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Minireview

Ribosome as a molecular machine

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Abstract General principles of structure and function of the ribosome are surveyed, and the translating ribosome is regarded as a molecular conveying machine. Two coupled conveying processes, the passing of compact tRNA globules and the drawing of linear mRNA chain through intraribosomal channel, are considered driven by discrete acts of translocation during translation. Instead of mechanical transmission mechanisms and power-stroke 'motors', thermal motion and chemically induced changes in affinities of ribosomal binding sites for their ligands (tRNAs, mRNA, elongation factors) are proposed to underlie all the directional movements within the ribosomal complex. The GTP-dependent catalysis of conformational transitions by elongation factors during translation is also discussed. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Ribosomal translocation; Conformational movement; Thermal ratchet mechanism; Locked and unlocked conformation; Elongation factor; GTP-dependent catalysis

1. Introduction

'Machine: device, having a unique purpose, that augments or replaces human or animal effort for the accomplishment of physical tasks... The operation of a machine may involve the transformation of chemical, thermal, electrical, or nuclear energy into mechanical energy, or vice versa, or its function may simply be to modify and transmit forces and motions. All machines have an input, an output, and a transforming or modifying and transmitting device' [1]. The term 'molecular machine' seems to require a somewhat modified and more precise definition. I would propose the definition that molecular machine is a naturally occurring molecular device (macromolecular complex) that involves the transformation of chemical or electrochemical energy into directional molecular motion. According to the definition, this class of machines includes DNA and RNA polymerases, ribosomes, intracellular transport systems of microtubules (tubulin–kinesin and tubulin–dynein) and microfilaments (actin–myosin), eukaryotic flagella and cilia, and myofibrils. All of them use the so-called 'high-energy compounds' – ATP, GTP and other nucleoside triphosphates (NTPs) – as an energy source for directional movements of macromolecules relative to each other. The cases where transmembrane ionic potential may be used as an energy source for movements are transmembrane ATP synthetases and bacterial flagella.

2. General principles of structure and function of the ribosome

Several basic principles of structural organization of the ribosome can be formulated as follows [2]. (1) The ribosome is built of two unequal sub-particles, or the large and the small ribosomal subunits, which are associated with each other in a labile manner (Fig. 1). (2) The core of each ribosomal subunit is formed by a high-polymer ribosomal RNA self-folded into a compact body of specific conformation. (3) Various ribosomal proteins are assembled on the RNA core, each protein recognizing its specific binding site.

The ribosome performs translation, i.e. it sequentially reads out a genetic message from mRNA chain and concomitantly synthesizes a cognate polypeptide chain. Thus, the ribosome can be considered as a three-functional entity: it is, at the same time, (1) a decoding device responsible for the arrangement of amino acids in accordance with the nucleotide sequence of a genetic message (genetic function), (2) a peptidyl transferase catalyzing the reaction of transpeptidation that results in polypeptide elongation (enzymatic function), and (3) a conveying machine moving along mRNA chain and passing tRNA molecules through itself during elongation (function of translocation) [2]. The genetic function and the enzymatic function of the ribosome are distinctly divided between the two ribosomal subunits – the small one and the large one, respectively. The function of movements (translocation) requires the two subunits to be in association.

There is a spacious cavity or hole (entrance–exit channel) between the associated subunits (see Fig. 1) where main functional sites of the ribosome, such as tRNA-binding sites, mRNA-retaining site of the small subunit and peptidyl transferase center (PTC) of the large subunit, are localized. During translation, aminoacyl-tRNA (Aa-tRNA) molecules sequentially enter this intersubunit space from one side (entrance channel), deacylated tRNAs leave the ribosome from the other side (exit channel), and the chain of mRNA is drawn through the hole in the direction from its 5′-end to 3′-end. It is the passing of tRNA together with a paired cognate

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Abbreviations: Aa-tRNA, aminoacyl-tRNA; Pept-tRNA, peptidyl-tRNA; PTC, peptidyl transferase center; EF-Tu, elongation factor Tu; EF-G, elongation factor G; GMPPCP, 5′-guanylyl methylene diphosphonate; GMPPNP, 5′-guanylyl imidodiphosphate; colons (:) in molecular complexes, such as Aa-tRNA:EF-Tu:GTP, EF-Tu:GDP and EF-G:GMPPNP, designate non-covalent multi-center bonding

