding (11) would be consistent with differential regulation of translation on distinct mRNAs and under different cellular conditions. This idea remains to be reconciled with biochemical data suggesting a preferred pathway (48). Nevertheless, the existence of parallel pathways is an attractive hypothesis for translation and other regulated cellular processes that depend on multiple factors. For example, ribosomal subunit biosynthesis—when the newly made rRNA transcript is chaperoned by ribosomal proteins—proceeds via different orders of protein binding rather than being an orderly assembly line-like process (49).

Eukaryotic initiation has diverged considerably from bacterial initiation. It involves dozens of polypeptides forming initiation factors and interacting in complex ways to regulate translation from 5'-m<sup>7</sup>G-capped mRNAs (reviewed in 50, 51). Yet, recent structural studies reveal mechanistic similarities, highlighting how the dynamics of the conserved small-subunit core determines the formation of the 80S initiation complex. Despite the absence of sequence homology, the functions of bacterial IF1 and the C- and N-terminal domains of IF3 are similar to those of the eukaryotic factors eIF1A, eIF1, and eIF2 $\alpha$ , respectively (Figure 2d) (52, 53). The eukaryotic 40S samples open and closed conformations, resulting in eIF1 dissociation upon tRNA recognition (54, 55), emphasizing the conserved mechanistic principles of initiation accuracy. Furthermore, the eukaryotic GTPase eIF5B recognizes the

aminoacyl moiety of initiator tRNA and facilitates subunit joining, resembling bacterial IF2 (Figure 2d) (56, 57). These similarities, along with the increased complexity of eukaryotic initiation, are consistent with the idea that eukaryotic initiation can proceed via multiple pathways, in keeping with the complex regulation of eukaryotic translation of different mRNAs.

### 3. ELONGATION

#### 3.1. mRNA Decoding and tRNA Proofreading

Translation elongation involves a periodic repetition of three steps, as the ribosome moves along sense codons until it reaches the stop codon (Figures 3 and 4). First, the ribosome recognizes an aa-tRNA cognate to the A-site codon and rejects noncognate aa-tRNAs (decoding) (Figure 3a–h). Second, the ribosome catalyzes formation of a peptide bond between the incoming cognate aa-tRNA and the P-site peptidyl-tRNA, resulting in peptidyl-tRNA in the A site and deacyl-tRNA in the P site (peptidyl transfer) (Figure 3i,j). Third, the A-site and P-site tRNAs translocate, along with the mRNA, to the P and E sites, respectively, to allow the next round of elongation (translocation) (Figure 4). These steps have been extensively discussed in reviews (16, 58–64), so the following discussion focuses on the recent structural studies that visualize the roles of ribosome dynamics and elongation factors EF-Tu and EF-G in maintaining elongation accuracy.

The high accuracy of decoding, with only  $10^{-3}$ – $10^{-5}$  errors per codon (65), is essential for all life. It is achieved through the two-step selection of cognate tRNAs involving initial decoding and kinetic proofreading (66, 67). The aa-tRNAs are delivered to the ribosome as part of the ternary complex with the universally conserved GTPase EF-Tu (eEF1A in eukaryotes) and GTP. When a cognate tRNA is successfully recognized, GTP hydrolysis releases EF-Tu. This irreversible reaction separates the biochemical steps of initial selection and proofreading, the latter allowing an incorrectly decoded near-cognate tRNA to be rejected prior to peptide bond formation.

Recent cryo-EM studies, supported by an extensive body of biochemical (e.g., 68–71), biophysical (e.g., 72–75), crystallographic (e.g., 12–14, 76), and computational (e.g., 77–79) studies, have visualized how decoding is driven by 30S dynamics. Both the initial selection and proofreading steps were recently resolved in structural ensembles formed with EF-Tu prior to GTP hydrolysis (80, 81) and by uninhibited, time-resolved reactions with GTP and authentic aa-tRNAs (82). These structural ensembles reveal that initial selection and proofreading rely on the same 30S rearrangements. As part of the ternary complex, EF-Tu lands on the 30S subunit shoulder, presenting the tRNA anticodon at a  $\sim 15$ -Å distance from the A-site codon (Figure 3a,f). This initial mode of binding prevents GTP hydrolysis because EF-Tu's GTPase active site is  $\sim 10$  Å away from the 50S GTPase-activating SRL. The 30S subunit adopts a domain-open conformation in the absence of base-pairing between the codon and anticodon. Stochastic tRNA dynamics kink the ASL, bringing the anticodon toward the codon and allowing the formation of a codon–anticodon helix. A Watson–Crick helix is stabilized by hydrogen bonds with universally conserved 16S nucleotide G530 at the tip of the shoulder domain of 30S (Figure 3g). The codon–anticodon RNA helix is further stabilized by transient interactions with decoding center nucleotides A1492–A1493 from the



30S body (Figure 3g). In the presence of a cognate codon–anticodon helix, G530 and the shoulder domain move  $\sim 3\text{--}5\text{\AA}$  closer to A1492–A1493, locking the tRNA in the decoding center of a domain-closed 30S subunit (Figure 3h). Thus, transient positioning of G530 next to A1492, in the presence of a cognate codon–anticodon helix (Figure 3g), shifts the equilibrium toward a domain-closed 30S (Figure 3h). This motion brings the shoulder-bound EF-Tu toward the SRL, activating the EF-Tu GTPase domain.

Cryo-EM studies that compare decoding with cognate and near-cognate tRNAs revealed different interactions and populations of particles in structural ensembles. With cognate tRNA, the largest population of structures features G530 fully engaged in the decoding center of a domain-closed 30S. By contrast, near-cognate tRNA (with mismatches in the first or second positions) (80, 81) shifts the population toward domain-open 30S with G530 disengaged from the distorted near-cognate codon–anticodon helix. Thus, near-cognate tRNAs shift the equilibrium toward the domain-open 30S, preventing GTP hydrolysis and favoring ternary complex dissociation. Cognate tRNAs shift the equilibrium toward the domain-closed 30S, favoring EF-Tu GTPase activation.

