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Minireview

Movement in ribosome translocation

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Abstract

Translocation of peptidyl-tRNA and mRNA within the ribosome during protein synthesis is promoted by the elongation factor EF-G and by the hydrolysis of GTP. A new study reports that EF-G binds to ribosomes as an EF-G•GDP complex and that GTP is exchanged for GDP on the ribosome. Together with cryo-electron microscopy, this unexpected finding helps clarify the role of GTP in translocation.

Small GTPases play central roles in catalyzing each stage of protein synthesis on the ribosome. In prokaryotes, the relevant GTPases are: initiation factor IF2, which delivers the initiator tRNA to the P (peptide) site of the 30S ribosomal subunit; elongation factor EF-Tu, which delivers the aminoacyl-tRNA to the 70S ribosome (composed of 50S and 30S subunits); elongation factor EF-G, which promotes the translocation of tRNAs and the mRNA within the ribosome; and peptide release factor RF3, which promotes the dissociation of the release factors RF1 and RF2 following peptide release. These factors have been assumed to resemble classical GTPases, with the active form of the protein being the GTP binary complex. For example, the active EF-Tu•GTP complex binds aminoacyl-tRNA and transports it to the ribosome, which then stimulates the GTPase activity of EF-Tu (functioning as a GTPase-activator protein, or GAP) upon detection of a correct codon-anticodon interaction [1]. Following dissociation of EF-Tu•GDP from the ribosome, the GDP is exchanged for GTP in a guanine-nucleotide exchange reaction catalyzed by an elongation factor (EF-T) acting as a guanine-nucleotide exchange factor (GEF). For the other three factors, it is thought that the ribosome also provides the GAP function, whereas the requirement for a GEF has not been defined. The Ehrenberg group [2] recently discovered that the ribosome is in fact a GEF for the RF3 GTPase. Now, in *Journal of Biology*, the same group reports that the active form of EF-G for ribosome binding is the EF-G•GDP complex, not the EF-G•GTP complex, and that the ribosome acts as a GEF for EF-G as well [3]. Together with a number of other recent publications from the Ehrenberg, Frank, Wintermeyer and van Heel groups [4-6], these results shed new light on the roles of GTP and EF-G during the translocation reaction.

In order to understand fully the function of the translation factors and the ribosome during each stage of protein synthesis, it is essential to recognize all of the dynamic conformations that they undergo. During the past decade, intensive studies using cryo-electron microscopy and X-ray crystallography have added significantly to our understanding of ribosome function and have provided a structural framework for viewing this large macromolecular machine

(reviewed in [7,8]). High-resolution atomic structures for most of the translation factors have also been solved. One of the most challenging problems regarding ribosome function is to determine how the coordinated movement of tRNA and mRNA in the ribosome is achieved during translocation.

We know that tRNAs bind to ribosomes by spanning the interface between the ribosomal subunits, with the aminoacyl end binding to the 50S ribosomal subunit and the anticodon loop binding to an mRNA codon within the 30S ribosomal subunit. The ribosome contains three distinct tRNA binding sites: aminoacyl-tRNAs bind to the A site; peptidyl-tRNA binds to the P site; and stripped tRNA binds to the E (exit) site (see Figure 1a). The movement of tRNAs between these sites during translocation is catalyzed by EF-G and GTP hydrolysis. Classical models of translocation, first proposed by Bretscher [9] and Spirin [10], suggest that immediately after peptide-bond synthesis a peptidyl-tRNA resides in the A site and an uncharged tRNA resides in the P site (Figure 1b). But there is a spontaneous movement of the aminoacyl ends of the tRNAs relative to the 50S subunit, resulting in peptidyl-tRNA in a P/A hybrid site (P in the large ribosomal subunit but A in the small subunit) and uncharged tRNA in an E/P hybrid site (Figure 1c) [11]. Translocation catalyzed by EF-G and GTP hydrolysis involves the movement of these tRNA derivatives within the 30S subunit (Figure 1d) to generate peptidyl-tRNA in the P/P site and uncharged tRNA in the E/E site, together with the movement of the associated mRNA by one codon (Figure 1e). Despite the apparent loosening of the bound tRNAs to allow movement, the bonds nevertheless must remain strong enough to retain a tight association between the peptidyl-tRNA and the mRNA to prevent slippage of the reading frame, and may not result in dissociation from the ribosome. As it is possible to observe translocation *in vitro* in the absence of EF-G and GTP [12,13], the translocation process appears to be an inherent property of the ribosome that is enhanced by the presence of EF-G and GTP. The classical model suggests that EF-G bound to GTP drives the translocation movement and that the subsequent GTP hydrolysis results in the dissociation of EF-G. Indeed, apparent translocation can occur with EF-G bound to the nonhydrolyzable GTP analog GMPPNP, when measured by the ability of the peptidyl-tRNA to react with the aminoacyl-tRNA mimetic