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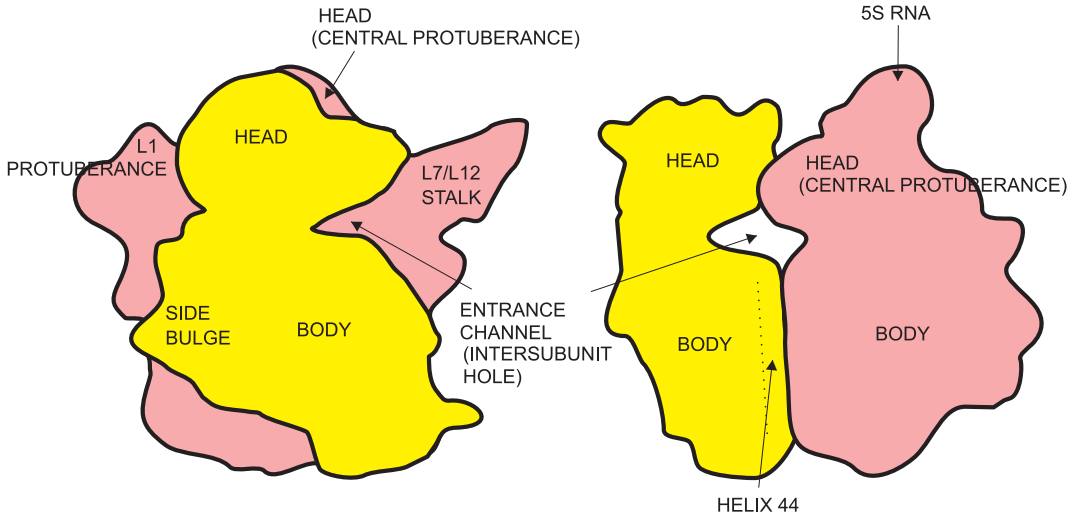


Fig. 1. Contours of the bacterial ribosome and ribosomal subunits in two projections (drawn by outlining the ribbon model of the *Thermus thermophilus* 70S ribosome [29]), with designations of the main lobes. The small (30S) subunit is in yellow, the large (50S) subunit is in pink.

codon (nucleotide triplet) of mRNA through the intersubunit channel that is provided by the translocation mechanism of the ribosome. The sequence of events is schematically presented in Fig. 2. The Aa-tRNA that enters the translating ribosome first binds to the A site and sets the amino acid terminus in *a* site of PTC, while the P site retains peptidyl-tRNA (Pept-tRNA) (A/*a*, P/*p* state). Then, the transpeptidation reaction between Pept-tRNA and Aa-tRNA takes place, so that the peptidyl residue is transferred from its tRNA to the Aa-tRNA, thus resulting in the formation of one-residue-elongated Pept-tRNA in the A site, with the peptide C-terminus shifted to *p* site of PTC (A/*p* state). The basic act of translocation consists in the movement of the tRNA residue of the elongated Pept-tRNA from the A site to the P site and the displacement of the deacylated tRNA from the P site. Due to the complementary coupling between tRNA anticodons and mRNA codons, mRNA is driven by the same movement.

Thus, the translocation includes two coupled conveying processes: passing of tRNA globules through the intersubunit space, or intraribosomal channel, and coordinated drawing of the chain of mRNA along the groove of the small subunit. Thus, the ribosome is a conveying molecular machine. The input of the machine is the chemical energy of Aa-tRNA and GTP. The output is tRNA and mRNA conveyance. The cardinal question arises: What is the transmitting device of the ribosome as a conveying machine? The following questions concern the structural and functional features relevant to this problem: Why is the ribosome built of two loosely associated subunits, and the association required for translocation? Why are decoding and enzymatic functions strictly divided between the subunits? Why are the functional events, first of all translocation, played out at the subunit interface?

3. Movable parts and intersubunit mobility of the ribosome

Lipmann with associates were the first who suggested the possibility of 'an alternate contraction and expansion of the ribosome' [3] or 'a pulsating ribosome contraction' [4] during translation. Continuing this idea, I proposed the model where mutual mobility of the two loosely associated ribosomal subunits was postulated and suggested to be an essential part of the transmitting mechanism for translocation [5,6]. About the same time, a hypothesis on mutual shifts of the ribosomal subunits relative to each other during translation was reported by Bretscher [7].

The first direct physical evidence that the ribosome can alternately change its conformation during translation, specifically at the translocation step, was obtained from the results of small-angle neutron scattering studies of isolated bacterial ribosomes prepared either in pre-translocation or in post-translocation states; it was shown that the post-translocation state ribosomes were slightly less compact than the pre-translocation state particles [8,9]. The results could be interpreted in terms of somewhat drawing the ribosomal subunits apart upon translocation. Further analysis of the pre-translocation and post-translocation state ribosomes with the same technique indicated the mobility of the head of the small subunit relative to its body [9,10]. The possibility of the change in mutual arrangement of the two ribosomal subunits depending on a functional state, specifically their bringing together and drawing apart, was confirmed in the in vivo study using electron-microscopic tomography technique [11].

