Dynamics of translation on the ribosome:  
molecular mechanics of translocation  

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Abstract  
The translocation step of protein elongation entails a large-scale rearrangement of the tRNA-mRNA-ribosome complex. Recent years have seen major advances in unraveling the mechanism of the process on the molecular level. A number of intermediate states have been defined and, in part, characterized structurally. The article reviews the recent evidence that suggests a dynamic role of the ribosome and its ligands during translocation. The focus is on dynamic aspects of tRNA movement and on the role of elongation factor G and GTP hydrolysis in translocation catalysis. The significance of structural changes of the ribosome induced by elongation factor G as well the role of ribosomal RNA are addressed. A functional model of elongation factor G as a motor protein driven by GTP hydrolysis is discussed. © 1999 Published by Elsevier Science B.V. All rights reserved.  

Keywords: Protein synthesis; tRNA; rRNA; Elongation factor; GTPase; Motor protein  

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1. Introduction

The elongation of protein synthesis consists of three major steps. Starting with the ribosome bound to mRNA and carrying peptidyl-tRNA (or initiator fMet-tRNA) in the P site, in the first step aminoacyl-tRNA binds to the A site in a codon-dependent manner. The reaction requires an elongation factor, EF-Tu in bacteria, and GTP which form a high-affinity ternary complex with aminoacyl-tRNA; it is the ternary complex which binds to the ribosomal A site. After codon recognition, GTP hydrolysis, and dissociation of EF-Tu-GDP from the ribosome, the 3’ end of aminoacyl-tRNA accommodates in the peptidyl transferase center and immediately enters the peptidyl transfer reaction, the second step of the elongation cycle. Formation of the peptide bond results in deacylated tRNA in the P site and peptidyl-tRNA in the A site. The third major step of the cycle, translocation, is catalyzed by another elongation factor, EF-G in bacteria, which hydrolyzes GTP during the reaction. During translocation, peptidyl-tRNA is displaced from the A site to the P site, while deacylated tRNA is transferred from the P site to the E site from where it dissociates. Thus, translocation restores the initial state of the ribosome which then enters the next round of elongation.

As far as structural rearrangements on the ribosome are concerned, translocation is the most complex step of elongation [85]. Two tRNAs together with the mRNA have to move rapidly from their respective pre-translocation sites, where they are bound with high stabilities, to their post-translocation sites. To allow the movement, tRNA-ribosome interactions have to be released temporarily, while keeping the tRNA-mRNA complex bound. Recent years have seen major advances in unraveling the mechanism of the process at the molecular level. A number of intermediate states have been defined and, in part, characterized structurally, and evidence is accumulating that suggests a dynamic picture of the ribosome and the ligands interacting with it during the process (for recent reviews see [26,96]).

The present article focuses on dynamic aspects of tRNA movements and on the role of EF-G and GTP hydrolysis by EF-G in promoting the reaction. Structural changes of the ribosome and of EF-G and their significance for the mechanism of translocation catalysis will be addressed. In this context, also the role of ribosomal RNA in translocation will be discussed. Finally, we will ask whether the GTPase paradigm is pertinent for EF-G, or whether there are functional parallels to force-generating proteins.

2. tRNA movement in translocation

In the pre-translocation state, A and P site-bound tRNAs are arranged such that the two anticodons and acceptor ends, respectively, are close to each other and the planes of the molecules enclose an angle of about 60°. This arrangement was suggested on the basis of distance measurements by fluorescence resonance energy transfer [34,62] and was confirmed by electron cryomicroscopy [89]. The detailed positions of the acceptor ends on the 50S ribosomal subunit depend on the functional state of the tRNA, as studied by chemical footprinting on 23S rRNA which revealed different footprinting patterns of deacylated tRNA in A, P, and E sites [47,48]. Interestingly, in ribosomal complexes carrying peptidyl-tRNA in the A site and deacylated tRNA in the P site, the A-site pattern on the 50S subunit was no longer observed, but rather the P-site and E-site patterns. These observations were interpreted as indicating that, upon forming peptidyl-tRNA in the A site, the acceptor end is moved out of the 50S A site towards, or into, the 50S P site, while the acceptor end of deacylated tRNA moves out of the P site towards the E site [47,48]. Thus, the tRNAs in this state are thought to be bound to the A (P) site on the 30S subunit by their anticodon domains and to the P (E) site on the 50S subunit by their acceptor ends, hence the designation A/P and P/E ‘hybrid states’. As the A site-bound state of peptidyl-tRNA is unreactive with puromycin and becomes reactive only by EF-G-catalyzed translocation, the pre-translocation hybrid state of peptidyl-tRNA is designated more precisely A/P* [96].

The P/E hybrid state of deacylated tRNA is formed when P site-bound peptidyl-tRNA donates the peptidyl residue to the A site-bound aminoacyl-tRNA, as indicated by the appearance of the E-site footprinting pattern on 23S rRNA [47,48]. The structural mobility is probably gained by releasing the
peptidyl residue which contributed to the fixation of the 3’ end in the P-site part of the peptidyl transferase center. This does not necessarily mean that the 3’ end of P site-bound deacylated tRNA establishes a strong interaction in the E site and is bound there exclusively. In fact, pre-bound E-site tRNA does not inhibit peptidyl transfer [72] and is not competed out of the E site when the peptide is formed (unpublished data). These results suggest that the observed P/E footprinting pattern reflects the formation of one of several possible structural states of the 3’ end of P site-bound tRNA. Therefore, the argument that the hybrid state model necessitated the ejection of E site-bound tRNA upon peptide bond formation in the next round of elongation [56] is not conclusive.

