Crystallographic studies of elongation factor G

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Abstract: The elongation factors G (EF-G) and Tu (EF-Tu) go through a number of conformation states in their functional cycles. Since they both are GTPases, have similar G domains and domains II, and have similar interactions with the nucleotides, then GTP hydrolysis must occur in similar ways. The crystal structures of two conformational states are known for EF-G and three are known for EF-Tu. The conformations of EF-G·GDP and EF-Tu·GTP are closely related. EF-Tu goes through a large conformational change upon GTP cleavage. This conformational change is to a large extent due to an altered interaction between the G domain and domains II and III. A number of kirromycin-resistant mutations are situated at the interface between domains I and III. The interface between the G domain and domain V in EF-G corresponds with this dynamic interface in EF-Tu. The contact area in EF-G is small and dominated by interactions between charged amino acids, which are part of a system that is observed to undergo conformational changes. Furthermore, a number of fusidic acid resistant mutants have been identified in this area. All of this evidence makes it likely that EF-G undergoes a large conformational change in its functional cycle. If the structures and conformational states of the elongation factors are related to a scheme in which the ribosome oscillates between two conformations, the pretranslocational and posttranslocational states, a model is arrived at in which EF-Tu drives the reaction in one direction and EF-G in the opposite. This may lead to the consequence that the GTP state of one factor is similar to the GDP state of the other. At the GTP hydrolysis state, the structures of the factors will be close to superimposable.

Key words: elongation factor G, elongation factor Tu, crystal structures, conformational changes, ribosomal conformation.

Résumé : Les facteurs d'élongation G (EF-G) et Tu (EF-Tu) prennent diverses configurations au cours de leur action. Puisque ce sont deux GTPases ayant des domaines G et des domaines II semblables et interagissant de façon analogue avec les nucléotides, les deux facteurs doivent hydrolyser le GTP de la même façon. Les structures des cristaux de deux configurations d'EF-G et de trois d'EF-Tu sont connues. Les configurations d'EF-G·GDP et d'EF-Tu·GTP sont très semblables. EF-Tu subit un important changement de configuration lors de l'hydrolyse du GTP. Ce changement de configuration est principalement attribuable à une modification de l'interaction entre le domaine G et les domaines II et III. Certaines mutations entraînant une résistance à la kirromycine sont localisées à l'interface des domaines I et III. L'interface du domaine G et du domaine V de l'EF-G est équivalent à cette interface dynamique de l'EF-Tu. Dans l'EF-G, la surface de contact est petite et les interactions se font principalement entre des acides aminés chargés se trouvant dans une portion qui subit des changements de configuration. De plus, certaines mutations entraînant une résistance à l'acide fusidique ont été identifiées dans cette région. L'ensemble de ces données indique que l'EF-G subirait un important changement de configuration lors de son cycle d'action. Si les structures et les configurations des facteurs d'élongation sont replacées dans un mécanisme où le ribosome oscille entre deux configurations, les états pré- et post-translocation, on obtient un modèle où la réaction est entraînée dans un sens par l'EF-Tu et dans le sens opposé par l'EF-G. Par conséquent, le complexe d'un facteur avec le GTP est semblable au complexe de l'autre facteur avec le GDP. Lors de l'hydrolyse du GTP, les structures des deux facteurs seraient presque superposables.

Mots clés : facteur d'élongation G, facteur d'élongation Tu, structures cristallines, changements de configuration, configuration du ribosome.

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Table 1. Structures of different conformational states of EF-G and EF-Tu.

Factor (species)	Complex	Resolution	Ref.
EF-G (*T. thermophilus*)	–	2.8 Å	Ævarsson et al. 1994
EF-G (*T. thermophilus*)	GDP	2.7 Å	Czworkowsky et al. 1994
EF-G (*T. thermophilus*)	GDP	2.4 Å	
EF-Tu (*E. coli*, trypsin modified)	GDP	2.6 Å	Kjeldgaard and Nyborg 1992
EF-Tu (*T. thermophilus*)	GMPPNP	1.7 Å	Berchtold et al. 1993
EF-Tu (*T. aquaticus*)	GMPPNP	2.5 Å	Kjeldgaard et al. 1993
EF-Tu (*T. aquaticus*)	GMPPNP + Phe-tRNA^{Phe}	2.7 Å	Nissen et al. 1995