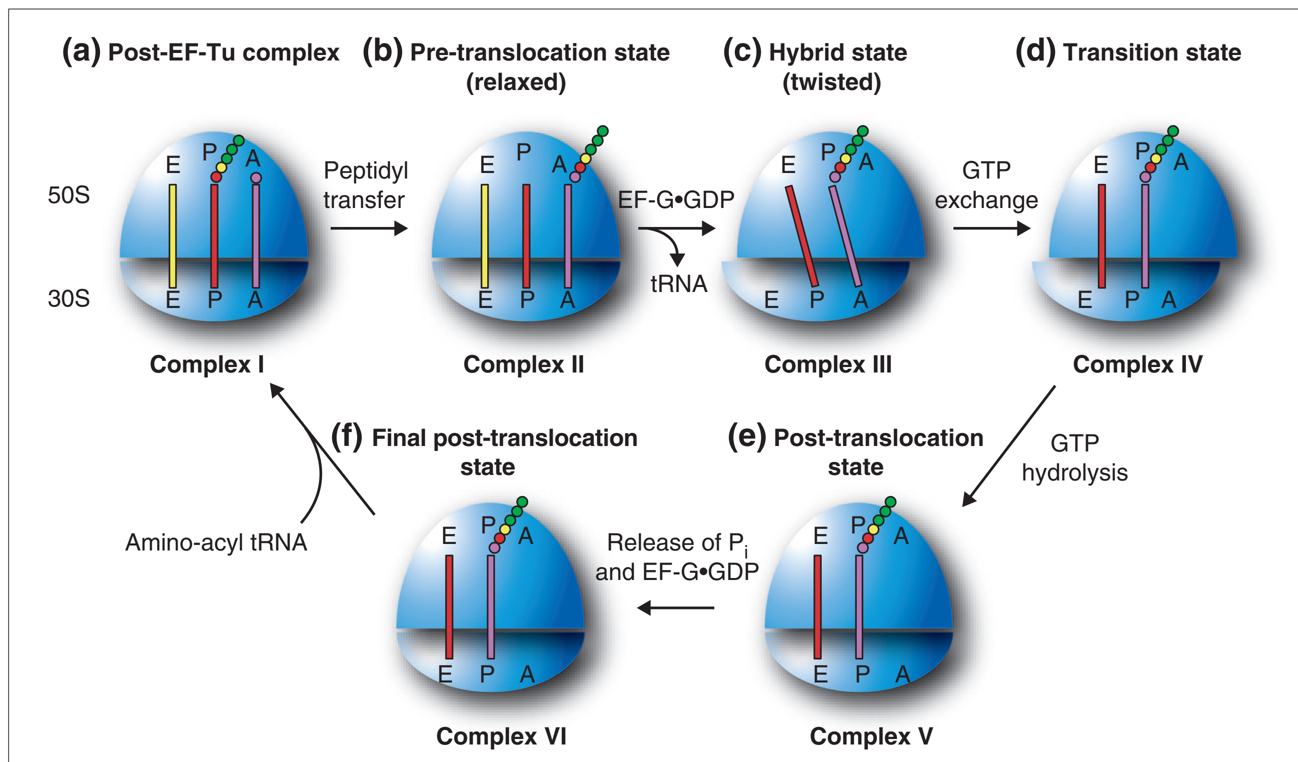

Figure 1
The pathway of translocation. The tRNAs are shown as colored bars, some with attached amino acids depicted as circles colored to correspond to their tRNAs. The reactions in the pathway are described in the text; the mRNA and bound EF-G are not shown.

drug puromycin [13,14]. More recently, a variation of the model proposed by the Wintermeyer group suggests that EF-G has two distinct functions in translocation [15,16]. The first function is to induce a conformational change that promotes 'unlocking' of the ribosome that must precede tRNA-mRNA movement; the second is to enhance the spontaneous forward movement which results from the unlocked ribosome. Even equipped with excellent knowledge of the ribosome structure, however, one is left with an incomplete understanding of the mechanism of translocation (for reviews, see [17-19]).

