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Current Opinion in Cell Biology

# Recent mechanistic insights into eukaryotic ribosomes

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Ribosomes are supramolecular ribonucleoprotein particles that synthesize proteins in all cells. Protein synthesis proceeds through four major phases: initiation, elongation, termination, and ribosome recycling. In each phase, a number of phase-specific translation factors cooperate with the ribosome. Whereas elongation in prokaryotes and eukaryotes involve similar factors and proceed by similar mechanisms, mechanisms of initiation, termination, and ribosome recycling, as well as the factors involved, appear quite different. Here, we summarize recent progress in deciphering molecular mechanisms of eukaryotic translation. Comparisons with prokaryotic translation are included, emphasizing emerging patterns of common design.

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**Current Opinion in Cell Biology** 2009, **21**:435–443

This review comes from a themed issue on
Nucleus and gene expression
Edited by Elisa Izaurralde and Phillip Zamore

Available online 23rd February 2009

0955-0674/$ – see front matter


**DOI** 10.1016/j.ceb.2009.01.023

## Introduction

Ribosomes are supramolecular RNA–protein assemblies that manufacture proteins in all cells. Bacterial 70S ribosomes consist of a small subunit (30S), where the decoding of mRNA by aminoacyl-tRNA takes place, and a large subunit (50S), which is responsible for the catalysis of peptide bond formation. Their eukaryotic counterparts are the small subunit (40S) and the large subunit (60S) that form the 80S ribosome. Archaeal ribosomes and their subunits are similar in size to bacterial ribosomes, although functionally they are closer to eukaryotic ones. A number of crystal structures of bacterial and archaeal ribosomes, their subunits, and their complexes with tRNAs, tRNA analogs, and translation factors have been obtained; a crystal structure of a eukaryotic ribosome is still not available. Cryo-electron microscopy (cryo-EM) provided first insights into the architecture of 80S ribosomes; structural models were obtained by docking atomic structures of bacterial and archaeal ribosomes into the cryo-EM density of eukaryotic ribosomes [1]. The current resolution of cryo-EM structures of yeast 80S ribosomes reaches 7.3 Å [2]. Ribosomes from bacteria, eukarya, and archaea have a high degree of sequence and structure conservation, indicating a common evolutionary origin, and share a 'common core', which is responsible for mRNA decoding, peptidyl transfer, and the translocation of tRNA and mRNA by one codon at a time. The eukaryotic ribosome contains additional ribosomal proteins and extra segments of rRNA, referred to as rRNA expansion segments, which are found predominantly around the periphery of the subunits. Translation of an mRNA into protein proceeds through four major phases: initiation, elongation, termination, and ribosome recycling (Table 1). Rather than providing a detailed view of each phase, the goal of this review is to summarize information published recently in the field and to focus on the differences as well as unexpected similarities in the mechanisms in prokaryotic and eukaryotic translation.

## Initiation

During initiation, initiator tRNA and the start codon are positioned in the P site of the ribosome, thereby establishing the correct starting point on the mRNA. Initiation entails two major steps. In the first step, initiator tRNA and mRNA bind to the small ribosomal subunit and the start codon is placed into the P site of the small subunit. In the second step, the large ribosomal subunit joins the complex to form the initiation complex that is ready to enter elongation. Although initiation differs in mechanistic detail in prokaryotes and eukaryotes (for reviews, see [3,4]), patterns of common design start to emerge (Figure 1).

Two groups of initiation factors, those involved in mRNA recruitment (eIF4, eIF3, PABP, and mRNA helicases) and those delivering Met-tRNA$_i$ to the 40S subunit (eIF2 and eIF5) in eukaryotes, are absent in bacteria. The lack of mRNA binding factors can be rationalized by differences in the structures of the translation initiation regions of mRNAs in prokaryotes and eukaryotes. The recruitment of the mRNA to the bacterial 30S subunit is independent of initiation factors, and the rate of mRNA binding depends mostly on the nature of the secondary structure in the translation initiation region [5,6]. The lack of prokaryotic homologs of eIF2 and eIF5 may be related to particular requirements of mRNA scanning in eukaryotic initiation or to differences in translational control. eIF2 in particular is a key target for translational control by phosphorylation, which does not take place in bacteria. In contrast to eukaryotic Met-tRNA$_i$, bacterial