Fig. 1. The conformational cycle for G proteins (Ævarsson et al. 1994). The G proteins change conformation as a response to the bound nucleotide. GAP, GTPase-activating component; GEF, G nucleotide exchange component.
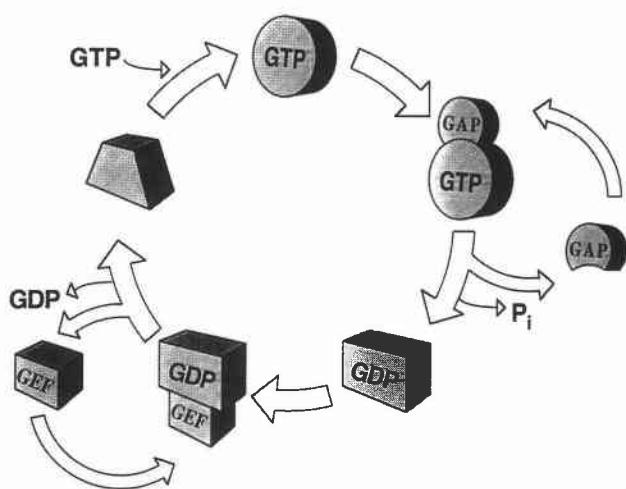
Introduction

Several G proteins or GTPases interact with the ribosome. These are primarily the translation factors IF-2, EF-Tu, EF-G, and RF-3. All G proteins are molecular switches and go through a number of conformational states in their functional cycle (Fig. 1; Bourne et al. 1990). These states are dictated by the bound nucleotide (GTP or GDP) or by the receptor. In the case of protein synthesis, the ribosome is the receptor. For an understanding of the function of the factors, we need to explore the structures of the different states. This review will discuss some functional aspects of the elongation factors that have been obtained from the structural work on EF-G and EF-Tu. Table 1 gives a summary of the states that so far have been characterized by structure determinations.

The ribosomal GTPases, and among them the elongation factors, are known to interact with overlapping sites on the ribosome (for a review see Liljas 1982). The ribosome has two different conformations, the pretranslocational and posttranslocational states (Spirin 1969, 1988; Möller 1974; Burma et al. 1986). The binding of EF-G to ribosomes in the pretranslocational state with a peptidyl tRNA in the A site causes translocation of both the tRNA and the mRNA (Kaziro 1978). Subsequently, EF-G is induced to hydrolyse the bound GTP and dissociate from the ribosome (Kaziro 1978). This step puts the ribosome in the posttranslocational state.

On one hand, EF-Tu interacts with posttranslocational ribosomes in binding a cognate tRNA to the A site. EF-Tu·tRNA interacts with ribosomes when the A site is empty, both with regard to the area for codon–anticodon interaction on the 30S subunit and the area at the peptidyl transfer site on the 50S subunit. EF-G, on the other hand, interacts with ribosomes where the codon–anticodon interaction remains but where the tRNA in the A site is associated with the nascent peptide close to the site for peptidyl transfer.

EF-Tu undergoes a drastic conformational change upon GTP hydrolysis (Berchtold et al. 1993; Kjeldgaard et al. 1993). Small changes in the G domain lead to totally different contacts between the G domain and the other two domains. It is remarkable that EF-G in complex with GDP has a conformation that is similar to EF-Tu in complex with GMPPNP (Czworkowski et al. 1994; Ævarsson et al. 1994). We will discuss whether EF-G undergoes a similar conformational change to that of EF-Tu but opposed to the conformational change in EF-Tu and in parallel to the oscillations of the ribosome between its two states.

The domain structure of EF-G

EF-G (Fig. 2) is a highly elongated protein with a maximal length of 120 Å (1 Å = 0.1 nm) (Czworkowski et al. 1994; Ævarsson et al. 1994). It has five structural domains, compared with the three domains in EF-Tu. The G domain has a core common to all GTPases and an additional subdomain, G'. This insert has also been observed through sequence analysis in different forms in some other G proteins that interact with the ribosome (Ævarsson et al. 1994; Ævarsson 1995).

Domain II of EF-G is a β-barrel structure that was first observed in EF-Tu where it also is domain II (Kjeldgaard and Nyborg 1992). From sequence comparisons it has been shown that all GTPases interacting with the ribosome have a domain II homologue (Ævarsson et al. 1994; Ævarsson et al. 1995). The possible function of this will be discussed below.

Domain III is currently only partly observed since it is a very flexible part of the molecule. From studies of crystals at liquid nitrogen temperature, it can be concluded that the mobility is not primarily due to thermal motion but most be due to several conformations that are present simultaneously in the protein (S. Al-Karadaghi, unpublished observations).

