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Review

Structural studies of elongation and release factors

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Abstract. The elongation and termination steps of protein synthesis are controlled by elongation and release factors, respectively. Elongation factors deliver the aminoacyl tRNA to the ribosomal A site, ensuring the elongation of the nascent polypeptide chain by one amino acid at a time, while release factors recognize the stop codons and trigger the release of the polypeptide from the ribosome. Recently, high-resolution crystal structures of ribosomes as well as translation factors on and off the ribosome have contributed a great deal to our understanding of the molecular basis of protein synthesis. This review concentrates on recent developments in our understanding of the elongation and termination steps of protein synthesis, particularly the roles of translation factors and their similarities and differences in the eukaryotic cytosol and prokaryotic systems, through a combination of structural and biochemical studies.

Keywords. Elongation factor, release factor, termination factor, ribosome structure, GTPase, protein synthesis.

Introduction

The ribosome is a huge molecular machine capable of translating the nucleotide mRNA sequence into amino acids. It consists of large and small subunits referred to as 50S and 30S, respectively, in prokaryotes. The 30S subunit contains the decoding center (DC) and is responsible for mRNA binding. It monitors the incorporation of the aminoacyl tRNA through the codon-anticodon interaction. The 50S subunit contains a peptidyl-transferase center (PTC) where peptide bond formation is catalyzed. During protein synthesis, transfer RNAs (tRNAs) bind to three distinct sites, the aminoacyl site (A site), the peptidyl site (P site) and exit site (E site), which are located at the interface between the ribosomal subunits [1].

Protein synthesis involves four different stages: initiation, elongation, termination and recycling, all of which are tightly controlled by translation factors [2]. Many of these proteins are GTPases, which are activated by binding to a universal site on the ribosome, called the GTPase-activating center (GAC). At initiation, the ribosome is assembled on the initiation codon in the mRNA with a methionyl initiator tRNA (fMet-tRNA^{fMet}) bound to its P site. During elongation, the GTP-bound form of elongation factor Tu/1A brings the new aminoacyl-tRNA (aa-tRNA) to the empty A site of the ribosome for decoding to occur. This triggers GTP hydrolysis and leads to the release of GDP-bound EF-Tu/1A from the ribosome. The elongating peptide is then transferred from the P site tRNA to the A site aa-tRNA, extending the polypeptide by one amino acid. Elongation factor G/2 then catalyzes the translocation of A and P site tRNAs to the P and E sites respectively, as well as movement of the mRNA by exactly one codon

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to allow a new round of elongation. When the ribosome encounters one of the stop codons, UAA, UAG or UGA, release factors are recruited to the ribosome to promote release of the newly synthesized polypeptide from the ribosome. After termination the ribosome is dissociated into its constituent subunits and the mRNA and deacylated tRNA are released, allowing the ribosome to begin another round of translation.

In the past few years, the structures of many elongation and release factors on and off the ribosome have been solved by crystallography and cryo-electron microscopy (cryo-EM) reconstruction. This structural information, combined with genetic and biochemical data, have improved our understanding of the mechanism of protein synthesis, particularly in relation to elongation and termination. In this review, we will focus on recent structural data that contribute to our understanding of the roles of elongation and release factors in protein synthesis. We will concentrate on prokaryotic elongation and release factors since the structures of many of these factors are known. We will also highlight the similarities and differences between these factors and their counterparts in the eukaryotic cytosol.

Elongation factor Tu/1A

After initiation, the ribosome particle contains both subunits bound to an mRNA with fMet-tRNA^{fMet} in the P site. The other tRNA sites are empty. Elongation is catalyzed by two GTPases, EF-Tu and EF-G (eEF1A and eEF2, respectively, in eukaryotes). The GTP-bound form of EF-Tu forms a complex with aa-tRNAs and brings them to the A site of the ribosome. The ribosome activates the GTPase activity of EF-Tu, hydrolyzing GTP to GDP. This causes EF-Tu to dissociate from the tRNA and allows the tRNA to fully enter into the A site [3], in a process known as accommodation. Once EF-Tu has dissociated, the GDP is exchanged for GTP by its guanine-nucleotide exchange factor (GEF), EF-Ts (EF-1Bα in eukaryotes).

The first structure of EF-Tu solved was its GTPase domain with a bound GDP [4]. Several years later, crystal structures of full-length EF-Tu were solved in complex with the non-hydrolyzable GTP analogue, GDPNP, so are thought to represent the GTP-bound conformation [5, 6]. Structures of the full-length EF-Tu with bound GDP were also determined [7–9]. These structures showed that EF-Tu consists of three domains. The GTPase domain, domain I or the G domain, is ~200 residues and is related to the GTPase domains of other G proteins. Domains II and III both consist of ~100 residues which form β barrels. These two domains are in the same relative orientation with respect to each other in both GDP- and GTP-bound forms of EF-Tu, suggesting that they function as a single rigid unit. The structures show that EF-Tu undergoes a large conformational change upon hydrolysis of GTP to GDP, particularly in the switch I and switch II regions of the G domain, causing a substantial rotation of the G domain with respect to domains II and III. The structures and this conformational change are likely to be well conserved between all species, since the structure of an archaeal GDP-bound EF1A shows that this protein has a similar overall fold to GDP-bound EF-Tu [10].

Structures have also been solved for EF-Tu bound to EF-Ts, helping us to understand how EF-Ts catalyzes the exchange of GDP for GTP [11, 12]. EF-Ts binds between the G domain and domains I and II and weakens the affinity of EF-Tu for the GDP by altering the GDP-binding pocket. This allows GDP to dissociate and for GTP to rebind. The structures of prokaryotic EF-Tu and the homologous eukaryotic elongation factor, eEF1A, are very similar, although eEF1A contains additional inserts within the G domain. Therefore it was surprising to find that the GEF for eEF1A, eEF1Bα, works by a different mechanism from that of EF-Ts. The crystal structure of eEF1A bound to the catalytic domain of eEF1Bα showed that eEF1Bα bound to eEF1A in an entirely different manner [13]. Mg^{2+} does not appear to be required for the exchange mechanism, in contrast to EF-Tu, since the structure of archaeal EF1A indicated that the GDP-bound form of eEF1A no longer coordinates Mg^{2+} in the nucleotide-binding site [10, 14], suggesting that eEF1Bα induces local conformational changes that cause GDP dissociation. It is also thought that eEF1Bα prevents eEF1A binding to an aa-tRNA, thus preventing it from forming a ternary complex until GTP is rebound.

It is likely that the interaction between EF-Tu and the ribosome is similar to the interaction between EF-Tu and EF-Ts. A cryo-EM structure of EF-Tu bound to the ribosome showed that the G domain of EF-Tu binds to the ribosomal protein L7/L12 [3]. A comparison of the structures of the C-terminal domain of L7/L12 and the N-terminal domain of EF-Ts shows that they have a similar shape and charge [15], indicating that both proteins interact with EF-Tu in a similar manner.

EF-Tu●GTP has high affinity for all 20 aa-tRNAs and is capable of discriminating those that are aminoacylated. Structures of complexes of EF-Tu bound to GDPNP and Phe-tRNA^{Phe} or Cys-tRNA^{Cys} show how EF-Tu recognizes common features of the aminoacyl tRNAs, rather than making tRNA-specific contacts

[16, 17]. EF-Tu binds to the aminoacyl end of the tRNA including the aminoacylated CCA end, the acceptor stem and the T stem. The T stem interacts with domain III of EF-Tu, and the acceptor stem with the switch regions of the G domain. Importantly, the aminoacyl ester bond is recognized in both complexes by the same motif, but in a somewhat different manner, indicating flexibility in the interaction with this region. The aminoacyl group itself is inserted into a cleft between the G domain and domain II, such that it can accommodate residues of different sizes.

Once the ternary complex of EF-Tu●GTP●aa-tRNA is bound to the ribosome, the GTPase activity of EF-Tu is activated by the GAC on the ribosome [18]. This only occurs if the anticodon binds to its cognate codon, since the GTPase activation and accommodation are accelerated in cognate compared to near-cognate codon-anticodon interactions [19]. Once the aa-tRNA is fully accommodated into the A site, the peptidyl-transfer reaction occurs rapidly [18]. This reaction is catalyzed by the rRNA of the large subunit [20, 21]. This transfers the peptidyl group from the P site tRNA onto the aa-tRNA in the A site, leaving a deacetylated tRNA in the P site, and extending the peptide by one amino acid.