The formation of the P/E state is essential for translocation, since modifications at the 3’ end of the tRNA that impair the interaction with the E site [40] strongly reduce the rate of translocation [41]. It has been shown that the 3’-terminal adenine 76 [40] and, to a lesser extent, cytosine 75 (unpublished data) of tRNA^Phe are important for the E site-binding affinity, possibly by base-pairing interactions with 23S rRNA. Potentially complementary positions are found in 23S rRNA in the region where E site-bound tRNA^Phe led to protection of bases against chemical modification (U2111, G2112, G2116 [47]). The importance of 50S E-site interaction of the tRNA leaving the P site is also borne out by the observation that an isolated anticodon arm inhibits translocation [35].

The P/E hybrid state of deacylated tRNA appears to be reinforced upon binding of EF-G to the pre-translocation complex, thereby initiating translocation. The role of the P/E state in the promotion of translocation can be attributed to lowering the energetic barrier for deacylated tRNA to leave the high-affinity P site by establishing a binding interaction in the E site [40]. Thus, we arrive at a model of translocation in which the tRNAs move in different steps on the two ribosomal subunits. The initial movement takes place on the 50S ribosomal subunit and entails the acceptor ends of deacylated tRNA and peptidyl-tRNA, and subsequently the anticodon arm-D arm domains are moved on the 30S subunit. Most likely, the latter movement is synchronous, since during the movement the arrangement of the two tRNAs relative to each other does not change, that is, both anticodons remain bound to contiguous codons [63] carrying the mRNA along by one codon.

Apart from the EF-G-independent formation of the hybrid A/P* and P/E states, discussed above, there is additional evidence supporting the idea of a sequential tRNA movement on 50S and 30S subunits during translocation promoted by EF-G. When translocation is induced by adding EF-G with a non-hydrolyzable GTP analogue, 50S translocation of peptidyl-tRNA, as followed by the puromycin assay, is faster than 30S translocation measured by the release of deacylated tRNA [69,74]. Unlike 50S translocation, 30S translocation is effectively inhibited by preoccupying the E site with deacylated tRNA [69]. Similarly, mutant EF-G lacking the G domain retains activity in 50S translocation, whereas it is inactive in 30S translocation [12]. It is likely, therefore, though not strictly proven, that 50S translocation precedes 30S translocation. Furthermore, these results indicate that the activity of EF-G in 30S translocation depends on GTP hydrolysis more than 50S translocation.

How EF-G, by binding to the pre-translocation complex and hydrolyzing GTP, may catalyze translocation is discussed in some detail below. For the present context of tRNA movement, it suffices to recall that the footprinting pattern of peptidyl-tRNA on 16S rRNA, which most likely reflects contacts of the anticodon region, changes upon translocation from the A to the P site [48]. At the same time, cross-linking contacts of the mRNA with 16S rRNA are changed [80,87]. Thus, 30S translocation entails a movement of the anticodon regions of the tRNAs, while bound to the mRNA, relative to the 30S subunit. In order to allow the movement, the pre-translocation interactions between tRNA (mRNA) and 16S rRNA have to be released, and new post-translocation interactions are formed. Hence the mRNA is kept in the complex, and the reading frame is maintained, mainly by the interactions with the tRNAs, that is, by codon-anticodon interactions. The codon-anticodon complex on the ribosome is kinetically more stable [61] than, for instance, the complexes of tRNAs with complementary anticodons free in solution [27,28]. This indicates that the decoding center of the ribosome provides an environment which stabilizes the interaction, presumably by binding interactions with the ribosome.
and, additionally, by the exclusion of solvent from the site of interaction, thus minimizing destabilization of hydrogen bonds by competing water [71]. The kinetic stability of codon-anticodon interaction appears to be high enough to maintain the complex throughout translocation, despite the release of binding interactions within the decoding center during the movement. In contrast, the codon interaction of the deacylated tRNA is destabilized upon transfer from the P site to the E site [42], possibly by exposing the codon-anticodon complex to the solvent [72] or by establishing an mRNA structure which is unfavorable for anticodon binding. As a consequence, E site-bound tRNA readily dissociates from the ribosome after the movement.

The movement of deacylated tRNA during translocation and subsequent dissociation from the ribosome takes place in several steps. From the P/E hybrid state, attained by donating the peptide to aminoacyl-tRNA, the tRNA is translocated to the E site to establish an intermediate E site-bound state with the anticodon still bound to the mRNA. The latter intermediate state, E', which could be demonstrated by kinetic experiments [63,70], is short-lived and rearranges to another state, E, most likely by moving the anticodon region away from the mRNA into a position about 35 Å apart from the anticodon of the tRNA in the P site [63]. In the E state, a substantial fraction of the binding energy is provided by hydrogen bonding interactions between the 3’ end of the tRNA and the ribosome, and the affinity to a large extent depends on the concentration of Mg$^{2+}$ [72] and of polyamines [25,78]. At any condition, however, the E site-bound tRNA is bound in a kinetically labile, that is readily chaseable, fashion [78]. An influence of E site-bound tRNA on aminoacyl-tRNA binding to the A site and vice versa, as put forward by Nierhaus’ group [55,83], could not be confirmed by several groups including our own ([78], and references cited therein).

In keeping with the indirect data, different structural arrangements of deacylated tRNA in the E site have been revealed recently by electron cryomicroscopy [6,83,89]. The heterogeneity of tRNA arrangements in the E site probably reflects the existence of several states of the tRNA leaving the ribosome during or after translocation, such as the two states discussed above. The extent to which the different states are populated seems to be very sensitive to the ionic conditions, which is probably the explanation for different observations reported in the literature.