More recently, the use of cryo-electron microscopy with three-dimensional reconstruction of images of ribosomes and their subunits confirmed the changeability of the small ribosomal subunit, in particular at the head-to-body junction and also at the side bulge (platform) region (see Fig. 1), as well as revealed potential mobilities of intersubunit bridges [12–19]. The analysis of the ribosome in various functional states suggested a small rotational movement of the small subunit with respect to the large subunit around the axis more or less perpendicular to the subunit interface (see below) [18]. Determinations of the atomic structure of ribosomal particles – the large subunit [20,21], the small subunit [22–27], and the complete ribosome [28,29] – using X-ray crystallography provided the most decisive insight into detailed conformation of the ribosome and its switches. As to the large-block mobility, it can be summarized that the small subunit is characterized by motions of all its four structural lobes – the body, the head, the side bulge (platform), and the so-called penultimate stem, or helix 44 (see Fig. 1) – relative to each other. The movement of the head relative to the body is especially noteworthy.

Some of these motions are interdependent. The motions are accompanied (or rather induced) by intradomain structural changes, in particular movements of some RNA helices. As to the large subunit, the most prominent is the mobility of two side protuberances – L1 bulge (protein L1 plus its 23S rRNA-binding site) and L7/L12 stalk (see Fig. 1).

The mobility of the intersubunit bridges has direct relevance to possible mutual movements of the two ribosomal subunits. There are three main areas of the contact between the subunits: the head, the side bulge (platform) and the penultimate stem of the small subunit connected to the head (central protuberance), the L1 ridge and the central height of the body of the large subunit, respectively [17,28]. The penultimate stem (or helix 44) of the small subunit is of special interest. It is a 100 Å RNA double helix that runs along the long axis of the particle from the head to the end of the body and contacts the large subunit about once per helical turn, thus contributing to about half of all the interface contacts between the subunits [28]. At the same time it is a movable element that exhibits relatively big displacements upon subunit association and depending on a functional state of the ribosome. The mobility of this intersubunit element, as well as other bridging elements in the ribosomal interface, indicates to their role in intersubunit movement. On the other hand, tRNA-binding sites of the ribosome directly abut on the central intersubunit bridges, including those of the penultimate stem and the platform of the small subunit [25,29]. Hence, tRNA binding and translocation, on one hand, and the subunit association and intersubunit movement, on the other, seem to be somehow coupled. Evidence for a direct connection between these functions of the ribosome also came from earlier studies with chemical probes [30,31]. Thus, the coupling of tRNA translocation with intersubunit movement, hypothetically proposed many years ago [5–7], is now gathering factual evidence [29].

4. How can movable macromolecular parts be involved in transmitting mechanisms?

Considering molecular machines, several important reservations are to be made. First, physical bodies of molecular size have small masses. Being subjected to intensive Brownian movement and their own thermal motion in a viscous environment, they would not be capable of accumulating and storing mechanical energy. Low inertia of molecular size bodies, random kicks of surrounding molecules and own thermal fluctuations must lead to dissipation of any form of mechanical energy at a picosecond time-scale. Thus, molecular machines can hardly use inertia of forward movement, momentum conservation, fly-wheels, pendulums, suspended weights, etc., for energy storage. Second, molecular bodies, such as proteins, nucleic acids and their complexes, are built from flexible polymers with movable side groups, and their structural elements