Domain IV is an elongated domain at the opposite end of the molecule from the G domain. It has an unusual fold where two parallel β-strands are connected by an α-helix forming a left-handed structure contrary to the common right-handed structure. A left-handed arrangement is usually related to function. The unusual fold of this domain has also been

Fig. 2. The structure of EF-G (Ævarsson et al. 1994; Czworkowski et al. 1994). The figure was produced using MOLSCRIPT (Kraulis 1991). The amino and carboxy termini are indicated and the domains are marked. Domain I consists of subdomains G and G′.
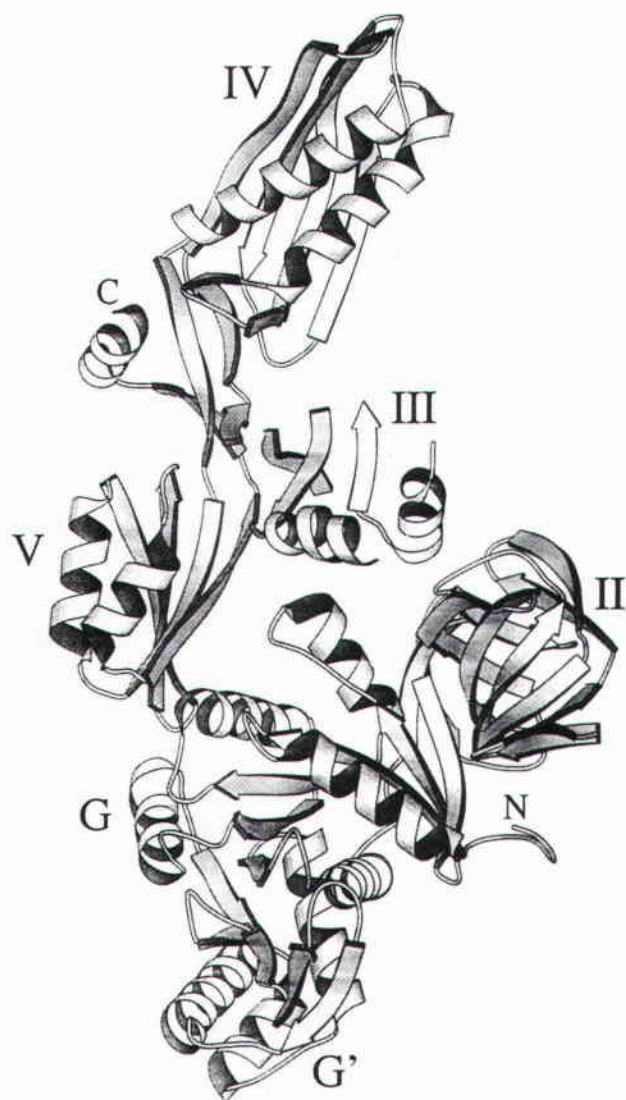

observed in ribosomal protein S5 (Murzin 1995). Whether this is coincidence or if there is an evolutionary or functional relationship is not known.

Finally, domain V has a structure that is closely similar to ribosomal protein S6 (Lindahl et al. 1994; Ævarsson et al. 1994; Czworkowski et al. 1994). This type of fold, called a double-split β–α–β, has been observed in a large number of proteins (Orengo and Thornton 1993). One large subfamily of these proteins is RNA binding proteins. Here the structure of the RNA binding domain of U1A has been determined both alone (Nagai et al. 1990) and in complex with a fragment of RNA (Oubridge et al. 1994). A large number of the ribosomal proteins for which the structure is known have closely similar folds (Ramakrishnan et al. 1995).

Domain V has an interface with the G domain. This interface is rich in charged amino acid residues, contrary with normal stable domain interactions. Primarily, Glu119 forms