3. Function of GTP hydrolysis in translocation

The potential for the molecular movements involved in translocation resides in the structure of the ribosome, and GTP hydrolysis is not required to drive the reaction thermodynamically, since the spontaneous, EF-G-independent reaction takes place, albeit very slowly, at certain conditions in vitro [23,85]. However, there is a large activation barrier to be overcome for translocation to proceed at the rate of protein elongation in vivo, or faster (on the average, about 10 amino acids per second are incorporated in Escherichia coli). The activation barrier is lowered by binding of EF-G-GTP to the ribosome in the pre-translocation state, as studied with non-hydrolyzable GTP analogues, and lowered further by GTP hydrolysis [74].

Until recently, the generally accepted model of translocation assumed that EF-G-GTP binds to the pre-translocation ribosome and induces a conformational change which allows translocation, and that, subsequently, EF-G hydrolyzes GTP, switches to a low-affinity GDP-bound conformation, and dissociates from the ribosome. The model was based on the observation that EF-G with non-hydrolyzable GTP analogues still enhances translocation relative to the spontaneous reaction, but is restricted to a single round [9,31,50], unless the factor is actively removed from the ribosome after translocation [9]. Alternative models in which GTP hydrolysis drives translocation were discussed [1,85], but were not generally favored.

Pre-steady-state kinetic experiments show that EF-G hydrolyzes GTP immediately after binding to the ribosome, prior to, and much faster than, translocation of peptidyl-tRNA (Fig. 1A) [74], contrary to the previous models and to our interpretation of previous experiments performed in a less well defined system [70]. The rate constant of single-round GTP hydrolysis measured at near-saturating concentration of EF-G is about 120 s$^{-1}$ (extrapolated to about 170 s$^{-1}$ at saturation), whereas the rate constant of peptidyl-tRNA movement is 25 s$^{-1}$. In these experi-
ments, translocation proceeds to the same extent as GTP is hydrolyzed, suggesting that fast translocation and prior GTP hydrolysis are related stoichiometrically. On the other hand, the rate of GTP hydrolysis is independent on the status of the ribosome with respect to the occupancy with tRNAs (pre-, post-translocation, or vacant) and is not affected when translocation is blocked by the antibiotic viomycin [74]. Thus, single-round GTP hydrolysis by EF-G on the ribosome does not depend upon subsequent translocation. It is likely, though, that the turnover of EF-G is coupled to translocation, that is, requires a free A site. This is suggested by results obtained with EF-G deletion mutants, as discussed below.

That rapid translocation depends on GTP hydrolysis is shown directly by the observation that GTP hydrolysis accelerates translocation more than 50 times relative to the reaction with non-hydrolyzable GTP analogues or GDP (Fig. 1B) [74]. The rate of the latter reaction still is very significant relative to that of the the factor-free reaction which hardly takes place on ribosomes programmed with near-natural mRNA. Thus, there seem to be two ways by which EF-G catalyzes translocation. In one, operating with non-hydrolyzable GTP analogues or GDP, the activation energy of translocation is reduced by the binding of EF-G to the pre-translocation complex, which is not very much affected by the nucleotide. Subsequent translocation is relatively slow and the turnover of EF-G even slower, because the transition state is reached by thermal motion only. By contrast, in the mechanism operating in the presence of GTP, the hydrolysis of GTP results in a substantial acceleration, presumably by inducing conformational transitions in EF-G and the ribosome that facilitate translocation. From the rates of GTP hydrolysis and translocation, a large time gap between the two reactions, about 35 ms, is calculated. The time lag suggests that conformational strain introduced by GTP hydrolysis is stored in the complex and can be released only in the forward direction, that is, by proceeding to the transition state and to translocation. We hypothesize that the conformational coupling is due to the formation of a kinetically stabilized EF-G-ribosome complex, and that, as outlined in more detail below, movements of domain 4 are important for both the forward reaction and the subsequent dissociation of the complex.

The evidence discussed so far is summarized in the translocation scheme depicted in Fig. 2. In the initial pre-translocation state, deacylated tRNA resides in
the P site with the 3′ end oriented towards or bound in the E site (P/E hybrid state) and peptidyl-tRNA in the A site with the peptidyl end towards the P site (A/P* hybrid state, P* indicating that this state is not yet puromycin-reactive). Immediately following the binding of EF-G-GTP (step 1), GTP is hydrolyzed (step 2). Most likely, GTP hydrolysis and/or subsequent release of P_i causes a conformational change of EF-G which, in turn, induces the formation of the transition state of the ribosome (step 3). In the transition state, the movement of the tRNA-mRNA complex takes place (step 4). This step comprises (i) the movement of the 3′ ends of the two tRNAs on the 50S subunit into their respective post-translocation positions, i.e., the E site for the deacylated tRNA and the puromycin-reactive position of the peptidyl end of the peptidyl-tRNA in the P site (A/P* state); (ii) the movement of the anticodon arms of both tRNA molecules together with the mRNA to their immediate post-translocation positions on the 30S subunit. In step 5, the ribosome returns to the ground state and EF-G assumes the GDP-bound conformation; the latter step is the one inhibited by fusidic acid binding to EF-G on the ribosome. Step 6 then comprises the dissociation of EF-G-GDP and deacylated tRNA (the order of dissociation is not known) to reach the final post-translocation state of the ribosome with peptidyl-tRNA in the P site and a free A site.