Time-resolved cryo-EM visualized EF-Tu dissociation after GTP hydrolysis, consistent with two tRNA proofreading steps: EF-Tu-dependent and accommodation-dependent (82). In the domain-closed 30S, EF-Tu's GTPase center docks at the SRL, resulting in GTP hydrolysis (82, 83). Upon inorganic phosphate ( $P_i$ ) release, the GTPase domain dissociates from the SRL, forming extended EF-Tu conformations (82) that resemble those in crystal structures of isolated EF-Tu•GDP (76). In the presence of EF-Tu•GDP, the shoulder of 30S continues to sample open and closed states, as the tRNA remains kinked to retain codon–anticodon interactions. The dynamics of the 30S subunit therefore suggests the possibility of tRNA dissociation with EF-Tu•GDP in a step proposed by biochemical studies to represent EF-Tu-dependent proofreading (Figure 3d) (70). Following EF-Tu release, the cognate tRNA relaxes and accommodates into the A site of the large subunit, traversing  $\sim 80\text{\AA}$  from its initial EF-Tu-bound state (Figure 3e,f). A slight rotation of the small subunit ( $\sim 2.5^\circ$ ) likely allows the accommodating tRNA to bypass elements that protrude from the large subunit [e.g., H89, as identified in molecular dynamics simulations (78, 84)]. Remarkably, cryo-EM classifications found that during accommodation, cognate tRNA is stably bound (locked) in ribosomes with a closed 30S subunit, suggesting that the ribosome has committed to accept the cognate tRNA. By contrast, ribosomes with near-cognate tRNA continue to sample the open 30S subunit with disengaged G530. The instability of interactions between near-cognate tRNA and 30S during accommodation likely favors dissociation, representing accommodation-dependent proofreading (Figure 3e). Thus, the same intrasubunit dynamics govern the initial tRNA selection and proofreading stages, accounting for high decoding accuracy.

### 3.2. Translocation of tRNA and mRNA

Accommodation of tRNA into the ribosomal A site results in peptidyl transfer from the P- to A-site tRNA. To prepare the ribosome for accepting the next tRNA, GTPase elongation factor (EF-G in bacteria and eEF-2 in eukaryotes) translocates the mRNA with deacyl-tRNA and peptidyl-tRNA (Figure 3i,j) (for reviews, see 60–64). It is critical to maintain the

open reading frame during translocation, when frameshifting may occur and result in a defective protein product (85, 86). Structural visualization of the fast GTP-catalyzed translocation has long remained a challenge. Numerous structural studies have captured EF-G with two tRNAs in the presence of EF-G mutations (87, 88), antibiotics (89–94), or nonhydrolyzable GTP analogs (95), but concerns have been raised that such approaches may capture off-pathway structures (96). A recent time-resolved cryo-EM study visualized translocation on the 70S ribosome by EF-G and GTP without inhibitors (97). Structures of pretranslocation and posttranslocation states along with three EF-G-bound intermediates yielded a dynamic view of the translocation trajectory, supported by an extensive array of structural, biochemical, and FRET studies.

After peptidyl transfer, the two tRNAs spontaneously sample the hybrid A/P and P/E states (9, 98), coupled with a 10–11° rotation of the small subunit (Figure 3k) (5, 99, 100). During these transitions, the elbow of the peptidyl-tRNA can shift ~20Å closer to the P site, adopting an A/P\* state (Figure 3l). EF-G•GTP binds to the rotated ribosome, placing the protein's translocase domain 4 near the A/P\* tRNA, while EF-G domain 5 is anchored at the L11 stalk of the large subunit (Figure 4a). During spontaneous reverse rotation of the 30S subunit (i.e., toward a nonrotated state), domain 4 sterically blocks peptidyl-tRNA. As a result, the tRNA and mRNA shift toward the P site on the small subunit, proceeding via intermediate stages of translocation (Figure 4b–d) in which the head swivels toward the large subunit by ~18° (97). The following discussion describes in more detail how EF-G and GTP catalyze translocation during reverse subunit rotation.

The initial binding of EF-G to the rotated ribosome (11.6° body rotation) places the tip of domain 4 (loop 1) next to G530, which, together with A1492 and A1493, stabilizes the peptidyl-tRNA in the A site (Figure 4e) (97). To initiate translocation, EF-G must unlock the decoding center. A recent cryo-EM study captured EF-G with translocation inhibitor spectinomycin and a slightly translocated tRNA–mRNA helix (93). In a marginally less rotated ribosome•spectinomycin conformation (10.9° body rotation), loop 1 of EF-G is shifted toward A1493, and the codon–anticodon helix is disengaged from G530. This intermediate resembles a yeast 80S translocation structure captured earlier with tRNA-like internal ribosome entry site (IRES) mRNA and eEF-2 stalled by sordarin (Figure 4f) (101). Although spectinomycin and sordarin could have perturbed the ribosome/elongation factor structures in these studies, their binding to different sites (the 30S and eEF2, respectively) yielded similar ribosome conformations. This suggests that the structures with disengaged G530 (G577 in yeast) likely mimic a conserved early intermediate of translocation.

Further reverse rotation of the 30S body (5°) shifts domain 4 deeper into the A site (Figure 4g), concurrent with a ~20-Å movement of peptidyl-tRNA into the P site and deacyl-tRNA into the E site of the 30S body, resulting in the ap/P and pe/E chimeric states, which could also be visualized in the presence of fusidic acid or nonhydrolyzable GTP analogs (91, 95). EF-G loops 1 and 2 shift closer to peptidyl-tRNA and mRNA, extending EF-G contact with the codon–anticodon helix. The 30S head rotates in the direction of tRNA translocation by ~17° while keeping contact with the tRNAs and mRNA. Concurrent movement of EF-G and the 30S head during translocation are likely important for preservation of the open reading frame and prevention of frameshifting. Indeed, recent cryo-EM and FRET studies



showed that frameshift-prone mRNAs and near-cognate tRNA can experience frameshifting during head swiveling in the course of EF-G-catalyzed translocation (86, 95). A crystal structure further corroborates the critical role of EF-G, demonstrating that without EF-G, the head-swiveled ribosome with a cognate tRNA fails to preserve Watson–Crick pairing of the codon–anticodon helix in the ap/P state (102).