The codon-anticodon interaction is only three base pairs so there must be additional levels of discrimination than just the base pairing alone. The DC in the 30S subunit recognizes the geometry of the matched codon-anticodon helix [22, 23], using three universally conserved bases (G530, A1492 and A1493 in *Escherichia coli*), which specifically recognize the correctly base-paired interaction. In the presence of tRNA and mRNA in the ribosome A site, G530 switches from the *syn* to *anti* conformation to interact with the bases of the anticodon at position 2 and codon at position 3. A1492 and A1493 interact with the minor groove side of the first and second base pairs of the codon-anticodon helix, respectively [22]. Importantly there are no specific interactions to the third base pair of the codon-anticodon helix, so that a wobble base pair can be accommodated in this position and base pairing is only required at positions 1 and 2. By recognizing the geometry of the codon-anticodon interaction, the ribosome is able to discriminate against non-cognate tRNAs, which would distort the helix.

The fidelity of translation is greater than can be accounted for by the three base-pair codon-anticodon interaction, indicating that there are greater levels of discrimination than simply recognizing the conformation of a cognate interaction. It is thought that the discrimination process consists of two separate steps, known as 'kinetic proofreading' [24]. Briefly, this process assumes that an initial reversible association of the aa-tRNA●EF-Tu●GTP complex to the ribosome, when the complex is selected based on the codon-anticodon interaction, is separated from a subsequent proofreading step by irreversible GTP hydrolysis by EF-Tu. Therefore the initial selection and subsequent proofreading steps are separated and the fidelity is the product of both discrimination steps. After GTP hydrolysis, the aa-tRNA can be accommodated or it can still dissociate if it does not form the correct codon-anticodon interaction.

Despite a number of structures of EF-Tu in various conformational states, the mechanism of GTP hydrolysis by EF-Tu is still not known. EF-Tu has low intrinsic GTPase activity, but it is considerably lower than that of the other families of G proteins, the small GTPases and the heterotrimeric G proteins [25]. For both of these other families, structures have been solved bound to the transition state analogue, GDP and AlF_4^-, providing detailed knowledge of the catalytic residues. However no structure of EF-Tu has been solved with a transition state analogue. In many heterotrimeric G proteins, conserved Arg and Gln residues in the switch regions stabilize the transition state [26], explaining their higher intrinsic GTPase activity. Many small GTPases have the Gln residue, but the Arg is provided in an 'arginine finger' by a GTPase-activating protein [27], so these small GTPases have a somewhat lower intrinsic activity. EF-Tu has a His residue in place of the conserved Gln found in the other families of G proteins, as well as a conserved Arg in the switch 1 region. Therefore it is not clear why EF-Tu has such a low intrinsic GTPase rate. Mutagenesis of the His residue reduces GTP hydrolysis by an order of magnitude [28], whilst mutagenesis of the Arg residue does not affect the intrinsic GTPase activity, but does reduce the ribosome-stimulated GTPase activity [29]. Therefore it is likely that the ribosome induces a particular conformation of EF-Tu that allows GTP hydrolysis, or it contributes catalytic residues to the GTPase active site. It is likely that answering this problem will require higher-resolution structures of EF-Tu bound to the ribosome.

EF-Tu is the target of at least four classes of antibiotics and antibacterials, which appear to work by two distinct mechanisms. Pulvomycin and the synthetic antibacterial, GE2270A, prevent EF-Tu binding to aa-tRNA to form the ternary complex. Recently, structures of both these compounds bound to EF-Tu●GDPNP were solved [30]. Despite binding in somewhat different manners, both compounds bind in a similar position between all three domains and block binding to the aminoacyl group of an aa-tRNA by steric interference. The other two classes of compounds, kirromycin and enacyloxin IIa, prevent the release of EF-Tu●GDP from the ribosome.

Structures of EF-Tu●GDP bound to the kirromycin derivative, aurodox [31], and of EF-Tu●GDPNP bound to enacyloxin IIa [32], show that these compounds also bind to a similar position on EF-Tu. Both compounds bind at the interface between the G domain and domain III and induce a conformation like that of GTP-bound EF-Tu. Therefore these compounds 'lock' EF-Tu in this state and prevent GDP release or release of EF-Tu from the ribosome.

Elongation factor G/2

After peptidyl transfer has occurred, the tRNAs in the A and P sites move to the P and E sites, respectively, and at the same time, the mRNA moves forward three nucleotides with respect to the ribosome so that a new codon is presented in the A site. This translocation reaction is catalyzed by the GTPase EF-G and a lot of work has gone into determining the exact mechanism. Binding of a non-hydrolysable GTP analogue to EF-G promotes translocation [33], so it was assumed that GTP hydrolysis is only required for recycling EF-G. However kinetic experiments indicate that GTP hydrolysis precedes translocation and therefore that the energy from GTP hydrolysis directly drives translocation [34]. Subsequent experiments showed that GTP hydrolysis drives a conformational change in the ribosome, referred to as an unlocking event [35]. EF-G has weak affinity for both GDP and GTP in solution, but GTP binding is stabilized by four orders of magnitude on binding to the ribosome, indicating that EF-G binds to the ribosome in the GTP-bound form [36].

Different possibilities for the translocation mechanism exist. If the movement occurred in discrete steps, with first one and then the other subunit of the ribosome moving, one would expect to see hybrid states for the tRNAs bound to the A and P sites of one subunit and the P and E sites of the other subunit [37, 38]. Another possibility is that the mRNA and tRNAs move together through the ribosome, driven by flexible regions of the ribosome itself [39]. Crystal structures of EF-G have been determined for the free protein and bound to both GDP and GTP [40–43]. EF-G consists of six domains, I–V and G′, but very little structural difference was seen between the structures, so initially it was not clear how EF-G caused the conformational changes required for translocation. Cryo-EM reconstructions of the pre- and post-translocation ribosomes bound to EF-G indicate that EF-G binding to the ribosome causes a large conformational change in both EF-G and the ribosome [44–46]. The ribosome undergoes a ratchet-like conformational change in which the small subunit moves with respect to the large subunit [38]. This ratcheting movement causes the tRNAs to move into A/P and P/E hybrid states bound to the A and P sites in the 30S subunit and the P and E sites in the 50S subunit [47]. Importantly the ratchet only occurs once the peptide bound to the tRNA in the P site has been removed [47]. This unlocks the ribosome and allows the conformational changes induced by EF-G to occur. Therefore these data support the hybrid state model of translocation, although as discussed below, this only accounts for part of the required movement.

Recently the structure of EF-G-2, a functional homologue of EF-G has been determined bound to GTP (Fig. 1) [48] and this adopted a very different conformation from that of a previous GDPNP-bound structure of EF-G [42]. The authors also determined the structure of EF-G●GDPNP bound to the ribosome using cryo-EM. The high-resolution structure of EF-G-2 adopted the same conformation as that of EF-G bound to the ribosome [48]. This suggests that this new structure is the active GTP- and ribosome-bound conformation of EF-G. The cryo-EM structure also allows the visualization of the switch regions of EF-G which are frequently disordered in both cryo-EM and crystal structures. The data agree with previous cryo-EM structures in which the largest conformational change was shown to occur to the tip of domain IV of EF-G, which inserts into the A site [47, 49]. Mutation of a conserved His residue at the tip of this domain decreases the rate of translation by over two orders of magnitude, indicating the importance of this region [50].

The ratchet movement is clearly extremely important for the movement of the tRNAs and mRNA, but this is not the whole story. The conformational change induced by EF-G in which domains III–V move in a hinge-like motion with respect to domains I, II and G, accounts for movement of about 8 Å, whereas the full translocation step requires a movement of 20 Å. Intriguingly, recent cryo-EM reconstructions provide further insight into the entire movement [51]. Using the 80S ribosome from the eukaryote *Thermomyces languginosus* the authors took advantage of the fact that eEF2 is ADP-ribosylated and used this as a marker to look at the movement of domain IV of eEF2. They suggest that movement of this domain of eEF2 breaks the interaction of the codon-anticodon complex with the decoding center, allowing the tRNA to move to the P site. By comparing structures before and after GTP hydrolysis, they show that the small subunit rotates about the neck with respect to the large subunit, and that this could account for the additional 12 Å movement required to move the 20 Å between the A, P and E sites.