4. Molecular mechanics of EF-G

To study the role of EF-G domains for translocation catalysis, domain deletion mutants were prepared which were truncated from the N- or the C-terminus. The activities of the deletion mutants of EF-G on the ribosome were tested with respect to GTP hydrolysis, translocation catalysis, and turnover. The isolated G domain (domain 1) of EF-G was able to bind guanine nucleotides, but was inactive in all other functions [12]. A C-terminal fragment of EF-G comprising only domains 4 and 5 was inactive in all ribosome-dependent functions [12]. Interestingly, a larger C-terminal fragment lacking only the G domain exhibited partial translocation activity [12]. The mutant factor catalyzed the transfer of the 3′ end of peptidyl-tRNA to the P site on the 50S ribosomal subunit into a puromycin-reactive state. In contrast, it did not bring about translocation on the 30S subunit, since (i) deacylated tRNA was not released from the P site and (ii) the A site remained blocked for aminoacyl-tRNA binding after partial translocation. The fragment bound to
the ribosome with an affinity comparable to that of intact EF-G (0.8 μM), and was active in turnover. The implications of this finding are: (i) the 50S phase of translocation probably represents the first EF-G-dependent step of translocation that follows the spontaneous formation of the A/P state that is not puromycin reactive [48], and (ii) the G domain has an important role, presumably exerted through interactions with other domains of EF-G, in the promotion of translocation on the small ribosomal subunit.

Most interesting are the effects of deleting domain 4. The comparison of the structure of EF-G with the structure of EF-Tu [2,10,37,64] and of the ternary complex EF-TuGDPNP-Phe-tRNA^phe [57] shows that domain 4 of EF-G mimics the anticodon domain of the ternary complex in relative spatial position, shape, and distribution of negative charges, suggesting a functional parallel (‘molecular mimicry’, [15,39,52,59]). Our data show that truncated EF-G lacking domain 4 retains low, GTP-dependent activity in translocation, while the single-round GTPase is unaffected [74]. The mutant factor was able to promote translocation on both ribosomal subunits, albeit quite slowly. The activity was restricted to a single round, indicating tight binding of the mutant factor to the ribosome after GTP hydrolysis. The data suggest that domain 4 is essential for coupling the conformational change of EF-G induced by GTP hydrolysis to the rearrangement of the ribosome required for rapid tRNA translocation and for subsequent release of the factor. An important functional role of domain 4 is also suggested by mutational studies on the homologous eukaryotic EF-2 in yeast [36] and by the fact that the inactivation of mammalian EF-2 by diphtheria toxin is due to ADP-ribosylation at the tip of domain 4 [21].

The same activity in ribosome-dependent functions was observed with a deletion mutant of EF-G lacking both domains 4 and 5 (unpublished results). Again, the ability to perform one round of rapid GTP hydrolysis was not affected by the deletion, whereas multiple rounds of GTP hydrolysis were completely suppressed. Thus, truncated EF-G lacking domains 4 and 5 behaved like the mutant lacking only domain 4 [74]. The unchanged GTPase activity of the truncated EF-G on the ribosome shows that the contact of domain 5 with domain 1 seen in the crystal structure [4,18] is not involved in GTPase activation by the ribosome.

The effect of GTP hydrolysis on the structure of EF-G is not known, since only the structures of EF-G and EF-G-GDP have been determined (Fig. 3) [4,7,18]. By analogy to the well-characterized structural change of EF-Tu brought about by GTP hydrolysis [2,10,37,64], it is likely that the loss of the γ-phosphate of GTP introduces a conformational change in the G domain of EF-G. This change, in turn, may affect interactions with neighboring domains (2 and 5), thereby changing the relative arrangement of the domains. Although the effect on the overall structure of EF-G is probably less extensive than in the case of EF-Tu [17], the presumed rearrangement may nevertheless suffice to induce the structural change of the ribosome discussed below, which leads to translocation. It is appealing to assume that an active movement of domain 4 is involved. It may be induced by a change of the interaction of domain 1 with domain 5, which is probably rigidly connected to domain 4. The interactions of domain 1 with domain 5 (and 2) indeed seem to be functionally important, since numerous fusidic acid resistance mutations and revertants have been found at the domain interfaces [32,33]. Further support comes from the finding that deletion of domain 1

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*Fig. 3. Structure of EF-G-GDP. The domains are indicated. The figure was produced by Rasmol using the atomic coordinates of the 2.4-Å structure of [7], PDB 1.DAR.*
abolishes the ability of EF-G to promote translocation of the tRNAs on the small ribosomal subunit [12].

As suggested by the structural homology of domain 4 of EF-G with the anticodon arm of the ternary complex, the main target of domain 4 function is likely to be the small ribosomal subunit. It may promote translocation by inducing a rearrangement either within the small subunit or in the relative arrangement of small and large subunits [84] which leads to a structural state of the ribosome ('translocation state') in which the tRNAs can move together with the mRNA. Another possibility is that domain 4, by the movement discussed above, is actively displacing the peptidyl-tRNA from the 30S A site [1]. During or after the movement of the peptidyl-tRNA out of the A site, domain 4 may well move into the A site; this may be functional in closing the A site for peptidyl-tRNA moving backwards from the transition state of translocation. It was also suggested that a direct interaction between the domain 4 and mRNA may play an active role in translocation [3, 38, 39].