The final EF-G bound state features a nearly nonrotated 30S body ( $1^\circ$ ) with the most swiveled head ( $18^\circ$ ) and tRNAs translocated further by  $\sim 4 \text{ \AA}$ , yielding the ap\*/P peptidyl-tRNA (Figure 4c) (97). While the translocase domain of EF-G remains in the 30S A site, its GTPase superdomain (domains 1–2) has detached from the ribosome, suggesting an EF-G dissociation stage. Indeed, a similarly rotated ribosome state without EF-G has been detected in the same cryo-EM data set with translocation intermediates, consistent with EF-G dissociation from a head-swiveled ribosome. Reverse swivel of the 30S head restores the ribosomal A and P sites, preparing the ribosome for the next decoding event (Figure 4d).

Recent advances in biochemical approaches and time-resolved cryo-EM have clarified the long-debated role of GTP in translocation. Since nonhydrolyzable GTP analogs and catalytically dead EF-G mutants accelerate translocation by more than three orders of magnitude, similar to GTP (87, 103, 104), the hydrolysis of GTP does not substantially contribute to tRNA movement. Instead, GTP hydrolysis enables EF-G dissociation, yielding a functional posttranslocation ribosome with a vacant A site. Cryo-EM structures revealed that GTP or GTP analogs facilitate EF-G binding to the pretranslocation ribosome by enabling structural complementarity of the EF-G GTPase center to the SRL and rotated 30S subunit (94, 97). The GTPase domain of EF-G coordinates GTP via ordered switch loops, one of which (sw-1) bridges the SRL of the 50S subunit and the platform of the 30S subunit. Docking of EF-G at the SRL results in rapid GTP hydrolysis (105). The phosphate, however, remains in the catalytic pocket due to stabilization of the switch loop by the rotated 30S subunit. Reverse rotation separates the 30S from sw-1, resulting in phosphate dissociation, consistent with the kinetic coupling of translocation with phosphate release. Movement of EF-G into the 30S A site coincides with dissociation of the GTPase domain from the SRL (Figure 4c), ultimately resulting in EF-G release and formation of the nonrotated posttranslocation ribosome. By contrast, the inability to hydrolyze GTP in studies with GTP analogs and catalytically inactive EF-G mutants yields EF-G attached to nonrotated posttranslocation ribosomes (87, 95). The inability of EF-G to dissociate modestly slows the reverse 30S rotation (8), consistent with a modest—twofold to 50-fold—decrease in translocation rate compared with GTP-catalyzed translocation (104, 106, 107). Thus, GTP hydrolysis and release of the phosphate allow EF-G dissociation, while EF-G catalyzes translocation by converting spontaneous ribosome fluctuations into tRNA translocation.

Recent cryo-EM studies emphasize the structural similarity of elongation in bacteria and eukaryotes, implicating a similar mechanism of elongation accuracy in eukaryotes. High-resolution 80S decoding complexes (108) and lower-resolution dynamics of eEF1A and the 40S subunit in decoding complexes (18) have been reported, although the detailed dynamics of codon recognition and proofreading remain to be visualized. A high-resolution understanding of eukaryotic translocation is also emerging (109), highlighting similarities between bacterial and eukaryotic translocases and small-subunit dynamics.

## 4. TERMINATION

Translation terminates when a stop codon arrives at the A site. Unlike sense codons, stop codons are recognized by proteins called release factors (RFs). RF1 and RF2 in bacteria terminate translation on the UAA/UAG and UAA/UGA stop codons, respectively. These bifunctional enzymes recognize the stop codon on the 30S subunit and catalyze hydrolysis of the peptidyl-tRNA ester linkage in the PTC, thus releasing a newly made protein from the ribosome. Biochemical, cryo-EM, and high-resolution crystallographic studies have revealed that RFs bind in the ribosomal A site and span the decoding center and the peptidyl-transferase center. The codon-recognition superdomain interacts with all three nucleotides of the stop codon on the 30S subunit. The catalytic domain crowned with the universally conserved GGQ motif is placed in the PTC. Here, the backbone NH group of the glutamine catalyzes the nucleophilic attack of a water molecule on the peptidyl-tRNA's ester linkage, resulting in peptide dissociation from the ribosome. The catalytic and codon-recognition domains are bridged by a switch loop, whose binding to the decoding center was proposed to be important for termination accuracy. The detailed mechanisms of stop-codon recognition and peptidyl-tRNA hydrolysis have been extensively reviewed (110, 111).

Two long-standing questions, however, remained unresolved until recent structural studies of RF and ribosome dynamics: (a) how is the RF activated by the stop codon? and (b) how does the RF dissociate after termination? The strict coordination between codon recognition and peptidyl-tRNA hydrolysis was proposed to be achieved via a large conformational change of the RF as it binds the A site. Keeping the GGQ away from the PTC during initial sampling of the A-site codon would allow the RF to dissociate without peptide release on a sense codon and thus prevent premature termination. Compact RFs were initially visualized in crystallographic structures of isolated RFs (112, 113) and in the studies of alternative ribosome-rescue factor ArfA, which acts with RF2 to recycle ribosomes stalled on a truncated mRNA, i.e., without a stop codon (114, 115). More recently, a hyperaccurate mutant of RF1 was crystallized with the PTC-binding antibiotic blasticidin S, which decouples codon recognition from insertion of the catalytic domain into the PTC (116) and suggests the presence of compact RF on the ribosome. Yet, capturing the canonical termination complex with a compact RF without inhibitors remained a challenge due to the fast rate of the conformational rearrangement (117, 118). After hydrolyzing the ester linkage of peptidyl-tRNA, the RF must dissociate from the A site to allow ribosomal subunit and deacyl-tRNA dissociation and recycling. Translational GTPase RF3, present in many bacteria, was shown to mediate RF1 release; however, RF2 can dissociate spontaneously (119). The function of RF3 is not essential for cell viability, indicating that both RFs can dissociate without RF3 (120, 121). One clue to the mechanism of RF dissociation is that RF3 stabilizes a rotated ribosome conformation (122, 123), arguing that intersubunit rotation is necessary for RF dissociation.