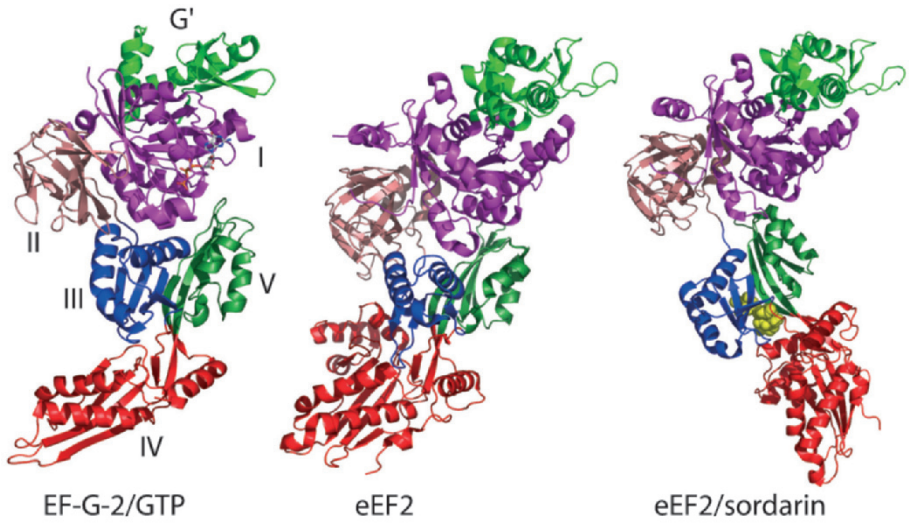

Figure 1. The structures of EF-G-2, eEF2 and eEF2 bound to sordarin. The equivalent domains of each structure are shown in the same color and labeled on EF-G-2. The GTP bound to EF-G-2 is shown in stick representation bound to domain, I. The sordarin molecule bound to eEF2 is shown in yellow in space-filling representation.

EF-G is the target of the antibacterial compound fusidic acid, which binds to EF-G and prevents the release of EF-G●GDP from the ribosome. Therefore it has been used extensively in cryo-EM studies to lock EF-G●GDP onto the ribosome [47]. No structure exists for fusidic acid bound to the free EF-G, since it only binds with high affinity to EF-G on the ribosome after GTP hydrolysis, so the binding site has been inferred from the positions of resistance mutations. The structures of resistant and hypersensitive mutants have been solved and agree with the interfaces between domains III, V and the switch II region of the G′ domain forming the binding site [52]. Fusidic acid binding seems to be intimately related to GTP binding, with many resistance mutations having a decreased affinity for GTP [53]. The structures suggest that the mutations affect the conformation of the switch II regions. In particular, a Lys residue in the P loop of EF-G was suggested to play a role in both GTP hydrolysis and sensitivity to fusidic acid [52].

Structures of yeast eEF2 alone and bound to the antifungal compound, sordarin, have also been determined (Fig. 1) [54]. Sordarin blocks the release of eEF2●GDP from the eukaryotic ribosome in a manner analogous to the antibiotic fusidic acid. The structure of eEF2 is similar to that of EF-G, except that eEF2 contains a larger G domain. Sordarin binds to the interface between domains III, IV and V, and binding causes a conformational change in the orientation of these three domains indicating that the domain boundaries are more flexible than previously thought. Recent structures of sordarin derivatives bound to eEF2 show that the ligand-binding pocket adopts a similar conformation, but that the orientation of domains III, IV and V with respect to domains G, I and II is altered, suggesting that these sordarins lock eEF2 in a different conformation [55].

Specialized elongation factors

Protein synthesis in fungi requires an additional translation factor, eEF3, that is not present in prokaryotes or higher eukaryotes. eEF3 has ATPase activity that is stimulated by the ribosome, and ATP hydrolysis is required for each addition of an aa-tRNA to the polypeptide chain. eEF3 is thought to be required for both aa-tRNA●eEF1A●GTP binding to the ribosome and for release of the tRNA from the E site [56]. With no atomic resolution structures of fungal ribosomes, it is not clear why protein synthesis in fungi requires this additional elongation factor. Recently the structure of eEF3 from *Saccharomyces cerevisiae* was solved alone, with ADP or with the non-hydrolyzable ATP analogue, ADPNP (Fig. 2) [57]. The structure confirmed previous sequence alignment indicating that it is a member of the ATP-binding cassette (ABC) family of ATPases. The structure consists of several N-terminal HEAT repeats, a four-helix bundle and two ABC-type ATPase domains, one of which contains an additional chromodomain insert. The authors also looked at the binding of eEF3 to the 80S ribosome using cryo-EM and showed that eEF3 interacted with both ribosomal subunits, with the chromodomain positioned next to the E site. Surprisingly, all three crystal structures of eEF3 adopted a similar conformation; however, the ribosome-bound eEF3 required movement of the chromodomain to fit the electron density, indicating that this region may undergo a conformational change on ribosome binding. The authors suggested that during the ATPase cycle, the chromodomain moves the L1 stalk of the ribosome to open the E site and allow tRNA release. Another specialized elongation factor is SelB, which is required to insert the atypical amino acid, selenocysteine (Sec), into the UGA stop codon when the Sec

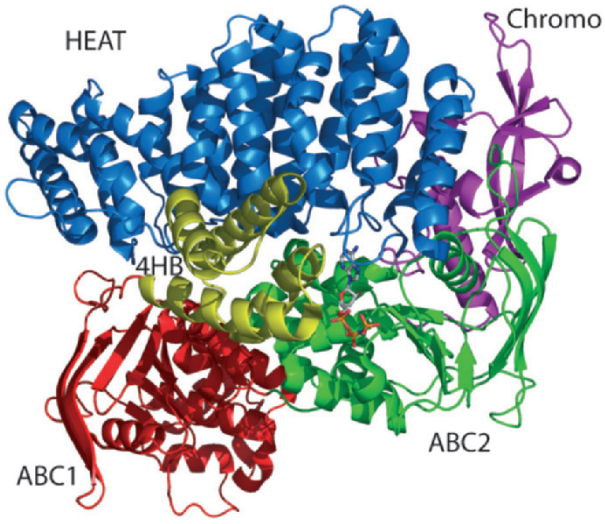

Figure 2. The structure of, *S. cerevisiae* EF3 bound to the non-hydrolyzable ATP analogue, ADPNP. The individual domains are coloured separately and labeled on the figure. The ADPNP is shown in a stick representation.

insertion sequence is present further downstream on the mRNA. SelB binds to Sec-tRNA^{Sec}, unlike EF-Tu, which binds to the other 20 aa-tRNAs. It is found in both prokaryotes and eukaryotes, although its domain organization differs [58]. Prokaryotic SelB is a single protein consisting of an EF-Tu-like domain and an N-terminal RNA-binding domain, whereas eukaryotic SelB only contains the EF-Tu-like domain and the RNA-binding role is performed by a second protein, Sbp2.

The structure of archaeal SelB has been determined in the apo, GDP- and GDPNP-bound forms, confirming that it is an EF-Tu-like GTPase protein and that it functions in a similar manner [59]. The Sec insertion sequence forms a hairpin structure in the mRNA and several structures of the C-terminal domain of prokaryotic SelB, both free and in complex with the RNA hairpin, have been determined [60–63]. These structures show that the RNA-binding domain consists of four winged-helix (WH) motifs and that the linkers between the WH motifs are flexible so that they undergo large conformational changes on RNA binding. The RNA hairpin binds between WH3 and WH4, inducing a sharp kink in the protein, possibly so that the mRNA can be wrapped around the 30S subunit of the ribosome. WH2 and WH3 may also interact with RNA and have been suggested to interact with the 16S rRNA on the ribosome [62].

Class I release factors

The elongation phase of protein synthesis continues until a stop codon is translocated into the ribosomal A site. Stop codons are recognized by class I release factors (RFs) that bind to the ribosomal A site and induce hydrolysis of the ester bond linking the peptide chain to the tRNA in the P site. In prokaryotes, there are two class I release factors, RF1 and RF2. Both recognize UAA, while only RF1 recognizes UAG and only RF2 recognizes UGA [64]. Genetic and biochemical analyses have identified highly conserved tripeptide motifs in the bacterial class I RFs, PAT in RF1 and SPF in RF2, that are directly involved in stop codon recognition in the decoding center of the small ribosomal subunit [65]. Eukaryotes and archaea have only one class I RF, eRF1 or aRF1, respectively, that recognizes all three stop codons. Archaeal and eukaryotic class I RFs are homologous, but are largely unrelated to prokaryotic RFs. Class I RFs release newly synthesized proteins from the ribosome by triggering hydrolysis of the ester bond in peptidyl-tRNA, presumably through contact between the universally conserved GGQ motif and the peptidyl-transfer center of the ribosomal 50S subunit [64, 66]. Methylation of the Gln residue in the GGQ motif has been shown to stimulate peptide release [67].