**Figure 1**

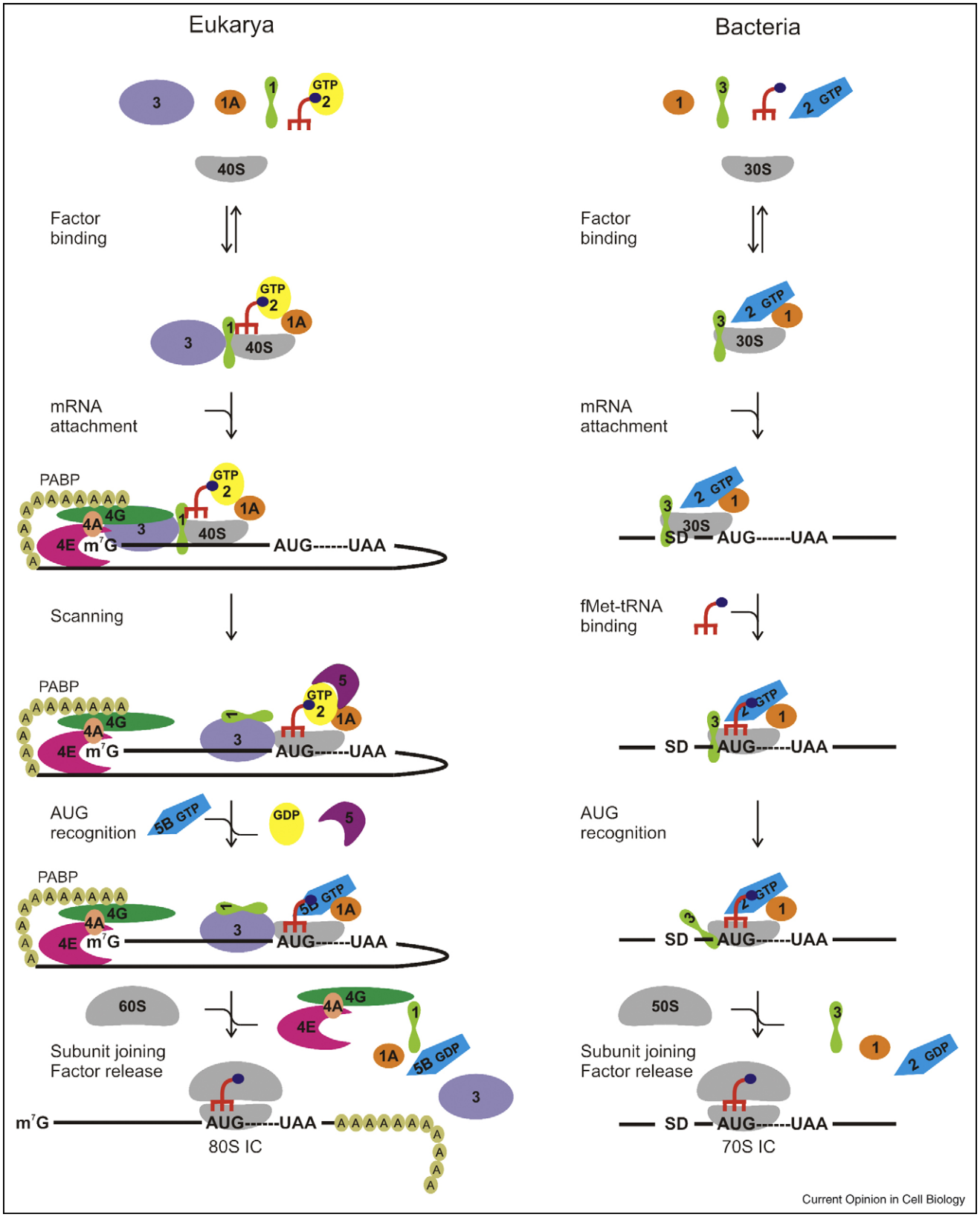
Eukarya
Bacteria
3
1A
1
GTP
2
40S
30S
Factor binding
mRNA attachment
PABP
4A
4G
4E
m7G
AUG------UAA
SD
Scanning
fMet-tRNA binding
AUG recognition
5B GTP
GDP
5
60S
50S
Subunit joining
Factor release
5B GDP
m7G
80S IC
70S IC
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**Table 1**