Fig. 3. The conformational changes in the G domain of EF-G upon dissociation of GDP. (*a*) The conformation of the P loop (residues 19–23) with (green) and without (red) bound GDP. In the empty form, the loop is closed. The nucleotide binding requires an open conformation of the loop. In the open form (green), His20 is located in a position occupied by Gln117 in the closed form (red). To allow room for Gln117 in the GDP conformational state, helix C_G has to move along its axis. (*b*) In its GDP-bound conformation (green), Gln117 occupies space where Val88 is located in the empty form (red). Val88, which is part of the switch II loop, obviously has to move. The conformational changes at the nucleotide binding site can thus be transmitted to other domains through the switch II loop and helix C_G.
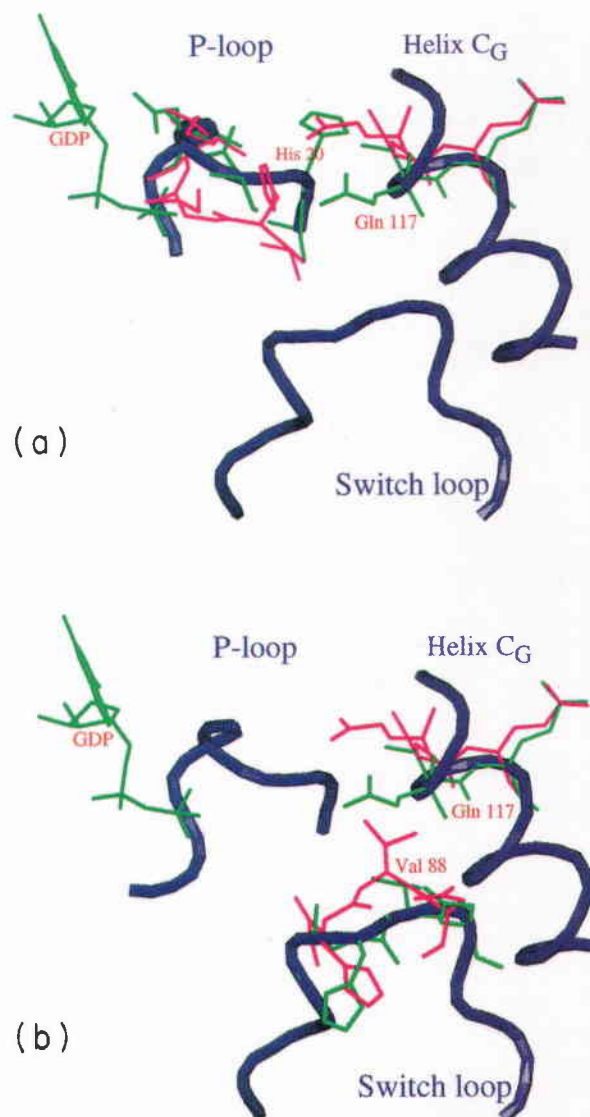
hydrogen bonds with three arginines and there are several additional arginines and lysines in close proximity.

Two particularly interesting parts of the structures of all G proteins are the effector region and the switch II region (Bourne et al. 1990). In the present structures of EF-G, the

Fig. 4. The arrangement of the homologous domains (I and II blue) is closely similar in EF-G·GDP and EF-Tu·GDPNP but very different in EF-Tu·GDP. Thus, the inactive form of EF-G resembles the active form of EF-Tu. The locations of fusidic acid and kirromycin resistant mutations in the two elongation factors are illustrated (red). The occurrence of mutations around helix C_G at a domain interface in both proteins may indicate that this surface is a site of structural rearrangements in both proteins (*a*) EF-G·GDP (left) and EF-Tu·GDPNP (right). (*b*) EF-Tu·GDP.

(a)

(b)
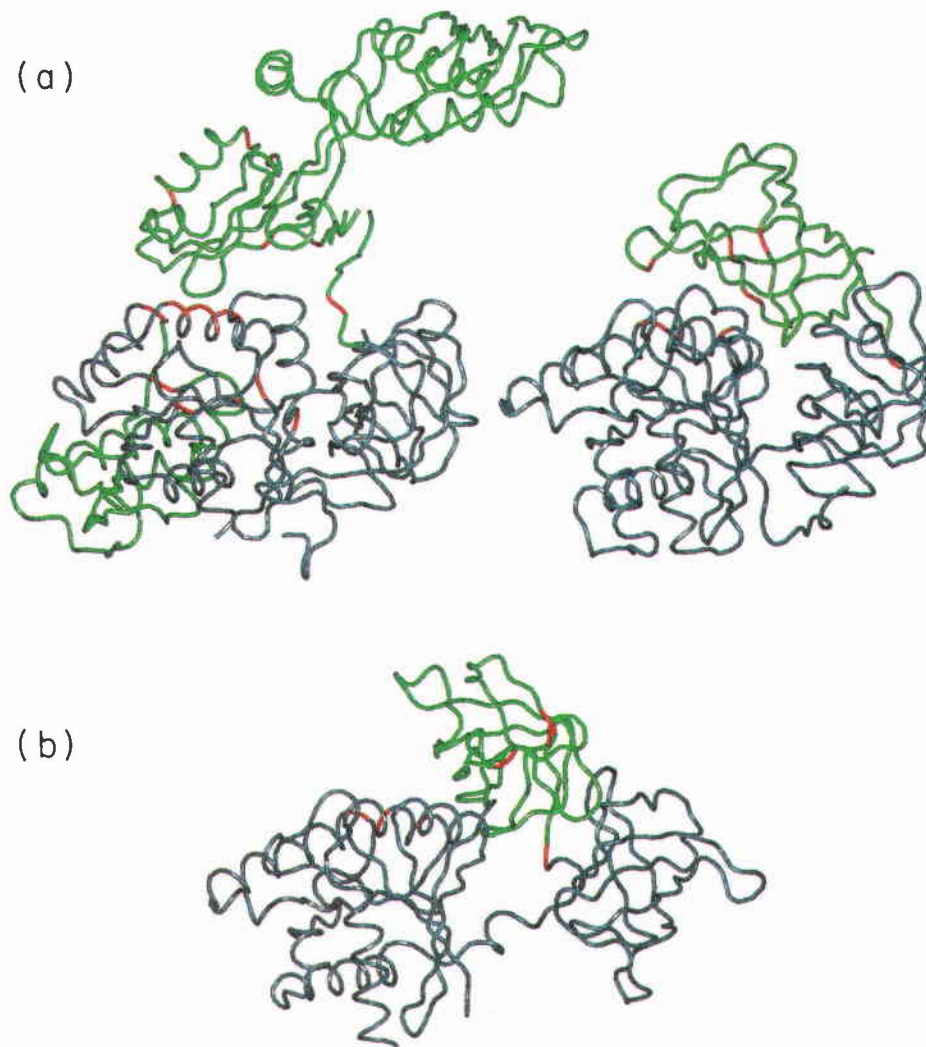
effector region is not observed, as a result of structural disorder. The switch II region of EF-G has an interesting location in contact with four of the domains but is still accessible for external interactions. Generally, in G proteins, this region has essential interactions with the receptors and controls the conformational states of the proteins.