The crystal structure of human eRF1 showed that it has a three-domain architecture with an overall shape reminiscent of the letter 'Y' [68]. Domain 1 contains a conserved groove including the motif NIKS, which has been proposed to be the stop codon recognition site. The universally conserved GGQ motif is at the tip of domain 2, and the eRF3 interaction site is within domain 3. The overall shape and dimensions of eRF1 resemble a tRNA molecule with domains 1, 2 and 3 corresponding to the anticodon loop, aminoacyl acceptor stem and T stem of a tRNA molecule, respectively. The distance from the proposed stop codon recognition site to the GGQ motif is 80 Å, slightly larger than the 75 Å between the anticodon bases and the aminoacyl group of aminoacylated tRNA molecules, suggesting that eRF1 undergoes a conformational change upon binding to the ribosome. The crystal structures of bacterial RF1 and RF2 are very distinct to that of eRF1 [69, 70]. Unlike the three domains of eRF1, RF2 and RF1 each have four domains. The GGQ motif is at the tip of domain 3, and the tripeptide motifs are at the tip of domain 2. Domains 2, 3 and 4 are packed together forming a compact structure that, unlike eRF1, does not appear to resemble a tRNA molecule. Surprisingly, the distance between the GGQ and tripeptide motifs is only ~25 Å, much shorter than the 75 Å between the DC and the PTC of the ribosome. This finding is

apparently incompatible with previous genetic and biochemical data for these factors, showing that they bound to the ribosomal A site. Subsequent cryo-EM structures of ribosomes with bound RF1 and RF2 showed that these factors undergo dramatic conformational changes in which domain 3, containing the GGQ motif, moves away from domains 2 and 4 [71–73]. The ribosome-bound RF1 and RF2 adopt 'open' conformations, similar to that of eRF1, in which the distance between the GGQ at the tip of domain 3 and the tripeptide motif at the tip of domain 2 is increased from 25 Å to 73 Å. These 'open' conformations allow simultaneous access of the tripeptide motif to the DC and the GGQ motif to the PTC. More recent small-angle X-ray scattering (SAXS) experiments showed that RF1 adopts a similar 'open' configuration in solution [74]. These data raise the question of what the biological significance of the compact conformation observed in the crystal structures of RF1 and RF2 is. The crystal structure of RF1 in complex with the methyl-transferase PrmC showed that RF1 adopts a similar compact structure, suggesting that the compact conformations are important for the methylation of Gln in the GGQ motif by PrmC [75].

The tripeptide motif, PAT in RF1 and SPF in RF2, has been shown to determine the specificity of stop codon recognition and therefore termed the tripeptide anticodon [65]. However, the exact mechanism of discrimination between stop codons and sense codons has remained elusive. Recently, crystal structures of the *Thermus thermophilus* ribosome complexed with RF1 or RF2 have been solved to ~6 Å resolution [76]. The structures showed that the four domains of RF1 and RF2 adopt an overall fold similar to those seen by cryo-EM, and predicted by SAXS. A loop between strands β4 and β5 containing the tripeptide motif, the anticodon loop, is in close contact with the stop codon. Intriguingly, the uridine in the first position of the stop codon is flanked by the tip of helix α5 of RF1 or RF2 in both structures, suggesting that it could discriminate against A, G and C in this position. If this is true, the anticodon loop, which is specific to RF1 or RF2, would recognize only the second and third bases. PAT in RF1 would recognize AA or AG and SPF in RF2 would recognize AA or GA. This indicates that elements other than the tripeptide motif are required for stop codon recognition.

The interaction between eRF1 and the stop codons is less obvious and a number of sequences contribute to codon recognition, allowing the eukaryotic protein to recognize more stop codons. Chimera experiments with an eRF1, which only recognizes UGA, and the yeast eRF1, which recognizes all three stop codons, show that the N-terminal domain is responsible for the stop codon specificity on mammalian ribosomes [77, 78]. At least two conserved regions within the N-terminal domain affect codon recognition, particularly the conserved sequences NIKS and YxCxxxF [78–81]. These data suggested that eRF1 recognizes stop codons not by a simple peptide-codon interaction, but rather through a three-dimensional network formed by conserved residues. It is likely that fully understanding the specificity of eRF1 will require a high-resolution structure of eRF1 bound to a stop codon within the ribosome.

One of the fundamental questions of translation termination is how the binding of class I RFs results in hydrolysis of the ester bond linking the peptide to the tRNA. The universally conserved GGQ motif has been shown to be essential for triggering hydrolysis of the peptidyl-tRNA bond [82, 83]. Two hypotheses have been proposed to explain the role of the GGQ motif in peptide release. One suggested that the Gln is directly involved in catalysis by co-ordination of a water molecule in the PTC that nucleophilically attacks the ester bond of the peptidyl-tRNA [68], whereas the other proposed that GGQ induces ester bond hydrolysis by activating a catalytic reaction performed by the PTC, possibly by allowing a water molecule access to the ester bond. The first hypothesis appears to be at odds with observations that Gln substitution mutants still retain significant release activity [84]. The recent low-resolution crystal structures of the ribosome bound to class I RFs showed that the GGQ motif is in close proximity to nucleotides C2063, A2451, U2506 and A2602 of 23S rRNA [76], and the last of these is the most essential nucleotide for hydrolysis, based on mutational data. However, due to the limited resolution of these structures, it is still not clear whether class I RFs function by directly co-ordinating a water molecule or by inducing conformational changes in the PTC. Recently, computer simulations predicted that the methylated Gln residue of the GGQ motif is involved in orientating a catalytic water molecule in good position for attack on the P site ester bond [85]. These data are supported by recent results examining the nucleophile specificity of RF1 [86]. The authors showed that RF1 is very specific for water and that mutation of the Gln residue in the GGQ motif removes this specificity. Together these data provide good evidence that the hydrolysis mechanism for peptide release does involve a water nucleophile co-ordinated by the Gln residue, as was originally proposed [68].

Class II release factors

Termination also requires class II release factors, RF3 in prokaryotes and eRF3 in eukaryotes. RF3 and

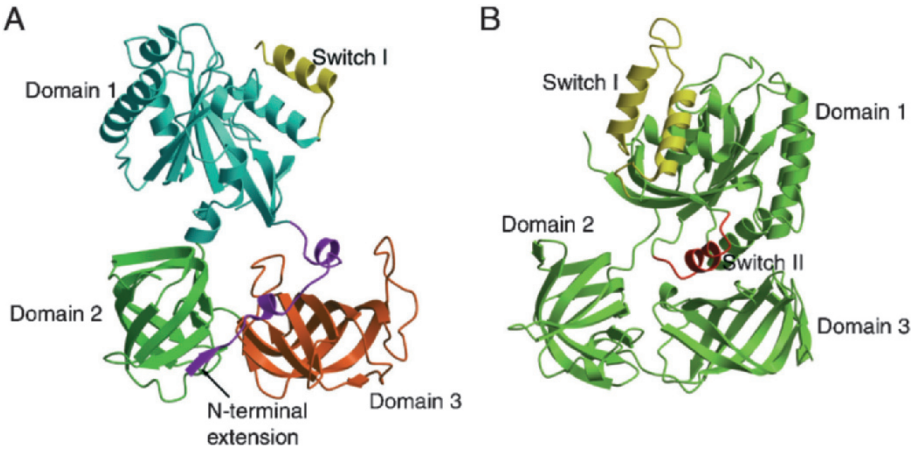

Figure 3. Crystal structure of eRF3c and comparison with eEF1α. eRF3c (*A*) and eEF1α (*B*) in the eEF1α-eEF1β complex. The ribbon diagrams are drawn with domains 2 and 3 in the same orientation. Domains 1, 2 and 3 and the N-terminal extension of eRF3c are colored as cyan, green, orange and magenta, respectively. Switch I and II regions are shown in yellow and red, respectively.

eRF3 are GTPases and sequence homology between them is limited to their GTP-binding domains. There are also significant differences in the mechanisms of eRF3 and RF3. eRF3 is essential for cell viability whereas RF3 is not. The GTPase activity of RF3 is dependent on the ribosome and is further stimulated by RF1 or RF2 in a codon-dependent manner [87], whereas the GTPase activity of eRF3 strictly requires both the ribosome and eRF1, independent of a codon [88]. Another significant difference between RF3 and eRF3 is that eRF3 forms a stable complex with eRF1 through mutual binding of their C-terminal domains [89–91], whereas there is no significant affinity between free RF1/RF2 and RF3 [92].