**Translation factors in the three kingdoms of life.**

| Translation step | Bacteria | Archaea | Eukarya |
|---|---|---|---|
| Initiation | IF1 | aIF1A | eIF1A |
| | IF2 | aIF5B | eIF5B |
| | IF3 | aIF1 | eIF1 |
| | | aIF2α | eIF2α |
| | | aIF2β | eIF2β |
| | | aIF2γ | eIF2γ |
| | | aIF2Bα | eIF2Bα |
| | | | eIF2Bβ |
| | | | eIF2Bγ |
| | | aIF2Bδ | eIF2Bδ |
| | | | eIF2Bε |
| | | | eIF3 (13 subunits) |
| | | aIF4A | eIF4A |
| | | | eIF4B |
| | | | eIF4E |
| | | | eIF4G |
| | | | eIF4H |
| | | aIF5 | eIF5 |
| | | aIF6 | eIF6 |
| | | | PABP |
| Elongation | EF-Tu | aEF1α | eEF1A |
| | EF-Ts | aEF1B | eEF1B (2 or 3 subunits) |
| | SelB | SelB | eEFSec |
| | | | SBP2 |
| | EF-G | aEF2 | eEF2 |
| Termination | RF1 | aRF1 | eRF1 |
| | RF2 | | |
| | RF3 | | eRF3 |
| Recycling | RRF | | |
| | EFG | | |
| | | | eIF3 |
| | | | eIF3j |
| | | | eIF1A |
| | | | eIF1 |

Orthologous or functionally homologous factors are aligned.

initiator tRNA, fMet-tRNA$^{fMet}$, is not delivered to the 30S subunit in a ternary complex with an initiation factor [7], although there is a weak ($K_d$ = 1–2 μM) interaction between the CCA-end of fMet-tRNA$^{fMet}$ and the C-terminal domain of IF2 (prokaryotic homolog of eIF5B) in solution. Rather, IF2 accelerates the binding of fMet-tRNA$^{fMet}$ to the 30S subunit containing the full set of initiation factors and (usually) the mRNA.

On the other hand, the functions of eIF1A and eIF1 and their bacterial analogs, IF1 and IF3, respectively, may be quite similar. eIF1A consists of a central domain, which is homologous to IF1, and random-coil N-terminal and C-terminal extensions, which are absent in IF1 [4]. Although there is little sequence conservation between IF3 and eIF1 (<15% identity), the factors have similar functions in initiation codon selection, bind to analogous regions on the small subunit, and can even function in heterologous initiation assays [8]. Similar to eIF1, IF3 is recruited to the ribosome already at the ribosome recycling step, when the post-termination ribosome is disassembled into subunits (see below). During initiation, eIF1A (IF1) and eIF1 (IF3) alter the structure of the small ribosomal subunit, which is important for the selection of the translation initiation region and the correct start codon [9,10••,11]. eIF1 (IF3) accelerates the dissociation of incorrect tRNA–mRNA complexes from the small ribosomal subunit and enhances sequence context discrimination in the translation initiation regions [10••,12,13]. eIF1A (IF1) plays a key role in determining the balance between different conformations of the 40S (30S) subunit [10••,14]. Recognition of the start codon lowers the affinity of eIF1 (IF3) for the ribosome and accelerates its release [10••,15]. eIF1 moves away from its binding site on the small subunit, most probably toward eIF3 [3,4]. Similarly, parts of bacterial IF3 may be displaced after codon recognition [16••], although the complete dissociation of the factor takes place only after joining of the large ribosomal subunit [10••]. Thus, the basic roles of eIF1 (IF3) and eIF1A (IF1) in start site selection may be conserved in prokaryotes and eukaryotes.

Also the roles of eIF5B and its bacterial homolog IF2 seem to be more similar than is usually realized. Both eIF5B and IF2 accelerate the rate of ribosomal subunit joining [17,18], which may be influenced by the conformation of the factor as mediated by the nucleotide