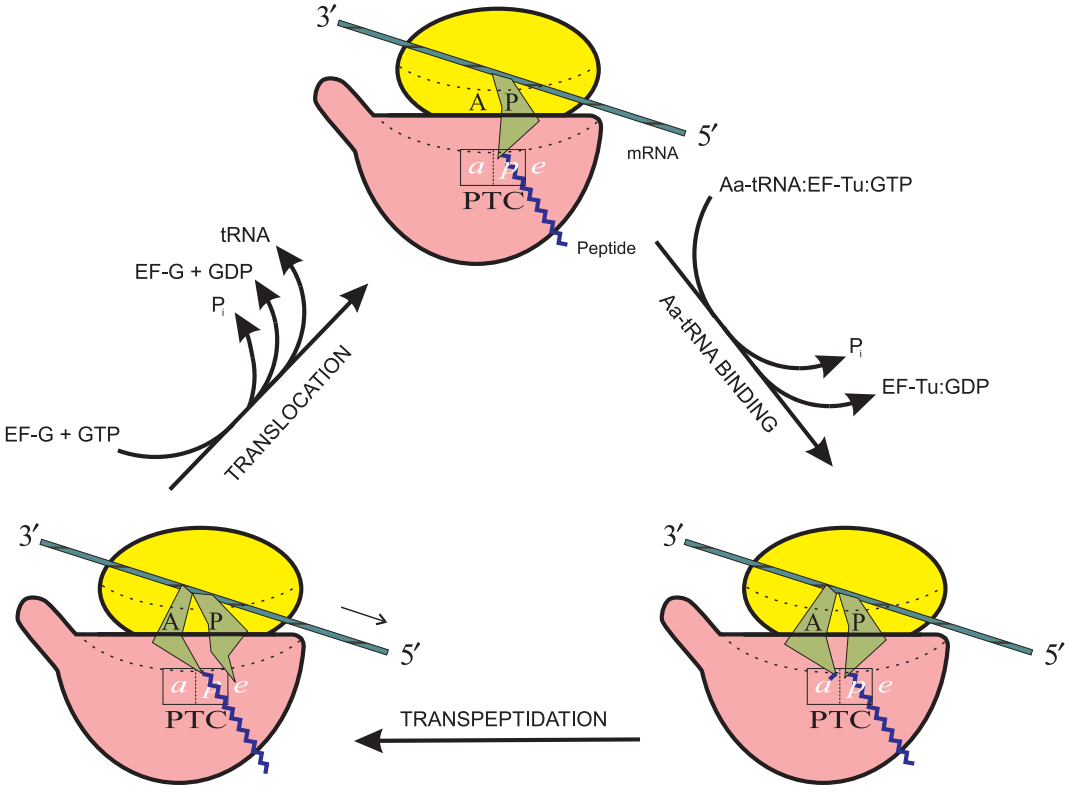


Fig. 2. The sequence of events during elongation cycle, resulting in the read-out of one mRNA codon and the synthesis of one peptide bond. The schematically represented ribosome is viewed from the side of the subunit heads. A and P, tRNA-binding sites on the small subunit; PTC, peptidyl transferase center with its acceptor and donor sites, *a* and *p*, respectively, on the large subunit; the adjacent exit site *e* is to trap the deacylated CCA terminus of tRNA produced by transpeptidation. Dashed lines trace the intersubunit entrance–exit channel. The curved arrows indicate the order of the release of products of the factor-catalyzed steps of Aa-tRNA binding and translocation. The small straight arrow shows the direction of mRNA shift during translocation. All tRNAs and mRNA go from left to right through the intersubunit channel. Conformational changes of the ribosome are not shown because of the uncertainty as to their participation in specific steps of the elongation cycle. The nomenclature of the functional states, such as (A/*a*, P/*p*) and (A/*p*, P/*e*), is adapted from [61].

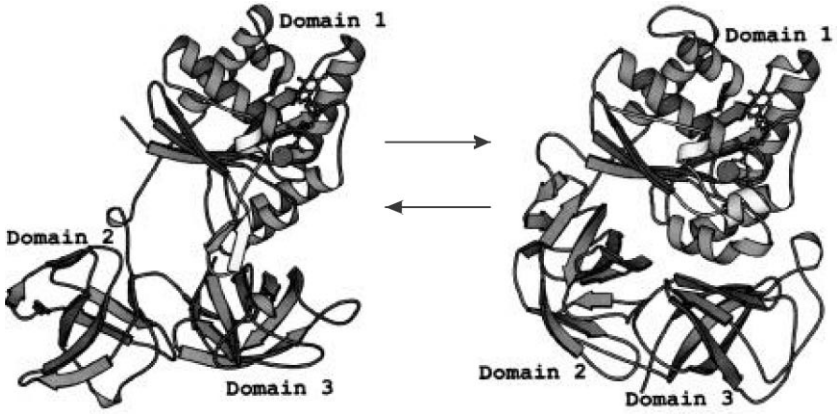


Fig. 3. Relaxed (unlocked) and tight (locked) conformations of elongation factor EF-Tu. Ribbon models are reproduced from [34] with permission.

are subjected to conformational fluctuations, so that they are hardly compatible with mechanical precision. This is the reason why it seems unlikely that the molecular machines can include the so-called 'simple machines' [1] – levers, wedges, wheels and axles, pulleys, and screws – as well as cranks and push rods, in their transmitting devices. Thus, if both mechanical energy storage and precise mechanics are unlikely at the molecular level, the molecular machines should be qualified as *conveying machines without mechanical transmission*.

What can molecular machines effectively use for transmission? The following features of macromolecules and their complexes may constitute a real physical basis for performance of the work at the molecular level: (1) non-covalent interactions with substrates and other ligands; (2) chemical reactions, either catalyzed by a macromolecule itself or induced at an attached enzymic factor; (3) flexibility (plurality of conformations) of single-stranded regions of linear biopolymers (polypeptide and polynucleotide chains); (4) possibility of some compactly folded (globular) structures to exist in a few (usually just two or three) alternative conformations; random oscillation, or equilibrium, between alternative conformational states; (5) fixation of one of the alternative conformations by a ligand attached; (6) Brownian motion and thermal mobility of macromolecular blocks and ligands within a macromolecule or macromolecular complex.