In addition RF3 and eRF3 have different functions. The function of RF3 is to recycle RF1 or RF2 and remove them from the ribosomal A site after hydrolysis of the peptidyl-tRNA bond [93]. The function of eRF3 is less clear. It was proposed that it might play a similar role to that of RF3 and just recycle eRF1. However, recent data have shown that GTP hydrolysis on eRF3 is coupled with stop codon recognition by eRF1 and with efficient peptide release [94–96], suggesting that the GTPase activity of eRF3 might have an additional function to enhance the accuracy of stop codon recognition by eRF1.

The structure of the eEF1A-like region of *Schizosaccharomyces pombe* eRF3 (eRF3c) was determined for the free protein and bound to GDP and GDPNP and shows an overall structure that is similar to eEF-1α (Fig. 3) [97]. In contrast to EF-Tu, GDP/GTP binding to eRF3 does not appear to induce conformational changes, and Mg^{2+} is not required for GDP binding to *S. pombe* eRF3. All three structures of eRF3 are similar to one another, like those observed for EF-G. Isothermal titration calorimetry experiments suggested that Mg^{2+} may modulate the guanine-nucleotide exchange of eRF3. However, this hypothesis is not consistent with similar experiments with human eRF3, which show that GDP binding to eRF3 is not significantly dependent on the Mg^{2+} concentration [98].

The mechanism by which RF3 dissociates RF1 or RF2 from the ribosome after polypeptide release is becoming increasingly understood. It is thought that RF3 binds to the ribosome in complex with GDP since the affinity of RF3 for GDP is three orders of magnitude higher than that for GTP [87]. The ribosomal post-termination complex containing RF1 or RF2 bound to a stop codon is the GEF for RF3. Therefore hydrolysis of the ester bond linking the peptide and tRNA by RF1 or RF2 leads to rapid exchange of GDP for GTP within ribosome-bound RF3. Formation of a high-affinity complex between the ribosome and RF3●GTP induces dissociation of RF1/RF2 because RF1 or RF2 and RF3●GDPNP destabilize each other's binding to the post-termination ribosome [87]. RF3 then hydrolyzes GTP and, since the GDP-bound form of RF3 has lower affinity for the ribosome, it dissociates.

Although this mechanism explains the mode of action of RF3 on the ribosome, the molecular details of how RF3 dissociates RF1/RF2 from the ribosome after polypeptide release is still not clear due to the lack of structural information for RF3. The cryo-EM structure of RF3 in complex with GDPNP was determined bound to a post-termination ribosome lacking a class I RF [99]. The complex was seen in two states differing in the conformations of both the ribosome and RF3, as well as in the location of the tRNA. In the first state, RF3 and RF2 can be simultaneously accommodated, whereas in the second they clash due to steric hindrance, implying that RF3 dissociates RF1/RF2 by direct competition [99]. However, the observation of two states contradicts the fact that RF3●GDPNP binds very stably to the ribosomal release complex [87], raising the question of whether the cryo-EM structures really represent GTP-bound RF3.

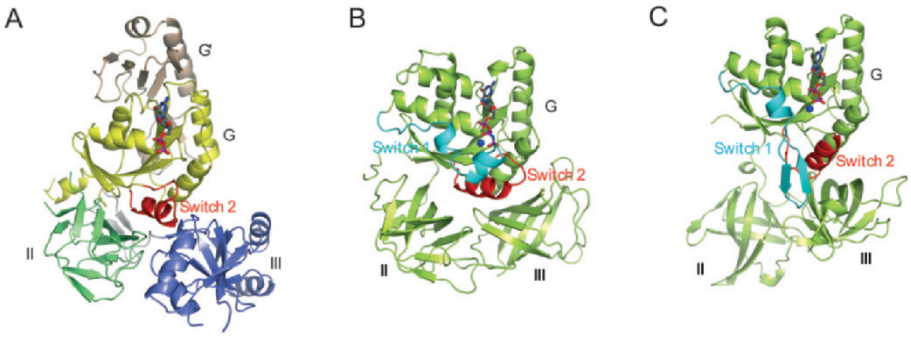

Figure 4. Crystal structure of RF3●GDP (*A*) and its comparison with EF-Tu●GTP (*B*) and EF-Tu●GDP (*C*). The ribbon diagrams are drawn with the G domain in the same orientation. Domains G′, G, II and III of RF3●GDP are colored in wheat, yellow, lime green and light blue, respectively, with the linker regions between domains in light gray. Switch 1 and 2 regions are colored in cyan and red, respectively. Bound nucleotide is shown as a stick model, and the Mg^{2+} ion as a sphere.

The crystal structure of *E. coli* RF3 bound to GDP was determined recently [100]. It has three domains and its conformation is remarkably similar to the crystal structure of EF-Tu●GTP, but distinct from those of EF-Tu●GDP (Fig. 4) or EF-G●GDP. The authors also presented a cryo-EM structure of the post-termination ribosome bound to RF3●GDPNP. Docking the crystal structure of RF3●GDP into the cryo-EM density for RF3 showed that RF3 undergoes a large conformational change in which domains II and III move relative to the G domain, forming a more extended structure. This change from a compact to a more extended conformation for RF3●GDPNP is reminiscent of that seen for EF-G●GDPNP on binding to the ribosome. The binding of RF3●GDPNP to the ribosome causes a ratchet-like movement of the small relative to the large ribosomal subunit accompanied by a swivel motion of the L1 stalk, as well as movement of the deacylated tRNA from the P to a hybrid P/E site. These conformational changes in the ribosome are also very similar to those previously observed for EF-G●GDPNP binding to an unlocked ribosome [47]. Formation of the high-affinity complex between RF3●GTP and the peptide-free ribosome breaks the interactions between the ribosome and RF1/RF2 in the DC and GAC regions, which may cause the dissociation of RF1/RF2 [100]. GTP hydrolysis would then reduce the affinity of RF3 for the ribosome and trigger its rapid dissociation. These results have increased our understanding of the molecular details of the mode of action of RF3, helping to explain previous biochemical data [87, 101, 102].

Concluding remarks

In recent years, our understanding of the mechanism of protein synthesis and its regulation by translation factors has improved dramatically. This has been helped enormously by a combination of X-ray crystallography to study individual translation factors in various conformational states and entire 70S ribosomes, as well as cryo-EM reconstructions of numerous ribosome conformational states. However, many challenges remain for the future, in particular, elucidating the exact differences between protein synthesis in prokaryotes and eukaryotes, and the daunting task of atomic-resolution structures of eukaryotic ribosomes. Another challenge is to determine atomic-level structures of prokaryotic ribosomes in different functional states. Now that crystals of bacterial ribosomes that diffract to atomic resolution can be produced, it should be possible to solve high-resolution structures of ribosomes bound to the various translation factors. While our knowledge of the structural basis for codon recognition and the peptidyl-transferase reaction during peptide elongation is increasing, much less is known about the mechanism of termination. How stop codons are recognized and how this triggers polypeptide release is still not clear. Resolving these issues will require high-resolution structures of the ribosome bound to release factors to support the biochemical data. Another question that remains is the nature of the co-operativity between eRF1 and eRF3. Understanding how the GTPase activity of eRF3 is activated by eRF1 and the ribosome may also require high-resolution structures.

1 Yusupbov, M. M., Yusupova, G. Z., Baucom, A., Lieberman, K., Earnest, T. N., Cate, J. H. and Noller, H. F. (2001) Crystal structure of the ribosome at 5.5 A resolution. Science 292, 883–896.
2 Ramakrishnan, V. (2002) Ribosome structure and the mechanism of translation. Cell 108, 557–572.
3 Stark, H., Rodnina, M. V., Rinke-Appel, J., Brimacombe, R., Wintermeyer, W. and van Heel, M. (1997) Visualization of elongation factor Tu on the *Escherichia coli* ribosome. Nature 389, 403–406.