Conformational changes in EF-G

The structures of EF-G in the empty form and in complex with GDP have been compared (S. Al-Karadaghi, A. Ævarsson, M. Garber, J. Zheltonosova, and A. Liljas, in preparation). All domains move to different extents in relation to the G domain. The movements on the whole become larger further away from the G domain and are maximally around 5 Å for main chain atoms. Some details are of considerable interest. In the empty from of EF-G, the P loop (residues 19–23) is closed (Fig. 3*a*). Upon binding of GDP the loop opens up. As a consequence of this, His20 moves into space previously occupied

by Gln117, which has to move (Fig. 3*b*). In its new location, Gln117 displaces Val88 of the switch II region. This leads to a new position for part of the switch II region, which may be part of the reason for the conformational rearrangements of the other domains.

Gln117 is part of helix C_G. The movement of this residue is partly due to a shift of helix C_G along its axis. Since helix C_G is located at the interface to domain V, it may have important consequences for the interactions between domains I and V and for the conformational changes of the molecule. In the two available structures, Glu119 does not alter its hydrogen bonding arrangement despite the movement of helix C_G, to which it belongs.

Comparisons of the structures of EF-G and EF-Tu

The structure of EF-Tu is known in three different states (Table 1). As has already been mentioned, two of the domains

Fig. 5. The charge distribution on the surface of EF-G is very distinct. Blue represents positive potential and red represents negative potential. The molecule is rotated 180° around its long axis compared with Fig. 2. Of particular interest are the inclined stripes of negative charges over domain IV (top narrow part of the molecule). This area then has similarities to the side with T loop of tRNA not only in its shape but also in its charge distribution. The figure was produced using GRASP (Nicholls et al. 1991).
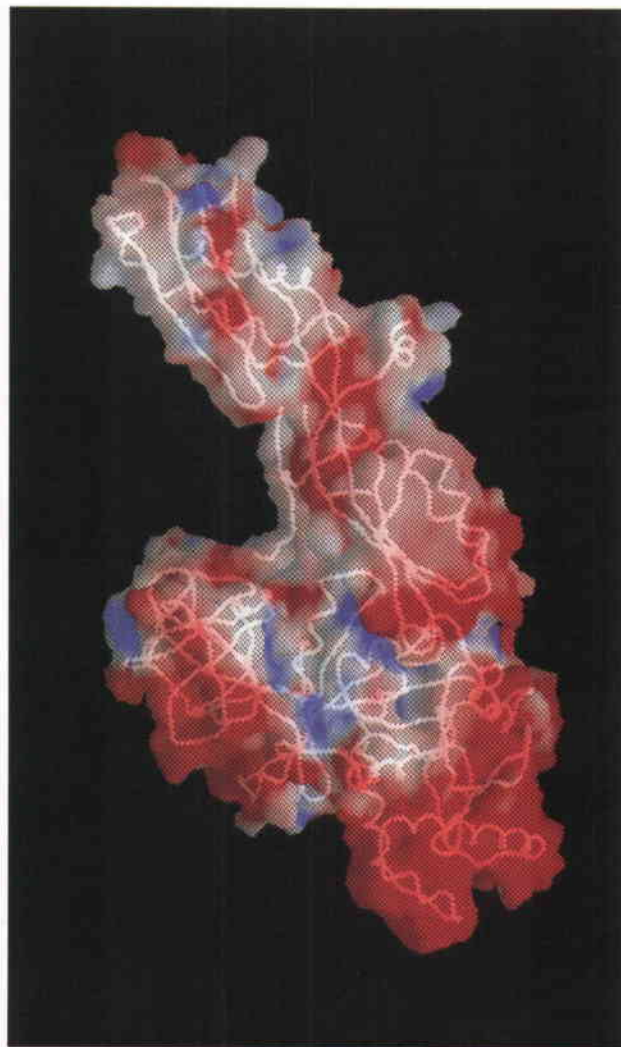
of EF-Tu (domains I (or G) and II) have homologous domains in EF-G. The third domain in EF-Tu has no structural correspondence in EF-G, but domain V of EF-G is located in a corresponding region in contact with the G domain. One remarkable observation (Czworkowski et al. 1994; Ævarsson et al. 1994) is that the GTP conformation of EF-Tu is closely similar to the GDP conformation of EF-G with regard to the domain arrangement, even though there are minor differences (Fig. 4a). On the other hand, the two GDP conformations are widely dissimilar both in the locations of domains II and the conformation of the switch II regions in both proteins (Fig. 4b).