Recent cryo-EM studies address both questions and, together with previous studies, allow a structural reconstruction of the mechanism of termination. Time-resolved cryo-EM proved necessary to visualize the elusive rearrangements of RFs—from compact to extended (active) conformations—that couple codon recognition with peptidyl-tRNA hydrolysis on a time scale of tens of milliseconds (Figure 5a,b,f,g) (118). RFs decode stop codons on the

nonrotated ribosome (both head and body rotation are less than  $1^\circ$ ). The inactive compact RF interacts with the stop codon with the catalytic domain  $\sim 50\text{\AA}$  away from the PTC (Figure 5f,i). The decoding center nucleotides are arranged to stabilize the mRNA (G530) (Figure 5i,j) and the switch loop of RF (A1492, A1493) (Figure 5g,h). To insert the catalytic domain into the PTC (Figure 5h), the codon-recognition domain moves  $\sim 3\text{\AA}$  to settle deeper in the decoding center (Figure 5j). The tip of the switch loop (Trp319 in *Escherichia coli* RF2) moves by  $20\text{\AA}$  to interact with A1492 in the decoding center (Figure 5g), consistent with the proposed essential role of the switch loop in termination (124). How RFs are rejected upon binding to a sense codon (Figure 5a,b), however, remains to be determined, and this question requires studies that compare transient binding of RF to stop codons and sense codons.

A recent cryo-EM study of 70S•RF2 complexes uncovered a novel conformation of the catalytic domain of RF2, implicating a local conformational change in peptide release from the ribosome (125). In the canonical catalytic complexes, the loop containing the GGQ motif forms a short  $\alpha$ -helix, which places the catalytic glutamine backbone group next to the ribose of the terminal P-tRNA residue A76 (Figure 5h), the leaving group of the deacyl-tRNA product. Upon dissociation of the CCA<sup>76</sup> tail from the PTC, however, the loop rearranges into a long  $\beta$ -hairpin, which extends into the peptide tunnel, as if plugging it to bias the newly formed protein to dissociate toward the 50S solvent surface (see the right side of Figure 5h). This rearrangement underscores the flexibility of the GGQ motif and its possible function in nascent-protein dissociation from the ribosome.

Upon peptide release, the deacylated tRNA can dissociate from the PTC (Figure 5h), allowing rotation of the small subunit (Figure 5b–d). Consistent with FRET studies (119), recent cryo-EM studies reveal that RF-bound ribosomes sample large-scale 30S rearrangements. RF1 was visualized in the presence of RF3 and the antibiotic Apidaecin 137, which stalls RF1 in the PTC (126). RF2 was captured without RF3 and inhibitors (125). Each study resolved structural ensembles showing that RFs can remain in the ribosomal A site, while the small subunit spontaneously adopts intermediate rotational states and the tRNA acceptor arm fluctuates between the P and E sites of the 50S subunit (Figure 5c,d). Partial 30S head swivel ( $3^\circ$  to  $7^\circ$  in the 70S•RF1•RF3 and 70S•RF2 structures) restructures the decoding center, disrupting interactions with the RF's codon-recognition superdomain. Both studies also observed a fully rotated 70S conformation with hybrid P/E tRNA but without an RF (Figure 5e). Thus, completion of intersubunit rotation is likely coupled with RF dissociation. The remarkable similarity between RF1- and RF2-bound ribosomes indicates the use of similar mechanisms for RF recycling and for preparation of the ribosome for subunit splitting and tRNA dissociation by ribosome recycling factor (RRF) and EF-G. Indeed, time-resolved cryo-EM visualized recruitment of RRF and EF-G•GTP to rotated ribosomes and suggested that reverse 30S rotation acts to catalyze tRNA dislodging and subunit splitting (127). In the RF-bound posttermination states, the 30S rotation and head swivel occur similarly to those during late initiation steps, featuring the same pivot points and similarly aligned axes of rotation (Figure 1e), highlighting the fact that RFs and initiation factors rely similarly on the inherent spontaneous ribosome dynamics.

Eukaryotic termination substantially differs from bacterial termination, yet they share the general principle of achieving termination fidelity via the opening of a RF. High-resolution

cryo-EM structures have revealed how eRF1 recognizes four mRNA nucleotides of the A site, highlighting the importance of a purine residue (i.e., G or A) following the canonical UAA, UGA, or UAG termination codons (128), consistent with biochemical, genomic, and cellular studies (129–131). Unlike bacterial RFs, eRF1 decodes the stop codons as a heterodimer with GTPase eRF3. The latter functionally mimics EF-Tu, so the eRF1•eRF3•GTP complex resembles the ternary complex during decoding. Cryo-EM structures revealed that eRF1 adopts a compact conformation in the presence of eRF3 and nonhydrolyzable GTP analogs (108, 132). Upon eRF3 dissociation, eRF1's catalytic domain extends to insert the GGQ motif into the PTC, so the chemical mechanism of peptidyl-tRNA hydrolysis is likely similar to that of bacterial RFs. The eukaryotic ATP-binding cassette protein ABCE1 then binds next to eRF1 (108, 133) to recycle the ribosome (134). Many dynamic steps, from eRF1 conformational rearrangements and eRF3 dissociation to ABCE1 binding and eRF1 dissociation, remain to be visualized. It is possible that, like the bacterial mechanisms, eukaryotic termination and recycling coopt 80S rearrangements, including intersubunit rotation, to facilitate posttermination recycling steps.