4 La Cour, T. F., Nyborg, J., Thirup, S. and Clark, B. F. (1985) Structural details of the binding of guanosine diphosphate to elongation factor Tu from, *E. coli* as studied by X-ray crystallography. EMBO J. 4, 2385–2388.

5 Berchtold, H., Reshetnikova, L., Reiser, C. O., Schirmer, N. K., Sprinzl, M. and Hilgenfeld, R. (1993) Crystal structure of active elongation factor Tu reveals major domain rearrangements. Nature 365, 126–132.

6 Kjeldgaard, M., Nissen, P., Thirup, S. and Nyborg, J. (1993) The crystal structure of elongation factor EF-Tu from *Thermus aquaticus* in the GTP conformation. Structure 1, 35–50.

7 Abel, K., Yoder, M. D., Hilgenfeld, R. and Jurnak, F. (1996) An alpha to beta conformational switch in EF-Tu. Structure 4, 1153–1159.

8 Polekhina, G., Thirup, S., Kjeldgaard, M., Nissen, P., Lippmann, C. and Nyborg, J. (1996) Helix unwinding in the effector region of elongation factor EF-Tu-GDP. Structure 4, 1141–1151.

9 Song, H., Parsons, M. R., Rowsell, S., Leonard, G. and Phillips, S. E. (1999) Crystal structure of intact elongation factor EF-Tu from *Escherichia coli* in GDP conformation at 2.05 A resolution. J. Mol. Biol. 285, 1245–1256.

10 Vitagliano, L., Masullo, M., Sica, F., Zagari, A. and Bocchini, V. (2001) The crystal structure of *Sulfolobus solfataricus* elongation factor 1alpha in complex with GDP reveals novel features in nucleotide binding and exchange. EMBO J. 20, 5305–5311.

11 Kawashima, T., Berthet-Colominas, C., Wulff, M., Cusack, S. and Leberman, R. (1996) The structure of the *Escherichia coli* EF-Tu.EF-Ts complex at 2.5 A resolution. Nature 379, 511–518.

12 Wang, Y., Jiang, Y., Meyering-Voss, M., Sprinzl, M. and Sigler, P. B. (1997) Crystal structure of the EF-Tu.EF-Ts complex from *Thermus thermophilus*. Nat. Struct. Biol. 4, 650–656.

13 Andersen, G. R., Pedersen, L., Valente, L., Chatterjee, I., Kinzy, T. G., Kjeldgaard, M. and Nyborg, J. (2000) Structural basis for nucleotide exchange and competition with tRNA in the yeast elongation factor complex eEF1A:eEF1Balpha. Mol. Cell 6, 1261–1266.

14 Vitagliano, L., Ruggiero, A., Masullo, M., Cantiello, P., Arcari, P. and Zagari, A. (2004) The crystal structure of *Sulfolobus solfataricus* elongation factor 1alpha in complex with magnesium and GDP. Biochemistry 43, 6630–6636.

15 Wieden, H. J., Wintermeyer, W. and Rodnina, M. V. (2001) A common structural motif in elongation factor Ts and ribosomal protein L7/12 may be involved in the interaction with elongation factor Tu., J. Mol. Evol. 52, 129–136.

16 Nissen, P., Thirup, S., Kjeldgaard, M. and Nyborg, J. (1999) The crystal structure of Cys-tRNACys-EF-Tu-GDPNP reveals general and specific features in the ternary complex and in tRNA. Structure 7, 143–156.

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

18 Pape, T., Wintermeyer, W. and Rodnina, M. V. (1998) Complete kinetic mechanism of elongation factor Tu-dependent binding of aminoacyl-tRNA to the A site of the, *E. coli* ribosome. EMBO J. 17, 7490–7497.

19 Pape, T., Wintermeyer, W. and Rodnina, M. (1999) Induced fit in initial selection and proofreading of aminoacyl-tRNA on the ribosome. EMBO J. 18, 3800–3807.

20 Ban, N., Nissen, P., Hansen, J., Moore, P. B. and Steitz, T. A. (2000) The complete atomic structure of the large ribosomal subunit at 2.4 A resolution. Science 289, 905–920.

21 Nissen, P., Hansen, J., Ban, N., Moore, P. B. and Steitz, T. A. (2000) The structural basis of ribosome activity in peptide bond synthesis. Science 289, 920–930.

22 Ogle, J. M., Brodersen, D. E., Clemons, W. M., Jr., Tarry, M. J., Carter, A. P. and Ramakrishnan, V. (2001) Recognition of cognate transfer RNA by the 30S ribosomal subunit. Science 292, 897–902.

23 Selmer, M., Dunham, C. M., Murphy, F. V., Weixlbaumer, A., Petry, S., Kelley, A. C., Weir, J. R. and Ramakrishnan, V. (2006) Structure of the 70S ribosome complexed with mRNA and tRNA. Science 313, 1935–1942.

24 Ogle, J. M. and Ramakrishnan, V. (2005) Structural insights into translational fidelity. Annu. Rev. Biochem. 74, 129–177.

25 Andersen, G. R., Nissen, P. and Nyborg, J. (2003) Elongation factors in protein biosynthesis. Trends Biochem. Sci. 28, 434–441.

26 Coleman, D. E., Berghuis, A. M., Lee, E., Linder, M. E., Gilman, A. G. and Sprang, S. R. (1994) Structures of active conformations of Gi alpha 1 and the mechanism of GTP hydrolysis. Science 265, 1405–1412.

27 Scheffzek, K., Ahmadian, M. R., Kabsch, W., Wiesmuller, L., Lautwein, A., Schmitz, F. and Wittinghofer, A. (1997) The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. Science 277, 333–338.

28 Cool, R. H. and Parmeggiani, A. (1991) Substitution of histidine-84 and the GTPase mechanism of elongation factor Tu. Biochemistry 30, 362–366.

29 Knudsen, C. R. and Clark, B. F. (1995) Site-directed mutagenesis of Arg58 and Asp86 of elongation factor Tu from *Escherichia coli*: effects on the GTPase reaction and aminoacyl-tRNA binding. Protein Eng. 8, 1267–1273.

30 Parmeggiani, A., Krab, I. M., Okamura, S., Nielsen, R. C., Nyborg, J. and Nissen, P. (2006) Structural basis of the action of pulvomycin and GE2270 A on elongation factor Tu. Biochemistry 45, 6846–6857.

31 Vogeley, L., Palm, G. J., Mesters, J. R. and Hilgenfeld, R. (2001) Conformational change of elongation factor Tu (EF-Tu) induced by antibiotic binding: crystal structure of the complex between EF-Tu.GDP and aurodox. J. Biol. Chem. 276, 17149–17155.

32 Parmeggiani, A., Krab, I. M., Watanabe, T., Nielsen, R. C., Dahlberg, C., Nyborg, J. and Nissen, P. (2006) Enacyloxin IIa pinpoints a binding pocket of elongation factor Tu for development of novel antibiotics. J. Biol. Chem. 281, 2893–2900.

33 Kaziro, Y. (1978) The role of guanosine 5′-triphosphate in polypeptide chain elongation. Biochim. Biophys. Acta 505, 95–127.

34 Rodnina, M. V., Savelsbergh, A., Katunin, V. I. and Wintermeyer, W. (1997) Hydrolysis of GTP by elongation factor G drives tRNA movement on the ribosome. Nature 385, 37–41.

35 Savelsbergh, A., Katunin, V. I., Mohr, D., Peske, F., Rodnina, M. V. and Wintermeyer, W. (2003) An elongation factor G-induced ribosome rearrangement precedes tRNA-mRNA translocation. Mol. Cell 11, 1517–1523.

36 Wilden, B., Savelsbergh, A., Rodnina, M. V. and Wintermeyer, W. (2006) Role and timing of GTP binding and hydrolysis during EF-G-dependent tRNA translocation on the ribosome. Proc. Natl. Acad. Sci. USA 103, 13670–13675.

37 Moazed, D. and Noller, H. F. (1989) Intermediate states in the movement of transfer RNA in the ribosome. Nature 342, 142–148.

38 Frank, J. and Agrawal, R. K. (2000) A ratchet-like intersubunit reorganization of the ribosome during translocation. Nature 406, 318–322.