A highly remarkable finding is that the overall shape of the ternary complex of EF-Tu·GMPPNP with Phe-tRNA[Phe] is very similar to EF-G·GDP (Nissen et al. 1995). Thus, the

tRNA part of the complex superimposes on domains III, IV, and V of EF-G. Here it seems as if the protein is imitating the tRNA. This is true to the extent that even the surface charge distribution on domains IV and V resemble the distribution of negative charges on the surface of tRNA (Fig. 5). However, this is true only for one side of the protein; the opposite side, the side with the effector region, has a more random distribution of charges. The anticodon part of the tRNA corresponds to the tip of domain IV, about 70 Å from the nucleotide-binding site of the factor. The surface with tRNA-like charge distribution corresponds to the side with the T loop of the tRNA and would be expected to interact with some components in the small subunit since the codon–anticodon–interaction occurs there. The side with the D loop of the tRNA would, from the same argument, face the large subunit. The function of the eukaryotic factor EF-II, which corresponds to EF-G, is inhibited by ADP ribosylation at the tip of domain IV.

Antibiotic resistance

Certain antibiotics can inhibit protein synthesis by locking the elongation factors in some functional state. In the case of EF-Tu, kirromycin prevents the transition to the GDP conformation after GTP hydrolysis. As a consequence, EF-Tu remains on the ribosome (Wolf et al. 1974). Kirromycin can also induce GTP hydrolysis by EF-Tu in the absence of ribosomes (Wolf et al. 1974). A number of mutants in EF-Tu that give resistance against kirromycin have been characterized. Several of these mutations are located on the interface between the G domain and domain III (Fig. 4; Abdulkarim et al. 1994). One possible explanation for the resistance is that the mutants destabilize the GTP conformation and permit the transition to the GDP conformation. Other possible explanations are that the mutants affect the binding site for the antibiotic or that they destabilize the factor interaction with the ribosome.

EF-G is inhibited by fusidic acid in a similar manner (Kaziro 1978). In the presence of this antibiotic, EF-G remains bound to the ribosome even after GTP cleavage. Several of the mutations leading to fusidic acid and kirromycin resistance are in both factors located in helix C_G, which is at the interface between the G domain and domain V or III in EF-G and EF-Tu, respectively (Fig. 4; Czworkowski et al. 1994; Ævarsson et al. 1994; Johansson and Hughes 1994). Unlike kirromycin, fusidic acid cannot induce EF-G to hydrolyze GTP off the ribosome. This may be due to the fact that fusidic acid binds very poorly in the absence of ribosomes.

The functional cycle of the elongation factors

It cannot be excluded that EF-G, like EF-Tu, goes through a large conformational change in its functional cycle. Since the antibiotics prevent the conformational transitions of the factors, it is possible that some of the resistant mutations at domain interfaces facilitate the transitions from the GTP to the GDP conformations. An alternative explanation is that these mutants have a weaker interaction with the ribosome. However, in such a case, a very significant conformational change is needed to expose these residues for interactions with the ribosome because they are buried between two domains. It is also unlikely that the hydrophobic antibiotics bind at this

highly polar surface and that this particular group of mutations would decrease their binding affinity. Thus, the most probable effect of the mutations at this interface is that they destabilize the GTP conformation or otherwise facilitate the transition to the GDP conformation, after which the factors fall off from the ribosome.