## 5. CONCLUDING REMARKS

### 5.1. Convergence of Mechanistic Insights from Cryo–Electron Microscopy and Other Methods

Rapid advances in cryo-EM technology have enabled the identification of many new structures. The convergence of insights from mutational, genetic, biochemical, and biophysical data has been essential for developing the mechanistic understanding of translation. Structure-based fluorescent techniques have been particularly complementary with structural techniques because they allowed the visualization and biophysical characterization of translation steps in solution, exploiting the structural positions of the reporter dyes. Perhaps the most notable example of convergence between cryo-EM and FRET has been the demonstration of large-scale intersubunit rotation, first by cryo-EM (2), then by bulk FRET in solution (122), and then with smFRET yielding the kinetics of intersubunit rotation and its coupling with tRNA and mRNA translocation (5). However, some cryo-EM structures that suggest mechanistic insights remain to be confirmed by FRET and vice versa. This gap could reflect the limitations of FRET studies (e.g., time resolution), differences in buffer conditions, off-pathway structures captured by cryo-EM, or other factors. Cryo-EM and FRET findings remain to be reconciled in all three steps of translation. In initiation, a fully rotated IF2-bound ribosome with GDPNP has been detected by cryo-EM, but only the semirotated 70S•IF2•tRNA state was reported by smFRET (42) and time-resolved cryo-EM with GTP (43). A fully rotated initiation-like complex with the initiator tRNA interacting with the L1 stalk, as in translocating P/E tRNAs, could be a short-lived intermediate of initiation that remains to be confirmed. Furthermore, capturing the complex interactions of all initiation factors during initiation remains a challenge for both techniques (11, 43) and is likely to be tackled by multi-dye FRET approaches (e.g., 135) and cryo-EM with deep classification (82) or other algorithms to deconvolute compositional and conformational heterogeneity (e.g., 136). In elongation, additional authentic states must exist with ribosome and EF-G rearrangements beyond the three translocation intermediates recently detected with EF-G and GTP (97). Future time-resolved cryo-EM studies will

also visualize the details of mRNA frameshifting in both directions during elongation (+1 and -1). In addition, the correlation of posttermination ribosome rotation with RF release, proposed by cryo-EM studies, remains to be demonstrated by smFRET or other approaches.

The steps of translation are best characterized for bacterial translation, which has historically been the most widely studied translation system. Nevertheless, fast progress is being made in the cryo-EM characterization of archaeal (e.g., 137, 138), eukaryotic cytoplasmic (e.g., 139, 140), and mitochondrial (e.g., 141–143) translation that will soon allow a complete visualization of translation steps in all kingdoms of life.

## 5.2. Ribosome Dynamics in Cellular Processes Beyond Translation

The ribosome is a key cellular sensor that interacts with numerous proteins or machineries beyond those directly involved in translation. Recent cryo-EM and biophysical studies revealed that inherent ribosome dynamics are remarkably similar among translation steps, underscoring the fact that distinct translation factors are used not to induce ribosome dynamics but to restrict (initiation factors and EF-Tu) or coopt (EF-G and RFs) spontaneous ribosome motions to achieve a high accuracy and efficiency of translation. The ribosome's interactions with many cellular macromolecules are also likely to involve similar dynamics to achieve their functional roles. High-resolution cryo-EM structures of large assemblies with RNA polymerase (reviewed in 144), mRNA-binding complexes (e.g., 145), diribosomes, and higher-order ribosome assemblies (146) have begun to uncover mechanisms that control transcription-translation coupling and mRNA quality control. They similarly implicate the dynamics of the small subunit, with distinct head tilts in the 30S complex with RNA polymerase (147) and 30S rotation in 70S•RNAP complexes and in the stalled ribosomes (146, 148). In future studies, in situ visualization of intraribosomal transitions and supramolecular ribosome complexes will be enabled by the rapid advances in cryogenic electron tomography (cryo-ET) and subtomogram averaging (149). Furthermore, structural features of individual macromolecules in the cellular environment will become identifiable with high-resolution approaches such as template matching that take advantage of databases of cryo-EM or X-ray structures (150).

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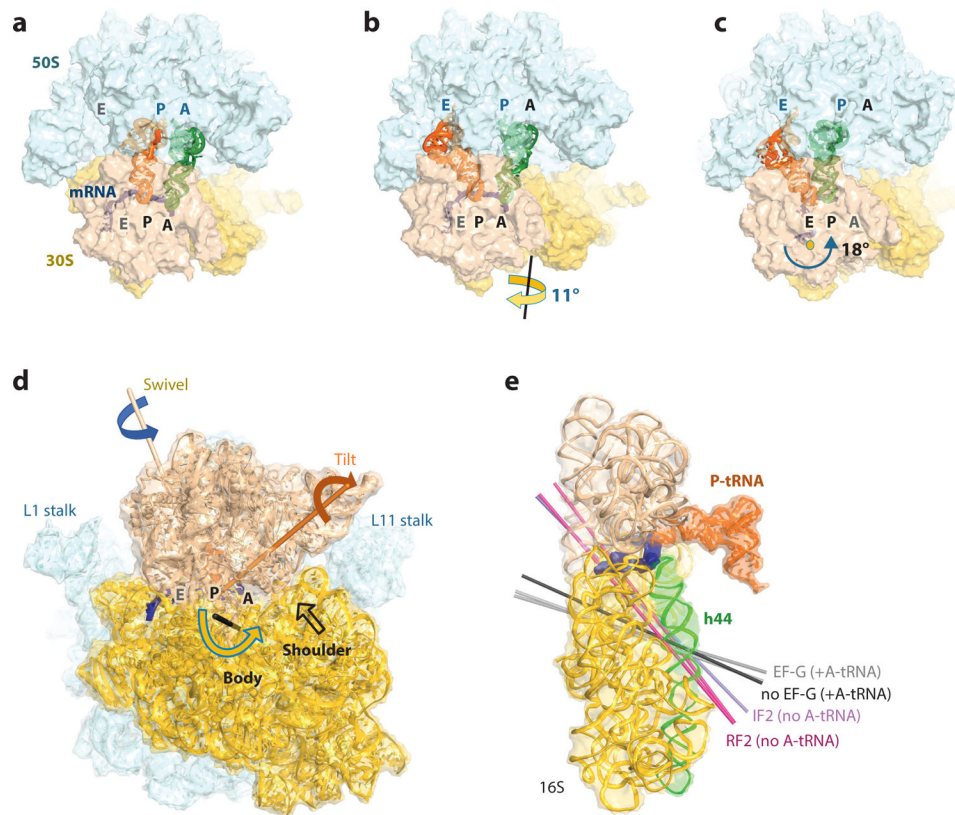
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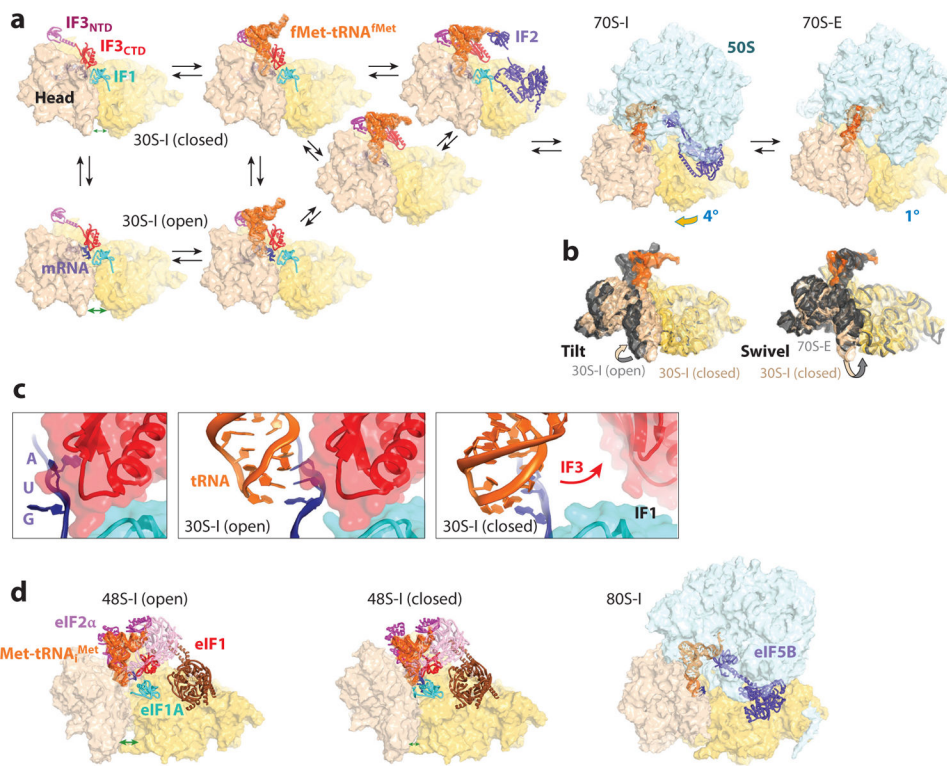