It seems evident that energy-induced periodical *conformational changes* within a macromolecular complex and resultant periodical *changes of the affinities* of binding sites for transported macromolecules underlie the conveying function of the complex as a molecular machine. In order to induce the changes in conformation and/or binding affinity, the energy contribution is required. There are two ways to change the mutual affinity of structural elements of a macromolecular machine (i.e. to induce conformational change), or the affinity for a ligand (binding affinity). One is an exergonic chemical reaction within the macromolecular complex that can result in alterations of contacts between the intramolecular elements and/or at a ligand-binding site. The other way is the attachment of a ligand (substrate or factor) that also can induce a change of the contact interface between macromolecule parts or the contact surface of another binding site. In both ways the changes of affinities are attained at the expense of the energy contribution from an inducing reaction, either a covalent chemical reaction, or a non-covalent reaction of the ligand attachment. In a typical situation, the ligand attachment fixes one of two most probable alternative conformations that are in equilibrium, whereas the chemical reaction destroys the ligand (substrate) and releases the conformation. On the other hand, conformational changes (fixation and release of conformational states) alter affinity properties of ligand-binding sites (and *vice versa*! It should be mentioned that, as affinity changes of ligand-binding sites are coupled with conformational changes, this must work in both directions: altered affinity properties of ligand-binding sites *require and induce* conformational changes, according to the third Newton law).

The paradigm of the molecular transmission mechanism with hierarchy from ligand attachment to large-block mobility is the elongation factor Tu (EF-Tu) [32–34]. The protein consists of two halves movably coupled to each other: domain 1 and the block of joint domains 2 and 3. Domain 1 has a GTP-binding site. In the absence of the ligand (GTP) the protein is in a loose or open conformation where the two halves are more or less apart and movable (Fig. 3, left). When GTP molecule binds to domain 1, its negatively charged γ-phosphate induces local atom rearrangements in its vicinity, resulting in some conformational shifts at the interface region of domain 1 (the region facing domain2/3). In particular, the interface α-helix somewhat rotates and exposes new groups. As a result, new contacts appear at the interface of domain 1, providing a stronger interaction with domain 2, and the two halves of EF-Tu (domain 1 and domain2/3) becomes drawn together (Fig. 3, right). Now, EF-Tu is in a closed or tight (locked) conformation. The closeness of the domains creates a strong binding site for another ligand of EF-Tu – the molecule of Aa-tRNA. On the other hand, the binding of GTP to domain 1 has induced one more local structural rearrangement (β→α transition of the 'effector loop') that contributed to the affinity for the ribosome. When the complex Aa-tRNA:EF-Tu:GTP binds to the ribosome, the hydrolysis of GTP on EF-Tu is induced, and the tight conformation of EF-Tu returns to the loose (unlocked) one; as a result, both the Aa-tRNA-binding site and the ribosome-binding site on EF-Tu are abolished, and EF-Tu is released from Aa-tRNA and the ribosome.

Thus, hierarchy of conformational changes in macromolecules and macromolecular complexes, including the ribosome, can be presented as follows:

Local "switch" – usually at the level of secondary structure:
one of alternative states is fixed by ligand binding or as a result of chemical reaction

↓

Resultant displacement of a movable element, such as helix or loop

↓

Large-block movement, e.g. shift of a compact domain or domains

↓

Overall change of the complex: alteration of compactness, shape, inter-domain space, etc.

As applied to the ribosome and different steps of its working cycle, the following transmitting chain may be postulated:

Binding of an energy-charged ligand
- Aa-tRNA, or Aa-tRNA:EF-Tu:GTP complex, or EF-G:GTP complex

↓

Induced local rRNA "switch"

↓

Displacement of movable elements, such as helix 44 or L7/L12 stalk

↓

Large block movement,
e.g. displacements of the head and platform of the small subunit, or an intersubunit shift

↓

Induced chemical reaction of the bound energy-charged ligand
(Aa-tRNA' + Pept-tRNA → Pept-tRNA' + tRNA,
or GTP + H_2O → GDP + P_i)

↓

Induced local rRNA "switches"

↓

And so forth…

What are the 'motive forces' that push molecular blocks to rearrange, move, and be drawn apart or together? Seemingly, in order to shift anything at the molecular level, nothing is required except Brownian motion and thermal mobility, and the general movement polarity is determined simply by an affinity difference between the previous site and the next site of binding along the conveyance way. This is a version of the so-called 'thermal ratchet' mechanism, as opposed to the mechanisms of macromechanical power-stroke motors (cf. [35,36]). In the thermal ratchet model, ligand binding or chemical reaction does not directly provide the energy for the power stroke – that comes from thermal fluctuations. Instead, the ligand binding and the chemical reaction play the role of a 'Maxwell's Demon': to rectify the random fluctuations to produce a net force in one direction [35].