All observations discussed above lead to the conclusion that EF-G may undergo a large conformational change. Since the GDP conformation of EF-G is closely related to the GTP conformation of EF-Tu, an interesting possibility is that EF-G·GTP is similar to EF-Tu·GDP and that the interfaces between the G domain and domains V, and III, respectively, play important roles in controlling these transitions. In this review, we discuss the consequences of the hypothesis that the conformational changes of the elongation factors are opposite in direction and related to the oscillation in ribosome structure between the pretranslocational and posttranslocational state.

As we have mentioned above, kirromycin alone can induce GTPase activity in EF-Tu (Wolf et al. 1974). This illustrates the fact that the factor alone has all of the essential components of the active enzyme. Furthermore, it has been found that an EF-Tu fragment lacking domain III has an increased rate of intrinsic GTP hydrolysis (M. Sprinzl, personal communication). This indicates that domains I and II constitute a complete GTPase and that domain III is regulating the activity to occur only as a response to specific signalling from the ribosome. The spatial relation between domains G and II defines the functional state of the factor. Domain II is a constant part of all ribosomal GTPases (Ævarsson et al. 1994; Ævarsson 1995) and may, therefore, be part of the GTPase activating system (GAP).

The large conformational change of the elongation factors could in principle occur after GTP hydrolysis. If, however, they go through the cycle in opposite directions, it is possible that one of the factors goes through the large conformational change upon binding to the ribosome and the other one after GTP hydrolysis. In such a case, we would propose that EF-G undergoes the large conformational change upon binding to the ribosome, since it is known that translocation occurs upon binding (Inoue-Yokosawa et al. 1974). It would seem natural that the factor changes its conformation in relation to the ribosomal conformation change. According to this description, the large conformational change in EF-G precedes GTP hydrolysis, whereas for EF-Tu, the GTP hydrolysis leads to a large conformational change.

In addition to the large conformational changes in the elongation factors it is obvious that smaller changes occur in the other steps in the functional cycle (Fig. 1).

The interplay between the ribosome and the elongation factors

As described in the previous section, EF-G·GTP promotes ribosomal translocation upon binding. GTP hydrolysis is not needed for translocation. It is less well known when the conversion to the pretranslocational state occurs. It would, however, seem unlikely to occur before the hydrolysis of GTP by EF-Tu. In such case, it would need to be converted back to the posttranslocational state, even for ternary complexes rejected. The differences between pretranslocational and posttranslocational ribosomes are presently not well described on a molec-

Fig. 6. Hypothetical scheme for the functional and structural relationship between the elongation factors and the ribosome. If one disregards the binding of tRNAs to the ribosome, its functional cycle can be described as an oscillation between two conformational states, the pretranslocational (pre) and posttranslocational (post) ribosome. The elongation factors catalyse the conversion between these two states. The free factors on the left-hand side of the figure related to the posttranslocational ribosomes are very similar in structure. Both factors interact with the same area of the ribosome in very similar manners and are induced to hydrolyze GTP by the same mechanism. Thus, the elongation factors follow parallel conformational developments in interacting with the ribosome. Thus, the large conformational change expected to occur for EF-G may be similar to what is seen for EF-Tu. aa, amino acid; conform., conformational.
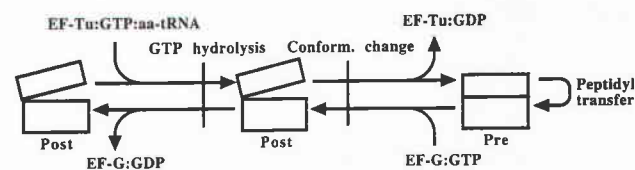

ular level. One interesting observation is that ribosomes, starved for aminoacyl-tRNA and, therefore, presumably in the posttranslocational state, have an open configuration (Öfverstedt et al. 1994).

Models based on the two main conformational states of the ribosome have been discussed earlier (see, e.g., reviews by Spirin 1969, 1988; Möller 1974, 1990; Mesters et al. 1994). Despite the scarcity of information, the new structural results provide the ground for a different model for the interaction between the ribosome and the factors (Fig. 6). According to this model, the ribosome oscillates between two states catalysed in opposite directions by the two elongation factors. The two transitions in ribosome conformation (post to pre and pre to post) are associated with the dissociation of EF-Tu·GDP and the association of EF-G·GTP, respectively.