**(Figure 1 Legend)** Initiation pathway in eukarya and bacteria. In eukarya, initiator tRNA (Met-tRNA$_i$) is delivered to the 40S ribosomal subunit in a ternary complex with eIF2 and GTP, assisted by eIF1, eIF1A, and eIF3, to form a 43S pre-initiation complex. Binding of the 43S complex to the mRNA near the 5′-7-methylguanosine (m$^7$G) cap is promoted by the cap-binding protein eIF4E, the scaffolding factor eIF4G, and the poly(A)-binding protein (PABP) to form the 48S complex. Subsequently, the complex scans along the mRNA with the help of mRNA helicases, such as eIF4A and possibly the newly discovered DHX29 [70•]. During scanning, eIF2 stimulated by eIF5 may reversibly hydrolyze GTP; inorganic phosphate (Pi) is not released until the start codon AUG is recognized [15]. Recognition of the start codon triggers events that commit the complex to initiate translation. These events include release of Pi from eIF2 [71], displacement of eIF1, eIF2, and eIF5, and binding of eIF5B GTP [4]. The resulting complex binds the 60S subunit, and – after ribosome-induced GTP hydrolysis by eIF5B – the initiation factors dissociate and Met-tRNA$_i$ accommodates in the P site of the 80S initiation complex (80S IC). In the bacterial system, IF3, IF1, and IF2·GTP bind to the 30S subunit that subsequently binds to the mRNA, in many cases guided by the interaction of the Shine–Dalgarno (SD) sequence upstream of the start codon and the anti-SD sequence near the 3′-terminus of 16S rRNA. Binding of the initiator tRNA (fMet-tRNA$^{fMet}$), which recognizes the AUG start codon, accurately positions the AUG codon into the P site of the 30S initiation complex; concomitantly, parts of IF3 may be displaced from the 30S subunit. Following subunit joining, GTP hydrolysis by IF2 alters the conformation of the 70S initiation complex and promotes the release of the initiation factors. GTP hydrolysis is followed by conformational changes of IF2 and fMet-tRNA$^{fMet}$ [19•,20,21] and the dissociation of IF3 [10••]. The release of IF3 initiates a cascade of further reactions, including IF1 dissociation [10••], Pi release from IF2 [20,21], further conformational changes of IF2, possibly IF2 dissociation from the ribosome, and the accommodation of fMet-tRNA$^{fMet}$ in the 50S P site [19•,72] of the 70S initiation complex (70S IC). The conformation of the ribosome and the position of the mRNA may change as well [73,74]. Orthologous factors are depicted in the same shape and color.

[19•,20]. The cryo-EM reconstruction of the 30S initiation complex suggested that IF2 positions the acceptor end of fMet-tRNA$^{fMet}$ for insertion into the 50S subunit [19•]. The largest part of IF2 in the complex is complementary in shape to the 50S surface, which explains how IF2 and fMet-tRNA$^{fMet}$ favor subunit association. Docking of the 50S subunit would place the GTP binding pocket of IF2 in contact with the sarcin–ricin loop, which may trigger immediate GTP hydrolysis [19•,20,21]. 50S subunit joining and the transition from the 30S to the 70S initiation complex provide an important kinetic control checkpoint for the accuracy of mRNA selection [10••]. Whether such a step exists in eukaryotic initiation has not been tested yet.