5. Factor-free translation

In order to get an understanding of the most basic principles of the ribosomal working cycle, the consideration of the so-called factor-free elongation seems to be helpful. It was demonstrated that the ribosome is able to translocate tRNAs and read out a message in the absence of elongation factors and GTP [37–39]. All three main steps of the factor-free elongation cycle – Aa-tRNA binding, transpeptidation and translocation (see Fig. 2) – were shown to duly response to specific inhibitors of these steps of the natural, factor-promoted elongation process (except inhibitors whose targets are elongation factors). These facts, as well as many other observations, give evidence that the factor-free cycle uses the same ribosomal machinery that operates in the factor-promoted elongation, and elongation factors, EF-Tu and EF-G, serve just as GTP-dependent catalysts of the Aa-tRNA binding and translocation steps, respectively [40,41]. As to the translocation step as the bottom of the conveying process during translation, the following deductions can be made. (1) Translocation mechanism is principally inherent to the ribosome (and not introduced by EF-G). (2) Translocation process is thermodynamically spontaneous. (3) EF-G can be considered as a GTP-dependent catalyst of translocation contributing to kinetics, rather than thermodynamics, of the process.

In an attempt to sketch out the scenario of the ribosome work as a conveying molecular machine, two points should be taken into account. First, movable parts of the ribosome are

to be involved in the conveyance process. Unfortunately, we do not know in detail yet which and when. Second, the movements during translation are underlain by induced changes of the affinities of the ribosomal binding sites for the transported macromolecules – tRNAs (including Aa-tRNA, Pept-tRNA and deacylated tRNA) and mRNA. The affinities can be changed in one or the other way discussed in the previous section. In the sequence of events described in Fig. 2, the incoming Aa-tRNA molecule binds to the vacant A site of the ribosome. This non-covalent interaction is an energy contribution that may induce a conformational change in the ribosome, via fixation of one of alternative states. Indeed, the binding of a codon–cognate tRNA results in a movement of the head and body shoulder of the small subunit toward each other, so *they close around the A-site tRNA* [25]. This and other conformational movements in the ribosome lead to the situation when the newly bound Aa-tRNA is found in the proper closeness and orientation with the retained Pept-tRNA. The situation can be considered as a closed, or locked, state favorable for transpeptidation reaction between the substrates. The chemical reaction takes place. The appearance of new product groups in the PTC – deacylated 3′-end of tRNA and newly formed peptide group – instead of the groups of the substrates alters the local chemical situation that induces the next series of conformational shifts in the ribosome. It may be that a somewhat open or loose (unlocked) conformation is established in the region of the PTC. The consequence is that the ends of the two tRNAs move along PTC to the sites of higher affinity, the newly formed ester group of the elongated Pept-tRNA being set in the *p* site, whereas the terminal nucleotides of the deacylated tRNA occupies the *e* site. These events of local rebinding may provoke further conformational changes. Now the ribosome is prepared for translocation of the tRNA residues. It is likely that the decrease of the affinity of the product tRNA residue for the A site and the deacylated tRNA for the P site of the ribosome as a result of transpeptidation reaction facilitates dissociation of the tRNA residues from their sites and the reassociation of the tRNA residue of the elongated Pept-tRNA with the higher affinity P site. At the same time, a conformational change may be required that leads to an unlocked state favorable for intraribosomal movements of tRNAs. Thus, the translocation is a concerted movement of the complex of the two adjacent tRNA residues and mRNA along the intersubunit space, so that the A-site residue shifts to the P site together with its codon of mRNA, while the P-site tRNA residue is displaced and released from its connection with mRNA.

6. What are the GTP-dependent elongation factors required for?

As well known, the transpeptidation between Pept-tRNA and Aa-tRNA is an exergonic reaction [2]. Hence, the step of transpeptidation in the elongation cycle is thermodynamically spontaneous. It has been experimentally shown that this reaction can be the only source of chemical energy for the elongation cycle (factor-free GTP-independent elongation) [36–39]. The codon-dependent binding of Aa-tRNA to the Pept-tRNA-carrying ribosome (to the A site) can proceed without EF-Tu and GTP ('non-enzymatic' binding) [42] and thus is also principally spontaneous. When the pre-translocation step ribosomes are prepared and used in experiments, the translocation can also be demonstrated to proceed spontaneously, without EF-G and GTP ('non-enzymatic' translocation) [36,37]; the fact that the affinity of an N-blocked amino acid residue-bonded tRNA to the P site of the ribosome is significantly higher than to the A site [43,44] seems to underlie the spontaneity of translocation. At the same time, participation of the elongation factors, EF-Tu and EF-G, with GTP in the codon-dependent Aa-tRNA binding and translocation steps, respectively, does contribute to these processes and to the whole elongation cycle: first of all, it makes them much faster. Hence, in view of thermodynamic spontaneity (downhill character) of the steps of the elongation cycle without elongation factors and GTP, the elongation factors with GTP should be regarded as catalysts of the corresponding steps of the cycle, Aa-tRNA binding and translocation [40,41]. In other words, EF-Tu and EF-G are enzymes catalyzing the non-covalent steps of the elongation cycle.

The peculiarity of the elongation factors as enzymes is that their catalytic (enzymatic) action is coupled with the cleavage (hydrolysis) of a 'high-energy compound', GTP. At that, both EF-Tu and EF-G were shown to be true hydrolytic GTPases cleaving GTP into GDP and orthophosphate by means of water, without intermediate chemical transfer of groups to substrates of the synthesis, to themselves or to ribosomal sites [45,46]. Hence, the energy role of NTP molecules in these cases is not an activation of a chemical group.