Unlike EF-G, the deletion mutants lacking domain 4 or domains 4 and 5 do not dissociate from the ribosome following GTP hydrolysis and translocation, contrary to what one might expect from deleting a domain which likely interacts with the ribosome. Thus, the presence of domain 4 is required not only for rapid translocation but also for the release of EF-G. This striking result can be explained by assuming that, early in the sequence of conformational changes induced by GTP hydrolysis, EF-G establishes additional interactions with the ribosome, thus enabling the factor to force the ribosome into the transition state of translocation. A movement of domain 4 into the A site, as discussed above, then may serve to resolve the tight interaction. There are two ways in which this could happen. A passive movement may induce a structural change in the body of the EF-G molecule that lowers the affinity for the ribosome and facilitates dissociation. Alternatively, an active movement of domain 4 may change the conformation of the ribosome towards low affinity for EF-G. Mutants lacking domain 4 would lack either activity and would, therefore, retain the high affinity gained by GTP hydrolysis, thereby preventing turnover.

5. Structural arrangement of EF-G on the ribosome

The interaction of EF-G with the ribosome has been studied by a variety of techniques. Studies using immunoelectron microscopy [24], cross-linking [82], chemical footprinting [49], and functional assays [29] have defined several EF-G-ribosome interaction sites mainly on the 50S ribosomal subunit. The factor binding site has been mapped to the base of the L7/12 stalk of the 50S subunit, and in 23S RNA the sarcin stem-loop (around residue 2660) as well as the thiostrepton binding site (around residue 1070) have been identified as presumed contact sites [54].

More detailed topographic information about the arrangement of EF-G on the ribosome was obtained by directed hydroxyl radical probing of the post-translocational ribosome complex from Fe^{2+}-EDTA tethered to various surface positions of engineered EF-G [95]. The complex was stabilized by fusidic acid, an antibiotic which inhibits the dissociation of EF-G-GDP from the ribosome after completion of translocation. Hydroxyl radical cleavages were found in both 23S and 16S rRNA. As expected, EF-G was found to bind in the proximity of the sarcin loop and thiostrepton binding region. Another cleavage site was the 1920 region of 23S rRNA, which in current models is placed close to decoding site of the small ribosomal subunit. In 16S rRNA, cleavage sites include three sites that are involved in tRNA binding, comprising the 790 and 1338 loops and the 1400 region, which is in the P-site part of the decoding center [45, 67]. Additional cleavages were observed around the binding site of protein S4. Because the probing positions of EF-G are known, the data provide sufficient constraints to place the factor on the ribosome. In the complex, EF-G lies across the 50S face of the 30S subunit, stretching from the S4 region of the body to a position near the base of the cleft. The globular end of EF-G (domains 1, 2 and 5) is bound between proteins S4 in the small subunit and L11 in the large subunit, with the GTP binding face of EF-G turned towards the 50S subunit. Domain 4 extends upwards towards the decoding site on 30S subunit. The overall orientation of EF-G in the model of [95] is very similar to that of the EF-Tu-tRNA complex visualized on the ribosome by electron cryomicroscopy [90].
This position and orientation of the factor on the ribosome was supported further when a similar ribosome-EF-G-fusidic acid complex, albeit without bound tRNA, was studied by electron cryomicroscopy [5]. The structural reconstruction places EF-G such that domain 5 appears to contact the base of the stalk, while the G domain (domain 1) is involved in an interaction with a structural element extending from the L7/12 stalk; the latter contact is reminiscent of a similar connection seen with EF-Tu [90]. Domain 4 reaches into the cleft of the 30S subunit at or near the decoding center, in keeping with the structural similarity between EF-G and the ternary complex. Thus, the concept of ‘molecular mimicry’ appears to apply for the post-translocation state, although the arrangement of EF-G does not provide us with an explanation about the function of EF-G in translocation catalysis.

The structures reported by Wilson and Noller [95] and Agrawal et al. [5] were obtained by blocking EF-G on the post-translocation ribosome by fusidic acid, that is at a stage late in translocation. In order to obtain other functional intermediates of translocation, we have recently studied the structures of EF-G-ribosome complexes stabilized by a different antibiotic, thiostrepton, before and after translocation. Based on evidence from biochemical and kinetic studies on translocation [73], thiostrepton blocks the system in a state after GTP hydrolysis and drastically decreases the rate of translocation. Therefore, the complex of the factor with the pre-translocation ribosome can be isolated. Upon longer incubation, translocation takes place, whereas subsequent dissociation of EF-G-GDP is practically blocked. Thus, from the same initial complex, both pre- and post-translocation EF-G-ribosome complexes can be isolated.

The structures of both complexes, as well as of a control ribosome complex without EF-G, were studied by electron cryomicroscopy and yielded structural reconstructions at 20 Å resolution [91]. The arrangement of EF-G in the two complexes was entirely different. In the EF-G complex with pre-translocation ribosomes, domains 1, 2 and probably 5 are oriented towards the 50S subunit, domain 1 making a strong contact with the L7/L12 stalk, while domain 4 interacts with the 30S subunit at the entrance to the inter-subunit cleft in the region where in the neutron map [14] protein S4 is located.

A strikingly different arrangement of EF-G was observed in the post-translocation state blocked by thiostrepton [91]. It is similar to that found by Wilson and Noller [95] and Agrawal et al. [5] in the presence of fusidic acid, with one important exception. In the thiostrepton-blocked state there are no contacts between EF-G and the 50S subunit other than the interaction with L7/L12, whereas in the post-translocation state of EF-G stabilized by fusidic acid the factor has more intensive contacts with the 50S subunit, in particular via domain 5 which interacts with the region at the base of the stalk [5]. It is likely, therefore, that the post-translocation state frozen by fusidic acid follows the state blocked by thiostrepton. In both post-translocation states of EF-G frozen by either fusidic acid [5] or thiostrepton [91], domain 4 of EF-G reaches into the decoding center close to or within the region which was occupied by the anticodon arm of the peptidyl-tRNA prior to translocation. In keeping with this position, hydroxyl radicals originating from surface positions at the tip of domain 4 hit 16S rRNA in the vicinity of the decoding center [95]. The positioning of EF-G in the post-translocation states, with domain 4 reaching into the cleft of the 30S subunit at or near the decoding center, supports the contention of a crucial functional role of domain 4 on the small subunit.