## Elongation

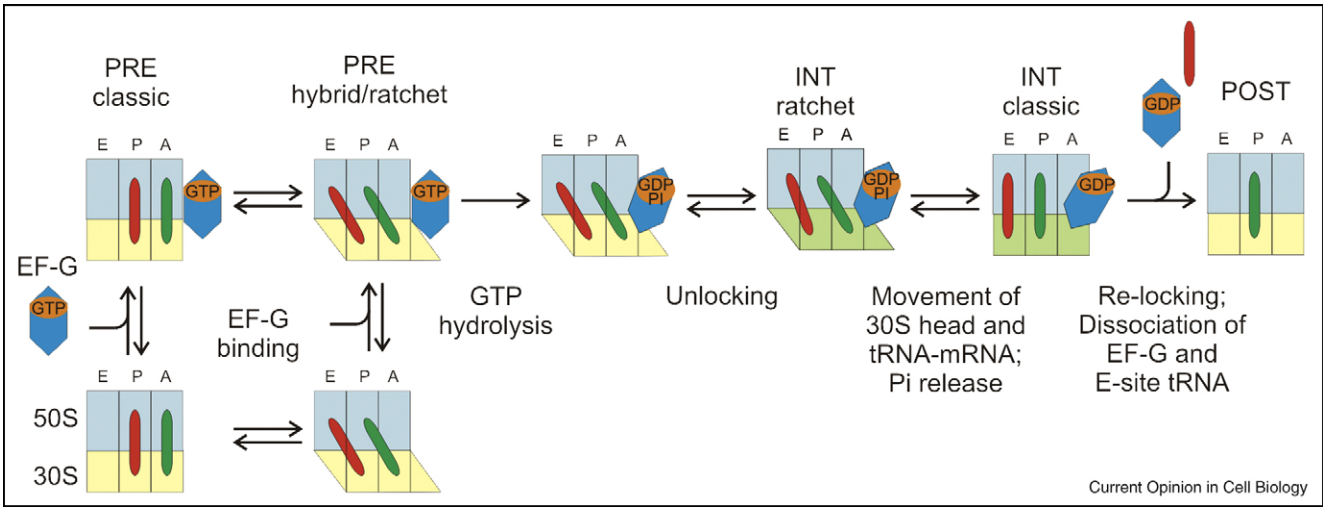
Elongation entails three steps, decoding of an mRNA codon by the cognate aminoacyl-tRNA, peptide bond formation, and translocation of the tRNA–mRNA complex, which moves peptidyl-tRNA from the A site to the P site and presents a new codon in the A site. The decoding and translocation steps are accelerated by elongation factors that are similar in prokaryotes and eukaryotes (Table 1). The eukaryotic elongation factor 1 (eEF1) comprises eEF1A and eEF1B [22]. eEF1A is homologous to prokaryotic EF-Tu and delivers aminoacyl-tRNAs to the A site of the ribosome. As EF-Tu, eEF1A is a member of the GTPase superfamily and binds and hydrolyzes GTP. The dissociation of GDP from eEF1A is accelerated by a guanine nucleotide exchange factor (GEF), eEF1B, which is composed of two subunits, eEF1Bα and eEF1Bγ, in yeast, or three subunits, eEF1Bα, eEF1Bγ, and eEF1Bβ, in mammals. eEF1Bα contains the catalytic domain necessary for nucleotide exchange and is thus the functional equivalent to the bacterial GEF of EF-Tu, EF-Ts, which acts as a single polypeptide. Although both eEF1Bα and EF-Ts function as GEFs, the two proteins do not exhibit any significant sequence homology and bind to their respective GTPase in different ways. eEF1Bα interacts with domains 1 and 2 of eEF1A, disrupting the switch 2 region of eEF1A, which forms part of the binding pocket for $Mg^{2+}$ and the γ-phosphate of GTP, and inserting the highly conserved Lys205 into the $Mg^{2+}$ and GDP/GTP binding sites of eEF1A. This prevents the binding of the β-phosphate and γ-phosphate to the P loop [23]. The structures of the sugar and base binding pockets of eEF1A are mostly unperturbed by eEF1Bα, in contrast to the EF-Tu·EF-Ts complex. Lys205 of eEF1Bα appears to be important for the mechanism of nucleotide exchange, as the Lys205Ala mutation is lethal owing to impaired GEF function [24]. However, *in vitro* the mutation reduced the rate of GDP release from eEF1A only about tenfold (at 1 mM $Mg^{2+}$) [24,25•], and removal of $Mg^{2+}$ increased the rate of eEF1Bα-induced GDP dissociation no more than sixfold [24]. This suggests that – as for EF-Tu·EF-Ts [26,27] – each of the contacts in the eEF1A·eEF1Bα complex contributes moderately to the destabilization of nucleotide binding, but together they act synergistically to bring about the overall acceleration of nucleotide exchange [25•].

Like prokaryotic EF-Tu, yeast eEF1A binds aminoacyl-tRNA in a GTP-dependent manner and promotes its binding to the mRNA-programed 80S ribosome. As EF-Tu, yeast eEF1A·GTP binds aminoacyl-tRNA with nanomolar affinity [25•]. After the ternary complex is formed, aminoacyl-tRNA delivery to the A site of the eukaryotic ribosome presumably follows the same pathway as described in detail for bacterial ribosomes [28–31], albeit decoding appears to take place somewhat more slowly and more accurately, particularly in higher eukaryotes.