The requirement for the hydrolysis of a NTP in the process of the catalysis of a non-covalent act can be deducted from the following consideration. So far as enzymatic catalysis implies a decrease of activation barriers of a reaction owing to the affinity of an enzyme for the transition state intermediate, the completion of the reaction and the vacation of the enzyme must be paid from the free energy change of the chemical reaction catalyzed. In other words, the exergonic chemical reaction is necessary in order to compensate the free energy gain, which has been attained during the formation of the enzyme–substrate complex. In the case of elongation factors, the catalyzed step is not a chemical reaction. Therefore, it is likely that if an elongation factor with GTP as a ligand also decreases the kinetic barrier of a certain step due to the affinity for an intermediate state (conformational transition state) of the tRNA–ribosome complex, the completion of the step and the desorption of the factor will require a significant energy compensation at the expense of an exergonic chemical reaction. It is the hydrolysis of GTP that can be such a reaction. Thus, catalysis in the given case is GTP-dependent because it is not a chemical reaction but a conformational change that is subjected to the catalysis. The prediction has been made that enzymatic catalyses of conformational changes of macromolecules and their complexes must be accompanied by NTP hydrolysis in other cases as well [47] (see also [48]).

From the above consideration, the conformational transition state during catalysis of Aa-tRNA binding or translocation is established in the ribosome when it is in a complex with a GTP-holding elongation factor, EF-Tu or EF-G, respectively, *prior to the hydrolysis of GTP*. This situation seems to be realized in the case of the ribosome associated with an elongation factor in the presence of an uncleavable GTP analog. Thus, the Pept-tRNA-retaining ribosome with bound ternary complex Aa-tRNA:EF-Tu:GMPPCP (5′-guanylyl methylene diphosphonate) can be assumed to be in the transition

state of the EF-Tu-catalyzed process of the binding of Aa-tRNA to the A site. Analogously, the ribosome with bound EF-G:GMPPNP (5′-guanylyl imidodiphosphate) complex is a model of the conformational transition state of the ribosome during translocation. Under normal conditions, when GTP is a ligand that has induced the affinity of an elongation factor for the ribosome, the hydrolysis of GTP leads to the removal of the elongation factor and thus releases the transition state; the ribosome falls down to a 'product state' of the corresponding step of the elongation cycle – either to the pre-transpeptidation state in the case of the EF-Tu-catalyzed step, or post-translocation state in the case of the EF-G-promoted act. All the free energy of GTP hydrolysis reaction dissipates into heat (no useful work is done).

Indeed, it has been proved by direct experiments that GTP hydrolysis by itself does not force translocation or a stage of it, but is required mostly for the release of EF-G: when EF-G with uncleavable GTP analog, GMPPCP, was attached to the pre-translocation state ribosome and then detached from it by a washing-off procedure, normal post-translocation state was attained [49–51]. Quantitative measurements of translocation via EF-G attachment–detachment without GTP hydrolysis demonstrated that high rates of translocation, just two times slower than those in the case of EF-G:GTP-promoted translocation, can be achieved at physiological temperatures and ionic conditions [52].

The question, which parts of the ribosome are involved and move during the elongation factor-driven conformational catalysis, appears principal for understanding its mechanism. On the background of the scarcity of authentic information, the side protuberance, called L7/L12 stalk, of the large ribosomal subunit is shown to be a movable module whose participation in EF-Tu- and EF-G-induced events, and especially in the EF-G-catalyzed translocation, seems real. For the first time the mobility of the globular protein L7/L12 block relative to the rest of the ribosome was demonstrated by Gudkov et al. using nuclear magnetic resonance spectroscopy [53] (later similar experiments and conclusions were made also by another group [54]). On the other hand, the binding sites of both elongation factors on the ribosome were directly visualized by immuno-electron microscopy in the immediate vicinity of the L7/L12 stalk [55,56]. It is remarkable that the attachment of EF-G with non-hydrolyzable GTP analog to the ribosome results in the immobilization of the L7/L12 stalk [57] and the sensitization of the L7/L12 protein to proteases, such as trypsin [58]. The hydrolytic cleavage of GTP on the ribosome-bound EF-G (in the presence of fusidic acid) leads to restoration of the resistance of the L7/L12 protein against proteases [59]. All this suggests conformational changes of the L7/L12 stalk, first upon the attachment of EF-G with GTP, i.e. presumably upon acquiring the transition state, and then as a result of GTP cleavage.