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**Figure 1.** Structure of the bacterial ribosome and large-scale 30S rearrangements. (a) Pretranslocation nonrotated 70S with the classical peptidyl-tRNA (green) and deacyl-tRNA (orange) in the P and A sites, respectively, observed without translation factor proteins. The tRNA-binding A, P, and E sites are labeled. (b) Pretranslocation 70S with hybrid-state P/E and A/P tRNAs with a fully rotated 30S body (10–11° relative to the nonrotated ribosome shown in panel a, as indicated by the arrow) and slightly swiveled 30S head (3–4°; not shown), observed without translation factor proteins. (c) 70S ribosome with nearly translocated chimeric ap/P and pe/E tRNAs (see Section 3) with a partially rotated 30S body (~4°; not shown) and highly swiveled 30S head (18°; indicated by arrow), observed without translation factor proteins (102). (d) Solvent side of the small ribosomal subunit, showing the four principal rearrangements: head swivel (beige axis), head tilt (orange axis), body rotation (black axis), and shoulder movement (black outlined arrow). Arrows denote the directions of forward rotation (curved) and shift (straight). (e) Axes of intersubunit body rotation in 70S complexes show similar pivot points (near residues 1,420–1,425 of 16S helix 44) for 30S body rotation without protein factors (black), with IF2 (violet), with EF-G (gray), and with RF2 (magenta). Two gray and two magenta arrows are derived from complexes obtained in different studies. The 50S subunit and ribosomal proteins are not shown. The 30S head and body are shown in beige and yellow, respectively, in all panels. The degrees of intersubunit rotation were measured relative to the previously published nonrotated posttranslocation 70S structure VII (97). Abbreviations: EF, elongation factor; IF, initiation factor; RF, release factor; tRNA, transfer RNA.



**Figure 2.**

Cryo-EM structures visualize initiation factors and 30S rearrangements underlying the fidelity of initiation. (a) Possible trajectories of initiation complex formation derived from ensemble cryo-EM (11) and time-resolved cryo-EM (43) studies. The existence of 30S-I in closed and open conformations in a single sample implies spontaneous rearrangements with different combinations of IF1 and IF3, whereas IF2 binds to 30S-I (closed), sampled upon acceptance of the correct tRNA. Double-ended green arrows indicate mobility of the head due to head tilt, resulting in different distances between the head (*beige*) and body (*yellow*) of the 30S subunit. The degrees of 30S body rotation in two 70S complexes are indicated. (b) Superposition of 30S structures shows the head tilt between open and closed 30S-I conformations and head swivel between 30S-I (closed) and 70S-E complexes, along with initiator-tRNA rearrangements. Superposition was performed by the alignment of the body domains of 16S rRNA (residues 930–1,385). Only 16S rRNAs are shown for clarity. (c) Close-up view of the P site, showing interactions of IF3 (*red*) and IF1 (*cyan*) with mRNA (*blue*) and initiator tRNA (*orange*) in the open and closed 30S-I states, which are implicated in the mechanism of tRNA acceptance (11). (d) Cryo-EM structures of the eukaryotic 48S initiation complex reveal that the small-subunit movements and tRNA interactions with initiation factors are overall similar to the bacterial ones, despite large mechanistic and sequence divergence. Initiation factors, whose positions are similar to bacterial release factors, are labeled. The degrees of intersubunit rotation were measured relative to the previously published nonrotated posttranslocation 70S structure VII (96). Protein Data Bank identifiers from left to right: 3jaq, 3jap, and 4v8z. Abbreviations: 30S-I, 30S initiation complex; 70S-E, elongation-competent 70S complex; 70S-I, 70S initiation complex; cryo-