39 Spahn, C. M. and Nierhaus, K. H. (1998) Models of the elongation cycle: an evaluation. Biol. Chem. 379, 753–772.

40 Evarsson, A., Brazhnikov, E., Garber, M., Zheltonosova, J., Chirgadze, Y., al Karadaghi, S., Svensson, L. A. and Liljas, A. (1994) Three-dimensional structure of the ribosomal translocase: elongation factor G from *Thermus thermophilus*. EMBO J. 13, 3669–3677.

41 Czworkowski, J., Wang, J., Steitz, T. A. and Moore, P. B. (1994) The crystal structure of elongation factor G complexed with GDP, at 2.7 A resolution. EMBO J. 13, 3661–3668.

42 Hansson, S., Singh, R., Gudkov, A. T., Liljas, A. and Logan, D. T. (2005) Crystal structure of a mutant elongation factor G trapped with a GTP analogue. FEBS Lett. 579, 4492–4497.

43 al Karadaghi, S., AEvarsson, A., Garber, M., Zheltonosova, J. and Liljas, A. (1996) The structure of elongation factor G in complex with GDP: conformational flexibility and nucleotide exchange. Structure 4, 555–565.

44 Agrawal, R. K., Penczek, P., Grassucci, R. A. and Frank, J. (1998) Visualization of elongation factor G on the *Escherichia coli* 70S ribosome: the mechanism of translocation. Proc. Natl. Acad. Sci. USA 95, 6134–6138.

45 Agrawal, R. K., Heagle, A. B., Penczek, P., Grassucci, R. A. and Frank, J. (1999) EF-G-dependent GTP hydrolysis induces translocation accompanied by large conformational changes in the 70S ribosome. Nat. Struct. Biol. 6, 643–647.

46 Stark, H., Rodnina, M. V., Wieden, H. J., van Heel, M. and Wintermeyer, W. (2000) Large-scale movement of elongation factor G and extensive conformational change of the ribosome during translocation. Cell 100, 301–309.

47 Valle, M., Zavialov, A., Sengupta, J., Rawat, U., Ehrenberg, M. and Frank, J. (2003) Locking and unlocking of ribosomal motions. Cell 114, 123–134.

48 Connell, S. R., Takemoto, C., Wilson, D. N., Wang, H., Murayama, K., Terada, T., Shirouzu, M., Rost, M., Schuler, M., Giesebrecht, J., Dabrowski, M., Mielke, T., Fucini, P., Yokoyama, S. and Spahn, C. M. (2007) Structural basis for interaction of the ribosome with the switch regions of GTP-bound elongation factors. Mol. Cell 25, 751–764.

49 Spahn, C. M., Gomez-Lorenzo, M. G., Grassucci, R. A., Jorgensen, R., Andersen, G. R., Beckmann, R., Penczek, P. A., Ballesta, J. P. and Frank, J. (2004) Domain movements of elongation factor eEF2 and the eukaryotic 80S ribosome facilitate tRNA translocation. EMBO J. 23, 1008–1019.

50 Savelsbergh, A., Matassova, N. B., Rodnina, M. V. and Wintermeyer, W. (2000) Role of domains 4 and 5 in elongation factor G functions on the ribosome., J. Mol. Biol. 300, 951–961.

51 Taylor, D. J., Nilsson, J., Merrill, A. R., Andersen, G. R., Nissen, P. and Frank, J. (2007) Structures of modified eEF2 80S ribosome complexes reveal the role of GTP hydrolysis in translocation. EMBO J. 26, 2421–2431.

52 Hansson, S., Singh, R., Gudkov, A. T., Liljas, A. and Logan, D. T. (2005) Structural insights into fusidic acid resistance and sensitivity in EF-G. J. Mol. Biol. 348, 939–949.

53 Martemyanov, K. A., Liljas, A., Yarunin, A. S. and Gudkov, A. T. (2001) Mutations in the G-domain of elongation factor G from *Thermus thermophilus* affect both its interaction with GTP and fusidic acid. J. Biol. Chem. 276, 28774–28778.

54 Jorgensen, R., Ortiz, P. A., Carr-Schmid, A., Nissen, P., Kinzy, T. G. and Andersen, G. R. (2003) Two crystal structures demonstrate large conformational changes in the eukaryotic ribosomal translocase. Nat. Struct. Biol. 10, 379–385.

55 Soe, R., Mosley, R. T., Justice, M., Nielsen-Kahn, J., Shastry, M., Merrill, A. R. and Andersen, G. R. (2007) Sordarin derivatives induce a novel conformation of the yeast ribosome translocation factor eEF2. J. Biol. Chem. 282, 657–666.

56 Triana-Alonso, F. J., Chakraburtty, K. and Nierhaus, K. H. (1995) The elongation factor 3 unique in higher fungi and essential for protein biosynthesis is an E site factor. J. Biol. Chem. 270, 20473–20478.

57 Andersen, C. B., Becker, T., Blau, M., Anand, M., Halic, M., Balar, B., Mielke, T., Boesen, T., Pedersen, J. S., Spahn, C. M., Kinzy, T. G., Andersen, G. R. and Beckmann, R. (2006) Structure of eEF3 and the mechanism of transfer RNA release from the E-site. Nature 443, 663–668.

58 Fagegaltier, D., Hubert, N., Yamada, K., Mizutani, T., Carbon, P. and Krol, A. (2000) Characterization of mSelB, a novel mammalian elongation factor for selenoprotein translation. EMBO J. 19, 4796–4805.

59 Leibundgut, M., Frick, C., Thanbichler, M., Bock, A. and Ban, N. (2005) Selenocysteine tRNA-specific elongation factor SelB is a structural chimaera of elongation and initiation factors. EMBO J. 24, 11–22.

60 Selmer, M. and Su, X. D. (2002) Crystal structure of an mRNA-binding fragment of *Moorella thermoacetica* elongation factor SelB. EMBO J. 21, 4145–4153.

61 Yoshizawa, S., Rasubala, L., Ose, T., Kohda, D., Fourmy, D. and Maenaka, K. (2005) Structural basis for mRNA recognition by elongation factor SelB. Nat. Struct. Mol. Biol. 12, 198–203.

62 Ose, T., Soler, N., Rasubala, L., Kuroki, K., Kohda, D., Fourmy, D., Yoshizawa, S. and Maenaka, K. (2007) Structural basis for dynamic interdomain movement and RNA recognition of the selenocysteine-specific elongation factor SelB. Structure 15, 577–586.

63 Soler, N., Fourmy, D. and Yoshizawa, S. (2007) Structural insight into a molecular switch in tandem winged-helix motifs from elongation factor SelB. J. Mol. Biol. 370, 728–741.

64 Kisselev, L., Ehrenberg, M. and Frolova, L. (2003) Termination of translation: interplay of mRNA, rRNAs and release factors? EMBO J. 22, 175–182.

65 Ito, K., Uno, M. and Nakamura, Y. (2000) A tripeptide 'anticodon' deciphers stop codons in messenger RNA. Nature 403, 680–684.

66 Nakamura, Y. and Ito, K. (2003) Making sense of mimic in translation termination. Trends Biochem. Sci. 28, 99–105.

67 Dincbas-Renqvist, V., Engstrom, A., Mora, L., Heurgue-Hamard, V., Buckingham, R. and Ehrenberg, M. (2000) A post-translational modification in the GGQ motif of RF2 from *Escherichia coli* stimulates termination of translation. EMBO J. 19, 6900–6907.

68 Song, H., Mugnier, P., Das, A. K., Webb, H. M., Evans, D. R., Tuite, M. F., Hemmings, B. A. and Barford, D. (2000) The crystal structure of human eukaryotic release factor eRF1 – mechanism of stop codon recognition and peptidyl-tRNA hydrolysis. Cell 100, 311–321.

69 Shin, D. H., Brandsen, J., Jancarik, J., Yokota, H., Kim, R. and Kim, S. H. (2004) Structural analyses of peptide release factor 1 from *Thermotoga maritima* reveal domain flexibility required for its interaction with the ribosome. J. Mol. Biol. 341, 227–239.

70 Vestergaard, B., Van, L. B., Andersen, G. R., Nyborg, J., Buckingham, R. H. and Kjeldgaard, M. (2001) Bacterial polypeptide release factor RF2 is structurally distinct from eukaryotic eRF1. Mol. Cell 8, 1375–1382.

71 Klaholz, B. P., Pape, T., Zavialov, A. V., Myasnikov, A. G., Orlova, E. V., Vestergaard, B., Ehrenberg, M. and van Heel, M. (2003) Structure of the *Escherichia coli* ribosomal termination complex with release factor 2. Nature 421, 90–94.