As soon as aminoacyl-tRNA is accommodated in the A site, it forms a peptide bond with the P-site peptidyl-tRNA. The peptidyl transferase center on the large ribosomal subunit is built up of highly conserved rRNA elements; most probably, the reaction mechanism is the same in prokaryotes and eukaryotes (for review see [32]). As revealed by the X-ray structures, the A-site and P-site substrates are precisely aligned in the active site by interactions of their conserved CCA sequences and of the nucleophilic α-amino group with residues of 23S rRNA in the active site. The reaction is driven by a favorable entropy change, while the enthalpy is small and unfavorable [33]. Catalysis seems to involve a six-membered transition state in which proton shuttling occurs via the 2′-OH of A76 of the P-site tRNA [34–36]. The reaction does not involve chemical catalysis by ribosomal groups, but may be modulated by conformational changes at the active site [37,38]. The ribosome appears to work by providing an electrostatic environment that reduces the free energy of forming the highly polar transition state, shielding the reaction against bulk water, helping the proton shuttle forming the leaving group, or a combination of these effects.

Following peptide bond formation, the pre-translocation ribosome can spontaneously assume a conformation in which the subunits have undergone a ratchet-like relative rotation and, concomitantly, the 3′ ends of tRNAs in the P and A sites have moved to the E and P sites, respectively, to assume hybrid states. There seems to be a dynamic equilibrium between the non-ratcheted conformation with the tRNAs in non-hybrid, classic states and the ratcheted conformation with the tRNAs in hybrid states [39•,40,41,42,43•,44,45••,46]. Subunit ratcheting and the ability of the P-site tRNA to interact with the E site on the 50S subunit are essential for EF-G promoted translocation [47•,48], suggesting that the combined hybrid-ratcheted state is an authentic early intermediate of translocation. However, the formation of this state as such is not sufficient to move the acceptor end of peptidyl-tRNA into the post-translocation position proper, as indicated by low reactivity with puromycin [49,50].

**Figure 2**




Translocation pathway. Following peptide bond formation, the pre-translocation complex (PRE) can have the tRNAs in the classic A/A (peptidyl-tRNA, green) and P/P (deacylated tRNA, red) states or in the respective hybrid A/P and P/E states. Hybrid state formation is correlated with the ratcheting movement of the subunits relative to one another. The hybrid-ratcheted state is stabilized by EF-G·GTP binding. Rapid GTP hydrolysis by EF-G drives a rearrangement of the ribosome ('unlocking') that precedes, and limits the rate of, tRNA–mRNA movement on the small ribosomal subunit from the hybrid pre-translocation (INT, hybrid/ratchet) to the non-hybrid post-translocation (INT, classic) intermediate complex [75]. Unlocking may allow for a movement of the head of the 30S subunit, which may be involved in the movement of the codon–anticodon complex from the A to the P site, as revealed by X-ray crystallography [76,77]. Pi release from EF-G, which takes place in parallel, induces another conformational change that is required for the ribosome to return to the locked state and promotes the dissociation of EF-G and the E-site tRNA from the ribosome to form the post-translocation complex (POST) with peptidyl-tRNA in the P site [78]. Further partial translocation steps were reported [79–81], but not assigned to particular structures.

The sequence of events leading to tRNA–mRNA movement through the ribosome to the post-translocation state, as deduced from kinetics, single molecule experiments, X-ray, and cryo-EM structures, is most probably very similar in eukaryotes and prokaryotes (Figure 2).

In the absence of EF-G, the tRNAs may move spontaneously in forward or backward direction through the ribosome [51••,52••]. This suggests that the formation of the unlocked state, as induced by EF-G and GTP hydrolysis, accelerates intrinsically spontaneous oscillations of the tRNAs between the A, P, and E sites. During translocation, eEF2 (EF-G) undergoes several large-scale conformational changes [42,53] that are probably required to provide the directionality of tRNA movement and prevent the backward sliding of tRNAs. Thus, EF-G may have several functions in translocation: stabilizing the hybrid/ratchet state, inducing the unlocked conformation of the ribosome, and acting as a Brownian ratchet that prevents backward movement of the tRNAs.