The entirely different positioning of the factor in pre- and post-translocation states implies a large-scale movement of EF-G during translocation. The factor initially binds across the inter-subunit cleft at the outer edge of the A site and, upon translocation, moves into the intersubunit space, domain 4 entering the decoding center and occupying approximately the position of the anticodon region of A site-bound tRNA.

6 Structural changes of the ribosome during translocation

Attempts to characterize structural changes of the ribosome related to translocation up to now were restricted to comparisons of pre- and post-translocation ribosomes. Scattering methods revealed slight differences [79,86], as did the direct visual compari-
son, by electron cryomicroscopy, of the pre- and the post-translocation state of the ribosome [89]. Apparently, the structural differences between pre- and post-translocation ribosomes are less extensive than often assumed. One reason for not observing any striking difference may be that the structure of the translocating ribosome does not prevail in the post-translocation state. Thus, in order to reveal structural changes related to translocation, the transition state of translocation should be studied, that is, the state in which the movement actually takes place.

The structures of ribosomes in the vacant state [22,88], with tRNAs bound to A and P or P and E sites [89], with ternary complex EF-Tu-GTP-Phe-tRNA_{Phe} bound in the A site [90], or with EF-G bound in the post-translocation state [5,91] are quite similar and reveal no significant differences between those functional states of the ribosome. By contrast, in the EF-G complex with pre-translocation ribosomes, studied by electron cryomicroscopy [91], the cleft between the head and the body of the 30S subunit is opened up significantly, and there are substantial additional structural changes in the 30S subunit on the side exposed to the solvent. The structure of the 50S subunit remains more or less unchanged in all structures available. The data suggest that EF-G binding to the pre-translocation ribosome substantially changes the conformation of the 30S subunit. We propose that this structure of the ribosome represents, or is closely related to, the transition state of translocation (Fig. 2) in which the movement of both tRNAs and mRNA takes place and which, after the movement, returns to the ground state.

According to these results, the ‘unlocking’ [85] of the ribosome that allows the movement of the tRNAs and the mRNA appears to take place largely on the 30S subunit, and less so on 50S, and does not involve a significant change of the relative arrangement of the subunits. At present, we can only speculate about how the contact of domain 4 with the 30S subunit induces the extensive conformational changes which are observed. The contact appears to be in the S4 region, thereby causing a local conformational change that may be transmitted to other parts of the 30S subunit. As discussed below, 16S rRNA is likely to be involved in the transmission of the conformational change.

7. The mechanism of translocation catalysis

On the basis of biochemical and kinetic evidence, as well as electron cryomicroscopic reconstructions, we propose the following model of translocation. The initial binding of EF-G to the pre-translocation ribosome positions the factor with its G domain tightly bound to proteins L7/L12. Domain 4 of the factor is oriented towards the 30S subunit and binds to 16S rRNA in the vicinity of the protein S4. GTP hydrolysis in EF-G that immediately follows the binding leads to a change of EF-G structure that forces a movement of the tip of domain 4 relative to the fixed G domain of the factor. Due to the interaction of domain 4 with the 16S rRNA, the S4 binding region is displaced, which gives rise to coupled structural transitions of the 30S subunit. In the transition-state conformation of the small ribosome subunit, a structure of the decoding region of 16S rRNA is induced that has low affinity to the codon-anticodon complex, both in the A and P sites, which facilitates the movement of tRNAs and mRNA. After the translocation, EF-G moves into the inter-subunit space, so that the tip of domain 4 is found close to the ribosome decoding region. The structure of the ribosome returns to the ground state. EF-G is stepwise released from the ribosome, via binding of the domains 1, 2 and 5 to the 50S subunit. The conformational change of the ribosome that allows tRNA-mRNA motion may occur spontaneously, resulting in slow spontaneous translocation. The interaction of EF-G with the ribosome stabilizes the ‘unlocked’ [85] structure of the ribosome, thereby accelerating the movement of the RNAs. Likewise, one might expect that the motion of EF-G that promotes the interaction with the 30S subunit may happen without GTP hydrolysis, and that, after GTP is cleaved, the catalytically active structure of EF-G becomes predominant and further facilitates translocation.

Given the fact that EF-G is binding at the A-site entrance of the pre-translocation ribosomes, a potential role for EF-G in translocation may be (i) facilitating the conformational changes of rRNA required for the tRNA-mRNA movement, or (ii) direct interaction with the A-site bound tRNA that actively displaces the tRNA-mRNA complex to the P site. The latter possibility seems to be less likely,
since a tRNA analogue comprising a minimally 15 nucleotides long anticodon stem-loop structure appeared to be a sufficient A-site substrate for EF-G-dependent translocation [35]. It is known that the anticodon stem-loop tRNA construct interacts exclusively with the small ribosomal subunit [45,76] and it binds to the decoding region of 16S rRNA [68], so that an additional contact with EF-G seems unlikely. Therefore the primary cause of tRNA translocation lies in EF-G-catalyzed structural changes of the 16S rRNA, rather than in active pushing of the tRNA out of the A site.