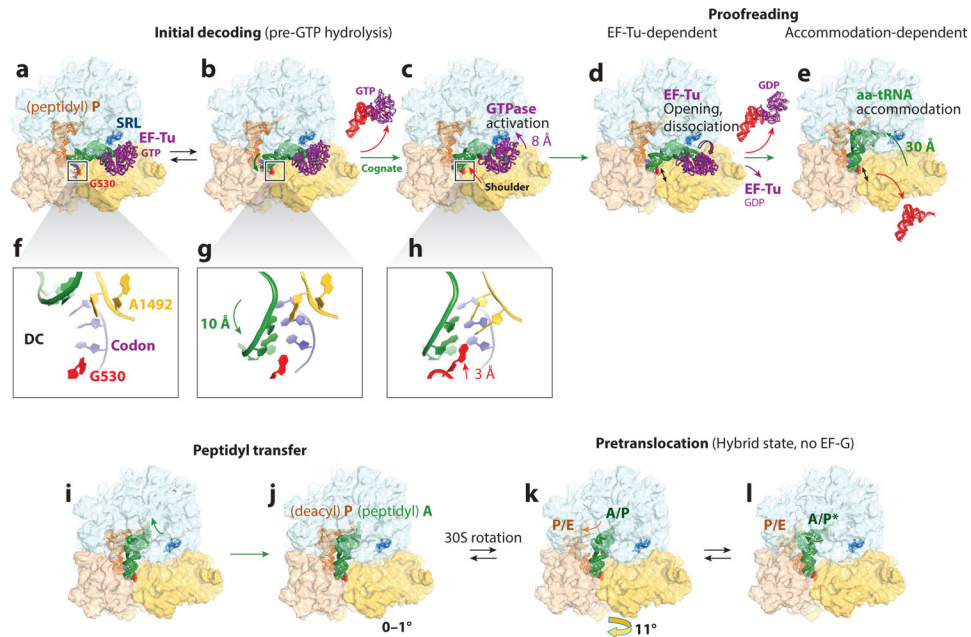
EM, cryo–electron microscopy; CTD, C-terminal domain; eIF, eukaryotic initiation factor; IF, initiation factor; NTD, N-terminal domain; rRNA, ribosomal RNA; tRNA, transfer RNA.

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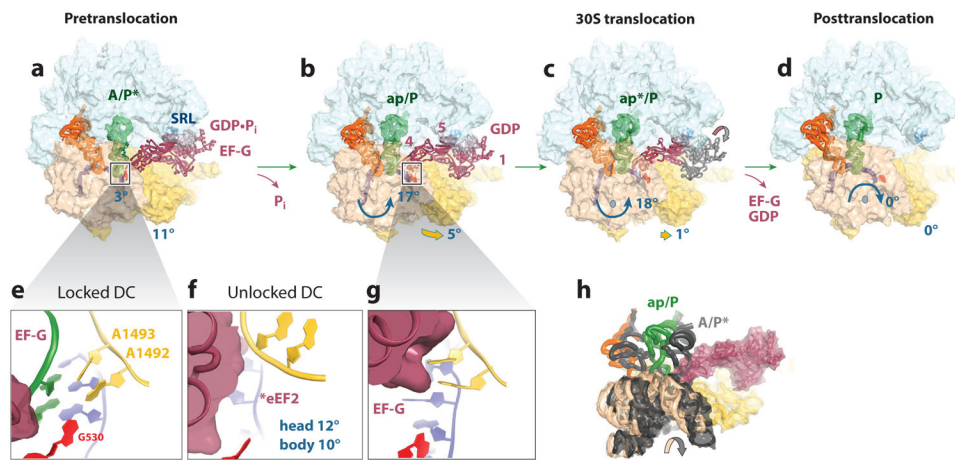
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**Figure 3.**

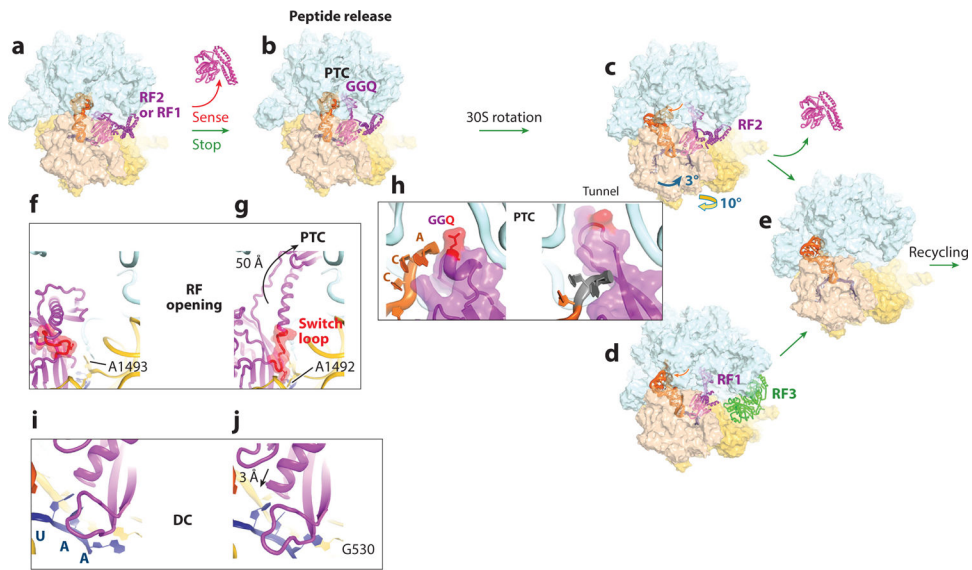
Ribosome, EF-Tu, and tRNA dynamics during mRNA decoding and tRNA proofreading, visualized by ensemble and time-resolved cryo-EM. (a) Binding of the aa-tRNA•EF-Tu•GTP ternary complex to the small subunit. (b) Codon recognition upon tRNA ASL tilting to base pair with the mRNA codon. (c) A shift in G530 (*red*) and the 30S shoulder brings EF-Tu toward the SRL (*blue*), resulting in EF-Tu GTPase activation (80). (d) Movement of the GTPase domain of EF-Tu, resulting in EF-Tu rearrangement and dissociation. The 30S subunit continues to sample the open and closed conformations (82). (e) Following EF-Tu dissociation, cognate tRNA (*green*) is stably held by the 30S (closed) and accommodated into the 50S A site, while near-cognate tRNA continues to sample open 30S conformations and is prone to dissociation via proofreading. (*f–h*) Conformation of the decoding center during recognition of the tRNA anticodon (location approximately shown within the square in panels *a–c*). G530 (*red*) at the shoulder of the open 30S subunit (*f*) transiently stabilizes the codon-anticodon helix (*g*) then moves toward A1492 (*yellow*), resulting in 30S domain closure and tRNA locking (*h*). (*i–j*) Insertion of the aminoacyl-CCA moiety into the peptidyl-transferase center leads to elongation of the polypeptide and is accompanied by small 30S subunit rotation. (*k–l*) Large-scale 30S rotation relative to the 50S subunit coincides with tRNA movement on the 50S subunit and preparation for translocation: A/P for peptidyl-tRNA and P/E for deacyl-tRNA. The elbow of A/P tRNA can spontaneously move toward the P site, yielding A/P\* tRNA, a substrate for EF-G binding to initiate translocation (panel *l*). The degrees of intersubunit rotation were measured relative to the previously published nonrotated posttranslocation 70S structure VII (97). Abbreviations: aa-tRNA, aminoacyl-tRNA; ASL, anticodon stem loop; cryo-EM, cryogenic electron microscopy; DC, decoding center; EF, elongation factor; SRL, sarcin-ricin loop.