## Termination

Elongation stops when the ribosome reaches the end of the coding region and a termination codon enters the decoding site. During termination in bacteria, release factors 1 or 2 (RF1 or RF2) bind to the ribosome in response to the termination codon displayed in the A site, recognizing UAG/UAA and UAA/UGA, respectively, and promote the hydrolysis of P-site peptidyl-tRNA. Recent crystal structures [54••,55••,56••] show that the binding of RF1 and RF2 induces conformational changes in the decoding center, allowing the factors to recognize their cognate termination codons with high specificity [57,58] and to place the universally conserved GGQ motif into the peptidyl transferase center where hydrolysis takes place. The GGQ motif is essential for peptide release, probably because it is involved in positioning the hydrolytic water or in the stabilization of the transition state [54••,55••] in a way similar to that observed during peptide bond formation [59•]. Another release factor, RF3, a GTPase, accelerates the dissociation of RF1 and RF2 from the ribosome, but is not required for peptide release and is not essential for bacteria. The current model for translation termination in bacteria implies that (i) peptide release precedes and regulates GTP hydrolysis by RF3 [60,61]; (ii) GTP hydrolysis is required for the dissociation of RF3 from the ribosome [60]; and (iii) the ribosomal post-termination complex containing RF1 or RF2 acts as a guanine nucleotide exchange factor for RF3 [62].

The mechanism of translation termination in eukaryotes is quite different. The termination machinery is limited to only two release factors, eRF1, which recognizes all

three termination codons, and eRF3, the GTPase. In contrast to the prokaryotic termination factors, eRF1 and eRF3 bind to one another with very high affinity and probably enter the ribosome as a complex [63•]. 80S ribosomes do not influence guanine nucleotide binding/exchange on the eRF1·eRF3 complex; rather, eRF1 acts as a GTP dissociation inhibitor for eRF3, promoting efficient ribosomal recruitment of its GTP-bound form [63•]. Thus, the mechanism of GDP–GTP exchange on eRF3, which depends on stimulation by eRF1, is unusual and entirely different from that on prokaryotic RF3. GTP hydrolysis by eRF3 is a prerequisite for peptide release [64,65••]; it couples stop codon recognition with peptidyl-tRNA hydrolysis mediated by eRF1 [64,65••,66]. eRF3 strongly enhances peptide release by eRF1 in the presence of GTP, but not GDP, and abrogates peptide release in the presence of non-hydrolyzable GTP analogs even when eRF1 is present in excess, that is, eRF1 turnover is not required [65••]. Thus, whereas bacterial RF3 increases the rate of RF1/RF2 release from the ribosome, eukaryotic eRF3 seems to ensure the rapid and efficient hydrolysis of peptidyl-tRNA by eRF1. Apparently, the binding of eRF1 and eRF3·GTP to pre-termination ribosomes leads to a complex that initially is not active in peptide release, and a rearrangement that is induced by GTP hydrolysis is required for activation [64,66]. Before GTP hydrolysis, the GGQ region of eRF1 may not be properly positioned in the peptidyl transferase center, and GTP hydrolysis could induce the correct accommodation and trigger peptidyl-tRNA hydrolysis. Thus, prokaryotic and the eukaryotic termination differ, in that in prokaryotes peptide release precedes, and is required for, GTP hydrolysis by RF3, whereas in eukaryotes GTP hydrolysis by eRF3 is necessary for peptide release.

## Ribosome recycling

Ribosome recycling, the last step of translation, is entirely different in prokaryotes and eukaryotes. In bacteria, ribosome recycling requires a specialized ribosome recycling factor (RRF) that acts together with EF-G to split the ribosome into subunits. Binding of IF3 to the 30S complex, which still contains mRNA and tRNA, promotes tRNA dissociation and destabilization of mRNA binding; subsequent mRNA dissociation or exchange with another mRNA is a spontaneous process [67,68]. Eukaryotic cells lack an ortholog of RRF, suggesting that ribosome disassembly involves other factors. Recently, it has been demonstrated that splitting of 80S ribosomes into 40S and 60S subunits is catalyzed principally by eIF3, the action of which is enhanced by eIF3j, eIF1, and eIF1A [69••]. eIF1 mediates the release of the tRNA from the 40S subunit, while eIF3j ensures subsequent mRNA dissociation. The exact role of each factor and the timing of events are not known. The reason for the divergence in ribosome recycling between prokaryotes and eukaryotes is not clear. Further mechanistic and structural analyses are required to delineate the detailed mechanism of the reactions.

## Acknowledgements

We thank Tatyana Pestova for critically reading the manuscript. The authors apologize to those whose work was not cited or discussed here because of space limitations. The work in our laboratories is supported by grants of the Deutsche Forschungsgemeinschaft and by the Max-Planck-Gesellschaft.

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- •• of outstanding interest

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