The current article by Ehrenberg and coworkers [3], together with two other recent publications [6,20], has significantly extended our understanding of the translocation reaction. Having shown recently that the ribosome is in fact a GEF for the RF3 GTPase [2], Ehrenberg and colleagues decided to investigate whether or not this could also be true for EF-G. On the basis of their new findings that EF-G binds to GDP 60-fold more tightly than to GTP, and that translocation with limiting EF-G is only modestly inhibited by GDP, they conclude that EF-G•GDP is the form that first binds to the pre-translocation ribosome (Figure 1b) [3]. This discovery is surprising, as it has been believed that EF-G binds to ribosomes as an EF-G•GTP binary complex. They propose that EF-G•GDP binding promotes the formation of the hybrid state (Figure 1c) that involves ratcheting of the 30S subunit relative to the 50S subunit, as has been shown by cryo-electron microscopy by Frank and colleagues [6]. EF-G•GDP binding to the pre-translocation complex requires that the uncharged tRNA be capable of entering the E/P hybrid site, as mutations that block such binding prevent EF-G•GDP binding. It remains to be clarified whether EF-G•GDP binding actually causes the rearrangement on the ribosome, or more simply stabilizes the hybrid/ratcheted state (Figure 1c) which may have formed spontaneously as a result of a favorable interaction of the peptidyl portion of the peptidyl-tRNA with the P site of the 50S subunit, as proposed by Moazed and Noller [11]. Upon EF-G•GDP binding, the 50S subunit undergoes a number of conformational changes, resulting in altered contacts with the 30S subunit [6]. In order to visualize possible mRNA movement by one codon during this process, Ehrenberg and colleagues [3] developed a new method that involves the specific cleavage by the bacterial toxin RelE of mRNA located in the A site of the ribosome. Ribosomes in the final post-translocation state (Figure 1f) exhibit cleavage because the A site is accessible, but RelE does not cleave mRNA within the hybrid-state complex with bound EF-G•GDP (Figure 1c) because the A site is blocked. The ratcheted state (Figure 1c) also does not react with puromycin, indicating that peptidyl-tRNA is not suitably positioned in the 50S peptidyl transferase center for the puromycin to be added to the growing peptide chain.

Ehrenberg and colleagues then hypothesize that the ribosome acts as a GEF to convert EF-G•GDP to EF-G•GTP [2,3]. Because the rate of exchange of GDP for GTP was not actually measured in the 70S complex, it is unclear whether the ribosome actually catalyzes the exchange reaction. Upon GTP binding, EF-G undergoes a substantial conformational change [6], thereby generating an altered 70S complex called the transition state (Figure 1d). The transition state allows puromycin to react with the peptidyl-tRNA and partially exposes the codon downstream from the peptidyl-tRNA codon to cleavage by RelE, indicating that this codon resides in or near the 30S A site. In addition, the transition state can be reversed by the addition of GDP but the post-translocation state cannot, indicating that the transition state and post-translocation states differ. Ehrenberg and colleagues [3] propose that the transition state serves as the GAP, promoting GTP hydrolysis. Unfortunately, a cryo-electron microscopy structure has not yet been obtained for the transition state, so the structure shown as complex IV in Figure 1d remains hypothetical and simplistic. Upon GTP hydrolysis and ejection of inorganic phosphate, the ribosome reaches the post-translocation state (Figure 1e), for which EF-G•GDP has low affinity, resulting in its dissociation and the formation of complex VI, the final post-translocation state (Figure 1f). It remains to be shown at which step - generation of the transition complex or the post-translocation complex - the movement of the mRNA relative to the 30S subunit occurs.

The combination of recent structural, kinetic and biochemical studies now provides a more satisfactory explanation than was possible previously for how the ribosome and its complexes cause directed movement of the tRNAs and mRNA. Yet a few critical details remain to be elucidated. Fast kinetic analyses with highly purified GDP and GTP are needed. Moreover, structures based on cryo-electron microscopy for the EF-G•GTP-bound transition complex and EF-G•GDP-bound post-translocation complex are lacking, and we still need to elucidate the precise time when the mRNA moves on the 30S subunit. Nevertheless, as the recent studies from the Ehrenberg and Frank laboratories amply show [2,3,6], surprises may yet emerge as we reach a greater understanding of the workings of the ribosome.

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