72 Rawat, U., Gao, H., Zavialov, A., Gursky, R., Ehrenberg, M. and Frank, J. (2006) Interactions of the release factor RF1 with the ribosome as revealed by cryo-EM. J. Mol. Biol. 357, 1144–1153.

73 Rawat, U. B., Zavialov, A. V., Sengupta, J., Valle, M., Grassucci, R. A., Linde, J., Vestergaard, B., Ehrenberg, M. and Frank, J. (2003) A cryo-electron microscopic study of ribosome-bound termination factor RF2. Nature 421, 87–90.

74 Vestergaard, B., Sanyal, S., Roessle, M., Mora, L., Buckingham, R. H., Kastrup, J. S., Gajhede, M., Svergun, D. I. and Ehrenberg, M. (2005) The SAXS solution structure of RF1 differs from its crystal structure and is similar to its ribosome bound cryo-EM structure. Mol. Cell 20, 929–938.

75 Graille, M., Heurgue-Hamard, V., Champ, S., Mora, L., Scrima, N., Ulryck, N., van Tilbeurgh, H. and Buckingham, R. H. (2005) Molecular basis for bacterial class I release factor methylation by PrmC. Mol. Cell 20, 917–927.

76 Petry, S., Brodersen, D. E., Murphy, F. V., Dunham, C. M., Selmer, M., Tarry, M. J., Kelley, A. C. and Ramakrishnan, V. (2005) Crystal structures of the ribosome in complex with release factors RF1 and RF2 bound to a cognate stop codon. Cell 123, 1255–1266.

77 Ito, K., Frolova, L., Seit-Nebi, A., Karamyshev, A., Kisselev, L. and Nakamura, Y. (2002) Omnipotent decoding potential

resides in eukaryotic translation termination factor eRF1 of variant-code organisms and is modulated by the interactions of amino acid sequences within domain 1. Proc. Natl. Acad. Sci. USA 99, 8494–8499.
78 Lekomtsev, S., Kolosov, P., Bidou, L., Frolova, L., Rousset, J. P. and Kisselev, L. (2007) Different modes of stop codon restriction by the *Stylonychia* and *Paramecium* eRF1 translation termination factors. Proc. Natl. Acad. Sci. USA 104, 10824–10829.
79 Bertram, G., Bell, H. A., Ritchie, D. W., Fullerton, G. and Stansfield, I. (2000) Terminating eukaryote translation: domain 1 of release factor eRF1 functions in stop codon recognition. RNA 6, 1236–1247.
80 Seit-Nebi, A., Frolova, L. and Kisselev, L. (2002) Conversion of omnipotent translation termination factor eRF1 into ciliate-like UGA-only unipotent eRF1. EMBO Rep. 3, 881–886.
81 Frolova, L., Seit-Nebi, A. and Kisselev, L. (2002) Highly conserved NIKS tetrapeptide is functionally essential in eukaryotic translation termination factor eRF1. RNA 8, 129–136.
82 Frolova, L. Y., Tsivkovskii, R. Y., Sivolobova, G. F., Oparina, N. Y., Serpinsky, O. I., Blinov, V. M., Tatkov, S. I. and Kisselev, L. L. (1999) Mutations in the highly conserved GGQ motif of class 1 polypeptide release factors abolish ability of human eRF1 to trigger peptidyl-tRNA hydrolysis. RNA 5, 1014–1020.
83 Mora, L., Heurgue-Hamard, V., Champ, S., Ehrenberg, M., Kisselev, L. L. and Buckingham, R. H. (2003) The essential role of the invariant GGQ motif in the function and stability in vivo of bacterial release factors RF1 and RF2. Mol. Microbiol. 47, 267–275.
84 Seit-Nebi, A., Frolova, L., Ivanova, N., Poltaraus, A. and Kiselev, L. (2000) Mutation of a glutamine residue in the universal tripeptide GGQ in human eRF1 termination factor does not cause complete loss of its activity. Mol. Biol. (Mosk.) 34, 899–900.
85 Trobro, S. and Aqvist, J. (2007) A model for how ribosomal release factors induce peptidyl-tRNA cleavage in termination of protein synthesis. Mol. Cell 27, 758–766.
86 Shaw, J. J. and Green, R. (2007) Two distinct components of release factor function uncovered by nucleophile partitioning analysis. Mol. Cell 28, 458–467.
87 Zavialov, A. V., Buckingham, R. H. and Ehrenberg, M. (2001) A posttermination ribosomal complex is the guanine nucleotide exchange factor for peptide release factor RF3. Cell 107, 115–124.
88 Frolova, L., Le, G., X, Zhouravleva, G., Davydova, E., Philippe, M. and Kisselev, L. (1996) Eukaryotic polypeptide chain release factor eRF3 is an eRF1- and ribosome-dependent guanosine triphosphatase. RNA 2, 334–341.
89 Ito, K., Ebihara, K. and Nakamura, Y. (1998) The stretch of C-terminal acidic amino acids of translational release factor eRF1 is a primary binding site for eRF3 of fission yeast. RNA 4, 958–972.
90 Ebihara, K. and Nakamura, Y. (1999) C-terminal interaction of translational release factors eRF1 and eRF3 of fission yeast: G-domain uncoupled binding and the role of conserved amino acids. RNA 5, 739–750.
91 Merkulova, T. I., Frolova, L. Y., Lazar, M., Camonis, J. and Kisselev, L. L. (1999) C-terminal domains of human translation termination factors eRF1 and eRF3 mediate their in vivo interaction. FEBS Lett. 443, 41–47.
92 Nakamura, Y., Ito, K. and Isaksson, L. A. (1996) Emerging understanding of translation termination. Cell 87, 147–150.
93 Freistroffer, D. V., Pavlov, M. Y., MacDougall, J., Buckingham, R. H. and Ehrenberg, M. (1997) Release factor RF3 in, *E. coli* accelerates the dissociation of release factors RF1 and RF2 from the ribosome in a GTP-dependent manner. EMBO J. 16, 4126–4133.
94 Salas-Marco, J. and Bedwell, D. M. (2004) GTP hydrolysis by eRF3 facilitates stop codon decoding during eukaryotic translation termination. Mol. Cell. Biol. 24, 7769–7778.
95 Alkalaeva, E. Z., Pisarev, A. V., Frolova, L. Y., Kisselev, L. L. and Pestova, T. V. (2006) In vitro reconstitution of eukaryotic translation reveals cooperativity between release factors eRF1 and eRF3. Cell 125, 1125–1136.
96 Hauryliuk, V., Zavialov, A., Kisselev, L. and Ehrenberg, M. (2006) Class-1 release factor eRF1 promotes GTP binding by class-2 release factor eRF3. Biochimie 88, 747–757.
97 Kong, C., Ito, K., Walsh, M. A., Wada, M., Liu, Y., Kumar, S., Barford, D., Nakamura, Y. and Song, H. (2004) Crystal structure and functional analysis of the eukaryotic class II release factor eRF3 from, S. pombe. Mol. Cell 14, 233–245.
98 Mitkevich, V. A., Kononenko, A. V., Petrushanko, I. Y., Yanvarev, D. V., Makarov, A. A. and Kisselev, L. L. (2006) Termination of translation in eukaryotes is mediated by the quaternary eRF1*eRF3*GTP*Mg2+ complex. The biological roles of eRF3 and prokaryotic RF3 are profoundly distinct. Nucleic Acids Res. 34, 3947–3954.
99 Klaholz, B. P., Myasnikov, A. G. and van Heel, M. (2004) Visualization of release factor 3 on the ribosome during termination of protein synthesis. Nature 427, 862–865.
100 Gao, H., Zhou, Z., Rawat, U., Huang, C., Bouakaz, L., Wang, C., Cheng, Z., Liu, Y., Zavialov, A., Gursky, R., Sanyal, S., Ehrenberg, M., Frank, J. and Song, H. (2007) RF3 induces ribosomal conformational changes responsible for dissociation of class I release factors. Cell 129, 929–941.
101 Zavialov, A. V., Mora, L., Buckingham, R. H. and Ehrenberg, M. (2002) Release of peptide promoted by the GGQ motif of class 1 release factors regulates the GTPase activity of RF3. Mol. Cell 10, 789–798.
102 Zavialov, A. V. and Ehrenberg, M. (2003) Peptidyl-tRNA regulates the GTPase activity of translation factors. Cell 114, 113–122.