Several of the translational GTPases (and maybe all of them) bind to overlapping sites on the ribosome (Liljas 1982). EF-G·GDP and EF-Tu·GTP·tRNA have structures that can overlap (Nissen et al. 1995) and are related to the same conformation (posttranslocation) of the ribosome. It seems highly unlikely that the two similar factors and the ribosomal binding site for them has evolved to hydrolyse GTP in two different ways. Thus, the transition states of the GTPase active conformations must be closely similar. In the GTPase-activated sates, the detailed conformations of domains I and II of the elongation factors are highly similar and the ribosomal GTPase activating component interacts with both factors in similar ways. It would then seem entirely possible that EF-G·GTP and EF-Tu·GDP are similar since they are related to the same conformational state (pretranslocation) of the ribosome and have, therefore, to adapt to the same sterical requirements of the binding site between the ribosomal subunits.

One observation that correlates well with this model (Fig. 6) is the accessibility of the ribosomal proteins to trypsin at different conformational states of the ribosome (Gudkov and Bubunenko 1989). Thus, on one hand, L7/L12 is very accessible to trypsin both when EF-Tu·GDP dissociation from the ribosome is inhibited by kirromycin and when EF-G is bound

Fig. 7. The possible mode of interaction for EF-G with the ribosome in the posttranslocational state. This then imitates the way in which the ternary complex of EF-Tu binds to the ribosome before GTP hydrolysis. In this state, the anticodon end of the tRNA is associated with the decoding site and the aminoacyl end of the tRNA is associated with the G domain close to the base of the L7/L12 stalk and far away from the peptidyl transfer site on the 50S subunit.
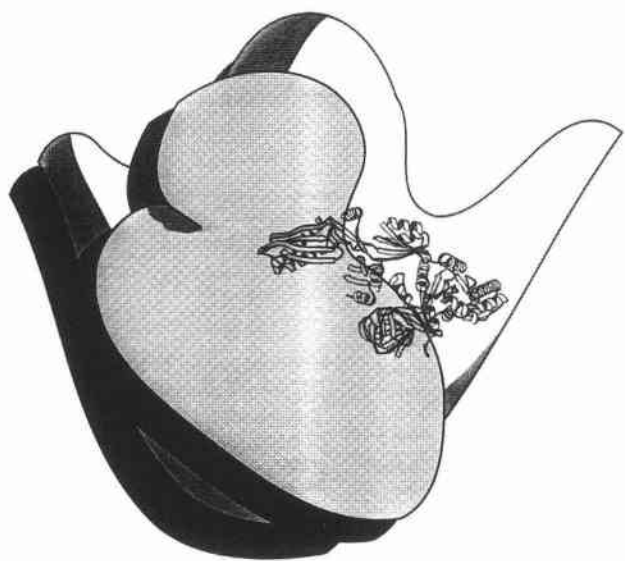
in complex with GMPPNP. These two conformations of the ribosome are found to the right of the GTP hydrolysis state in Fig. 6. On the other hand, L7/L12 becomes inaccessible to trypsin in the two cases of EF-Tu bound in complex with GMPPNP or EF-G·GDP bound in the presence of fusidic acid. According to our model, these two conformational states of the ribosome also correspond to each other and are just to the left of the GTP hydrolysis state.

The previous incompatibility of the factor and tRNA interacting sites on the ribosome may be reinterpreted from the elongated form of the ternary complex of EF-Tu and of EF-G. The anticodon of the tRNA is found in one end and the G domain in the other. This allows the anticodon to interact with the mRNA associated with the 30S subunit, probably in the region between the platform and the main body of the subunit (Fig. 7). At the same time, the G domain interacts with the 50S subunit at the base of the L7/L12 stalk (Stöffler and Stöffler-Meilicke 1986; Oakes et al. 1986; Möller and Massen 1986; Liljas 1982). The approximate distance between these sites agrees with the size of the ternary complex. In this binding mode, the aminoacyl end of the tRNA will be far from the peptidyl transfer site, which is below and on the L1 side of the central protuberance of the 50S subunit. When the tRNA dissociates from EF-Tu, the aminoacyl end will have to undergo a large shift in orientation. This shift in orientation may coincide with the transition to the pretranslocational state of the ribosome.

If EF-G·GTP has a different conformation from EF-G·GDP, it cannot be aligned with the ternary complex bound to the ribosome. It would then seem possible that the G domain binds in the vicinity of the base of the L7/L12 stalk. Translocation then occurs associated with a conformational

change in EF-G from the GTP conformation to the GTPase conformation. In this conformational change, domain IV replaces the peptidyl-tRNA in the A site (Czworkowski et al. 1994).

Acknowledgements

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