After the movement of peptidyl-tRNA from the A to the P site, domain 4 of EF-G occupies the A site on the 30S subunit, by moving to the site vacated by the translocated peptidyl-tRNA, closes the site and, by inhibiting the backward movement of the tRNAs, helps to stabilize the post-translocation state while the ribosome is in the transition-state conformation. The cause for this extensive EF-G movement is not known. It is possible that the factor is actively driven into the new location by the rRNA movement. In this case, a rotational movement in the 30S subunit may be involved, as has been suggested [96]. Another possibility is that the conformational change of 16S rRNA and tRNA-mRNA displacement to the P site provide the room to complete the structural transition of EF-G from GTP to the GDP form. At some stage during the movement of domain 4 into the decoding center, the initial contact to 16S rRNA is resolved, and the ribosome returns to its closed ground state structure. The structure of the decoding center changes towards high affinity conformation, resulting in tight binding of peptidyl-tRNA in the P site. The intermediate EF-G-GDP complex with the ribosome is thermodynamically unstable, but the dissociation by a pathway which is a reverse of the binding is kinetically disfavored, because there is no driving force to induce the transition state of the 30S subunit. Therefore, EF-G release requires a different set of ribosomal contacts, most prominently with the 50S subunit, which appears to form a last state of EF-G movement on the ribosome.

8. Involvement of rRNA in translocation

The site of tRNA-mRNA interaction is the decoding region of 16S rRNA, and the anticodon arms of both tRNAs interact with 16S rRNA. In the transition state of translocation, these interactions have to be released in order to allow tRNA-mRNA movement in translocation. There are numerous observations that relate structural changes of the decoding center, or their inhibition, to translocation. It is known that mutations both in protein S5 and in the vicinity of the S5 binding site (helix 34) in 16S rRNA confer resistance to spectinomycin [13], an inhibitor of EF-G-dependent translocation [11,81]. Furthermore, it has been reported that aminoglycoside antibiotics that bind to the decoding region on 16S rRNA and affect the fidelity of decoding, also inhibit translocation [19]. Finally, the antibiotic viomycin, which specifically inhibits translocation [51],
strongly protects position A1408 in the decoding center of 16S rRNA against modification by dimethyl sulfate, in addition to positions 913 and 914 in 23S rRNA [46].

The important question now is how the interaction of EF-G with the ribosome may affect the decoding center. We suggest that the structural changes in the small ribosomal subunit originate at the site of interaction of domain 4 of EF-G with the pre-translocation complex, which is in the vicinity of the binding region of protein S4 (Fig. 4). S4 binds to the rRNA junction of five helical elements (S4 junction; [30,66]) of which one comprises helix 18 of 16S rRNA, a region known to contain a pseudoknot (530 region pseudoknot [65]) and to be crucially important for ribosome function (for review see [60,65]). Therefore, motion at the S4 binding region may induce a conformational switch in the pseudoknot structure. It is known that the 530 loop is located close to helix 34 [53,58] and is conformationally coupled to the decoding region of 16S rRNA [45,53,65]. The switch in the 530 pseudoknot structure may induce a rearrangement in the 16S rRNA decoding region towards the conformation that has low affinity to the co-translational complexes. Destabilization of the 16S rRNA-codon-anticodon complex may reduce the activation energy barrier of translocation, thus facilitating movement. Such a model explains how changes in the stability of the 16S rRNA-codon-anticodon complex due to mutations or antibiotic binding affect the rate of translocation.

The role of helix 34 in translocation is less clear. According to the electron cryomicroscopic reconstructions of ribosome-EF-G complexes [5,91], a direct interaction of EF-G with helix 34 is unlikely. Rather, the structural rearrangement of helix 34 promotes an opening of the cleft between the head and the body of the 30S subunit, which may be important for the repositioning of the domain 4 of EF-G towards the decoding center.

The involvement of other ribosome regions in promoting translocation at present remains speculative. The 912 region may be particularly interesting, since it has been shown that a switch between two alternative base-paired arrangements in the 912 region is accompanied by structural rearrangements in 16S rRNA near the decoding region [43]. It seems possible, therefore, that structural changes of the 912 region contribute to translocation catalysis, in addition to the effect on decoding in the A site.

Comparatively less extensive conformational changes related to EF-G binding and translocation were observed in the large ribosomal subunit [91]. The interaction of EF-G with the α-sarcin stem-loop and the thiostrepton binding region is well documented [47,54,95]. According to electron cryomicroscopic reconstructions, EF-G binds to the region of the large ribosomal subunit that is supposed to comprise α-sarcin loop and thiostrepton binding region only after tRNA-mRNA translocation, before the factor leaves the ribosome [5]. It is likely that these interactions with 23S rRNA are involved in the release of EF-G from the ribosome.

9. EF-G: a switch or a motor?

Until recently, the functional cycle of EF-G was considered to follow the scheme that is common for GTP-binding proteins in general. In the active GTP-bound form the protein was thought to bind to its GAP (ribosome) and to catalyze a specific reaction (movement of tRNA and mRNA); subsequently, the protein is deactivated by GTP hydrolysis, which switches the affinity of the factor to the ribosome from high to low, thereby enabling the factor to dissociate from the ribosome. The picture emerging from the kinetic and mutational analyses is, however, inconsistent with the switch model. Moreover, the assumption of grossly different affinities with GTP and GDP, which forms the basis of the model, is not supported by the data. EF-G-GTP and EF-G-GDP bind to the ribosome with similar affinity [8], and association and dissociation rate constants of the initial complex of EF-G and the ribosome are the same in the presence of GTP, non-hydrolyzable caged GTP, or GDP (1.5×10^9 M^−1 s^−1 and 50–100 s^−1 at 37°C, respectively [74]. K_M values for EF-G, as determined in steady-state translocation experiments, are similar with caged GTP and GDP (0.3 and 1 μM, respectively; unpublished results). Finally, the kinetic analysis clearly shows that GTP is hydrolyzed immediately following the binding, and that the tRNA-mRNA movement takes place subsequently to and is accelerated by GTP hydrolysis. These features are not consistent with a classic G
protein model in which the ribosome is regarded as the downstream effector of EF-G-GTP.