**Figure 4.**

Cryo-EM structures of 70S translocation complexes visualized by ensemble and time-resolved cryo-EM with EF-G and GTP. The degrees of rotation are labeled for the small subunit body (*thick arrows*) and head (*thin arrows*). (a) Pretranslocation 70S ribosome with the fully rotated 30S bound with EF-G prior to Pi release (97). (b) 70S ribosome with extensive head swivel and intermediate body rotation, bound with EF-G with GDP (post Pi release) and chimeric tRNAs shifted by  $\sim 20$  Å relative to the A/P\* tRNA in panel a (97). The GTPase (1) and translocase domains (4 and 5) of EF-G are labeled. (c) 70S ribosome with extensive head swivel and low body rotation, with ap\*/P tRNA shifted by  $\sim 4$  Å relative to the ap/P tRNA in panel b. The GTPase superdomain of EF-G (*gray*) was not resolved in cryo-EM maps, consistent with dissociation from the SRL. (d) Posttranslocation nonrotated ribosome with tRNAs in the P and E sites. (e) The pretranslocation decoding center is locked by interactions between G530 and A1492-A1493, similar to the decoding structures shown in Figure 3 (pretranslocation structure from panel a is shown). (f) Eukaryotic 80S • IRES • eEF2 structure (101) illustrates an early step of elongation factor entry into the A site, coupled with separation of the decoding center nucleotides (unlocking). (g) Position of EF-G in the A site in a late translocation state shown in panel b. (h) Superposition of pretranslocation (*gray*) and nearly posttranslocated (colored as in panel b) complexes, showing that EF-G would create steric hindrance for tRNA during reverse head swivel (shown by arrow). Superposition was performed by structural alignment of the 16S rRNA body domains (i.e., excluding head nucleotides 930–1,385). The degrees of intersubunit rotation were measured relative to the previously published nonrotated posttranslocation 70S structure VII (96). Abbreviations: cryo-EM, cryogenic electron microscopy; DC, decoding center; eEF, eukaryotic elongation factor; EF-G, elongation factor G; IRES, internal ribosome entry site; Pi, inorganic phosphate; SRL, sarcin-ricin loop.





**Figure 5.**

Release factor and ribosome rearrangements during termination. (*a–e*) Recent time-resolved and ensemble cryo-EM structures reveal the mechanism of peptide release by RF1 and RF2, from RF opening (118) to ribosome intersubunit rearrangements leading to RF dissociation (125, 126). (*f–g, i–j*) A close-up view of the DC and stop codon in the structures with the inactive compact and catalytic open RF2 (*magenta*) demonstrates how the catalytic domain and stop codon settle in the DC, leading to peptidyl-tRNA hydrolysis. (*h*) The catalytic GGQ motif adopts distinct conformations in the PTC, with the catalytically engaged  $\alpha$ -helical (*left*; PDB ID: 4v67) and noncatalytic  $\beta$ -hairpin (*right*; PDB ID: 5u9f) plugging the peptide tunnel upon peptide release. The degrees of intersubunit rotation were measured relative to the previously published nonrotated posttranslocation 70S structure VII (97). Abbreviations: cryo-EM, cryogenic electron microscopy; DC, decoding center; PDB ID, Protein Data Bank identifier; PTC, peptidyl transferase center; RF, release factor.



**Table 1**Nomenclature for ribosomal positions of tRNA<sup>a</sup>

tRNA	Aminoacylation status	Position in ribosome structure
<b>Classical states (observed predominantly on the nonrotated ribosome)</b>		
A (also termed A/A) <sup>b</sup>	Aminoacyl or peptidyl	ASL and CCA are both in the A sites of the small and large subunits. (This state can also be sampled on the rotated ribosome)
P (also termed P/P)	Peptidyl or deacyl (or aminoacyl during initiation)	ASL and CCA are both in the P sites of the small and large subunits.
E (also termed E/E)	Deacyl	ASL and CCA are both in the E sites of the small and large subunits.
<b>Hybrid states (observed predominantly on the fully rotated ribosome)</b>		
A/P	Peptidyl	ASL is in the A site of the small subunit. CCA is in the P site of the large subunit.
A/P*	Peptidyl	ASL is in the A site of the small subunit. CCA is in the P site of the large subunit. The elbow of tRNA is shifted ~20 Å toward the P site relative to that in the A/P state.
P/E	Deacyl	ASL is in the P site of the small subunit. CCA is in the E site of the large subunit.
<b>Chimeric states (observed predominantly on the partially rotated ribosome)</b>		
ap/P	Peptidyl	ASL interacts with A-site elements of the small-subunit head domain and with P-site elements of the small-subunit body domain. CCA is in the P site of the large subunit.
ap*/P	Peptidyl	ASL interacts with A-site elements of the small-subunit head domain and is shifted ~4 Å further into the P-site of the small-subunit body domain relative to ap/P state. CCA is in the P site of the large subunit.
pe/E	Deacyl	ASL interacts with P-site elements of the small-subunit head domain and with E-site elements of the small-subunit body domain. CCA is in the E site of the large subunit.

Abbreviations: ASL, anticodon stem loop; CCA, 3' terminal cytidine, cytidine, adenosine.

<sup>a</sup> Alternative nomenclatures (such as A/ap, PRE1, CHI, or INT1) are used based on biophysical observations (e.g., see 64, 93, 151).

<sup>b</sup> The first part of the name denotes the position of the ASL on the small subunit; the last letter denotes the position of the acceptor arm on the large subunit.