The GTPase paradigm may nevertheless be useful for discussing the role of GTP hydrolysis for the function of EF-G and, similarly, of EF-Tu. Both elongation factors consist of several domains, and the parallel to other GTPases, like p21ras and the Gs subunit of heterotrimeric G proteins, is restricted to the G domain. The conformational switch of p21ras and Gs brought about by exchanging GDP for GTP (activation) and GTP hydrolysis (inactivation) changes their affinities to their respective downstream effector proteins,raf and adenylate cyclase, that is, it regulates intermolecular interactions. By contrast, in the elongation factors the GTP-GDP conformational switch in the G domains appears to change the interactions of the G domains with the neighboring domains within the same molecule, thereby changing the overall architecture of the molecule. For EF-Tu, this architectural change is well-documented by the crystal structures of the GTP and GDP forms [2,64]. The main consequence of the extensive conformational change in the G domain caused by GTP hydrolysis and loss of γ-phosphate is the release of domain 2 from the G domain. Thereby, the high-affinity binding site of the aminoacyl end of aminoacyl-tRNA, which is formed by those two domains [57], is destroyed, and the aminoacyl-tRNA becomes free to enter the peptidyl transferase center. It is important to note that EF-Tu-GDP dissociates from the ribosome due to the loss of the interaction with aminoacyl-tRNA, whereas the ribosome-binding affinity of EF-Tu alone, without aminoacyl-tRNA bound to it, is not much different with GTP or GDP.

Extending the example of EF-Tu to EF-G, we consider it likely that the conformation of the G domain of EF-G is different in the GTP- and GDP (or GDP/Pi)-bound form and that the difference regulates the interaction of the G domain with the neighboring domains which, in the GDP-bound and nucleotide-free structures are domains 2 and 5. One may envisage that a chain of coupled conformational changes of 16S rRNA which, as discussed above, may lead from the initial point of EF-G-ribosome interaction in the S4 binding region into the decoding center, provides a mechanical means to amplify an initially small conformational signal. This scenario bears interesting parallels to other systems where chemical energy is transformed into directed molecular movement, that is, to motor proteins.

The classic ATP-driven motor proteins, such as myosin and kinesin, are similar to G proteins in that the affinity of the motors to their protein partners depends on the nucleotide that occupies the active site [93]. The motor’s ADP form is the active species. For instance, both ATP and ADP-Pi forms of myosin are weakly bound to actin. P_i release and concomitant protein structural rearrangements allow myosin-ADP to bind 10000-fold tighter to the active filament and enable the motor to produce the force (‘power stroke’) and move unidirectionally along the filament [92]. Thus, the ATP to ADP transition converts the motor into its active state.

It has been pointed out [77,93] that there are interesting structural similarities between motor proteins and several GTPases as well as potential analogies in the switch mechanisms induced by NTP hydrolysis. While these similarities are confined to the nucleotide-binding domains, there are striking parallels between the mode of action of, for instance, myosin and the functional cycle of EF-G. Binding of EF-G-GTP to the ribosome is weak and readily reversible [74]. GTP hydrolysis by EF-G leads to the formation of the translocation transition state of the ribosome. It is possible that the GTP to GDP transition in EF-G produces the ‘power stroke’ that acts on 16S rRNA and promotes the movement of tRNA-mRNA. In this case, the motor function of EF-G is exerted on the rRNA and translocation is induced indirectly, due to coupled structural changes in the small ribosomal subunit. In keeping with a motor function of EF-G, GTP hydrolysis precedes translocation by a considerable time, indicating that the energy released by GTP hydrolysis is stored in the conformation of EF-G and/or the ribosome before the ‘power stroke’ of translocation is performed. These findings suggest that the function of EF-G is to translate chemical energy, derived from GTP hydrolysis, into directional molecular movement on the ribosome [16,74].

Details of the functional cycle seem to be different for myosin and EF-G. Myosin is tightly bound to actin in the ADP form (‘rigor complex’), and ADP-ATP exchange dissociates the myosin-actin complex which, after ATP hydrolysis, enables the motor to...
rebinding to a new actin subunit. Comparatively little is known about the mechanism of EF-G recycling. The affinities of EF-G-GTP and EF-G-GDP to the ribosome are similar ([8,74], unpublished results), suggesting that GDP-GTP exchange does not have a role in EF-G recycling. On the other hand, the intermediate EF-G-GDP-ribosome complex stabilized by fusidic acid binds GDP much more tightly than free EF-G, indicating that the factor in the complex has a conformation which differs from the one of EF-G-GDP in solution. The structure of the fusidic acid-stabilized EF-G-ribosome complex obtained by electron cryomicroscopy indeed suggests a difference in the arrangement of domains 4 and 5 relative to the body of EF-G [5].

In summary, the evidence suggests that EF-G acts by generating force from GTP hydrolysis. This is probably true also for its eukaryotic counterpart, EF-2, and, possibly, the homologous GTPase found in the spliceosome [20]. Another force-generating GTPase may be dynamin [44]. There is a growing list of proteins that are related to the classic motor proteins in that they promote directional molecular movement at the expense of the free energy of nucleoside triphosphate hydrolysis, such as RNA polymerase [97] and some DNA helicases [94]. The group of force-generating GTPases now seems ready to enter that list.

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