It is interesting that the changes in the L7/L12 stalk upon EF-G:GTP (EF-G:GMPPCP or EF-G:GMPPNP) binding and then during GTP cleavage seem to be accompanied by other conformational alterations in both ribosomal subunits. For example, when EF-G:GMPPNP is bound to the ribosome, the ribosomal proteins S15 and S18, normally protected against protease attack on the small subunit, become sensitive to trypsin [59]. Using the hot tritium bombardment technique for probing the surface of ribosomal particles [60], significant changes in the exposure of some ribosomal proteins upon binding of EF-G:GMPPNP were revealed; whereas the observed hiding of proteins L3, L6, L7/L12, L11 and L14 could be explained by directly shielding them when EF-G is bound at the base of the L7/L12 stalk, the reduced exposure of protein L1 at the opposite side of the large subunit and protein L19 at its bottom [21] suggested long-range conformational shifts within the large subunit (Agafonov and Zakeyeva, unpublished). A slight overall increase of the ribosome compactness upon EF-G binding (Agafonov and Kolb, unpublished) may indicate a tighter embracing of the ribosomal subunits – either just bending the side protuberances on the ribosome body, or a closer subunit association, or both.

Comparing cryo-electron microscopy maps of the ribosomes with bound EF-G:GMPPCP complex ('GTP state') and with EF-G:GDP in the presence of fusidic acid ('GDP state', after GTP hydrolysis, but before EF-G release), a number of conformational differences were also detected (see [18,19]). Among those was a bifurcation of the L7/L12 stalk of the large subunit after EF-G:GMPPCP binding and its reversal to a single, elongated form after GTP hydrolysis [13,14,18]. As to other movements in response to the binding of EF-G:GMPPCP, an intersubunit shift was noticed that was interpreted as a small (6°) counter-clockwise rotational movement of the small subunit relative to the large one; the GTP cleavage and EF-G release seemed to induce the back rotation [18]. Other effects of the EF-G binding were a widening of the cleft between the head and the body of the small subunit (entrance mRNA channel) and, perhaps, a somewhat narrowing of the space between the head and the side bulge or platform (exit mRNA channel) [18,19].

In general, the sense of all ribosomal movements during EF-Tu-catalyzed Aa-tRNA binding and EF-G-catalyzed translocation should be two-fold: first, the rupture, at least partial, of contacts of the ribosome-binding sites with their tRNAs, and second, the provision of a freedom and space for intraribosomal displacements of the tRNAs and mRNA. The details of these processes are vague. The main questions to be answered in the near future are the following. Which movements are induced by elongation factor binding and which are the result of GTP hydrolysis and/or elongation factor release? What is the conformational transition state of the ribosome during the elongation factor-promoted catalysis? How are the movements coupled with the attainment of the transition state and its release? What is hierarchy and kinematics of each series of the movements at the atomic level?

7. General scenario: The concept of locking–unlocking

A large molecular ligand, such as tRNA, binds to a macromolecular complex, be it the ribosome, by several points (multi-center binding). This implied very high kinetic barriers during both association and dissociation if simultaneous rupture-formation of the contacts were premised. Hence, intermediate steps are required in order to avoid the kinetic block. Molecular flexibility and intramolecular movements provide necessary conditions for driving the processes of association–dissociation (attachment–detachment) via intermediate states with partially disrupted–partially formed binding contacts. The binding of tRNA to the ribosome, its translocation, and its release must go through intermediate states involving intraribosomal movements in any case (including the case of fac-

tor-free elongation). The elongation factors with GTP seem to be destined to produce conformational transition states that allow the processes to avoid the slowest sub-steps (the highest sub-barriers).

A relevant problem to be solved is that both the binding of a large ligand (substrate) and its dissociation or the dissociation of a large product require space or freedom for its own movement relative to a macromolecule. At the same time, an interaction between two ligands or a chemical reaction between two bound substrates needs their tight fixation to provide a close atomic contact and a finely tuned mutual orientation. These two requirements are quite opposite, and the problem can hardly be solved without large-block mobility of the macromolecule. The concept is that any macromolecular complex operating with large ligands or substrates must oscillate between locked (closed) and unlocked (open) states. The complex is unlocked in order to accept a ligand or substrate, or to allow the movement of a ligand inside the complex, or to let a ligand or a product out. The complex is locked in order to immobilize and fix a ligand or substrate for performing a chemical reaction, as well as for a precise recognition. It is this idea that was put at the basis of the early hypothesis of locking–unlocking ribosomes [5,6]. Now it is becoming clear that the ribosome can be in more than two alternative conformational states, so that more than one locked and/or unlocked states may exist.

From the standpoint of the locking–unlocking concept, the general hypothetical scenario of the working cycle of the ribosome may be presented as follows.

1. Translating ribosome with Pept-tRNA in P site and vacant A site is in an *unlocked state* (unlocked state I), or fluctuates between unlocked and locked states. It allows the entering of an Aa-tRNA or a ternary complex Aa-tRNA:EF-Tu:GTP.
2. Binding of a cognate Aa-tRNA shifts the equilibrium and fixes a tighter (locked) conformation of the ribosome. When Aa-tRNA:EF-Tu:GTP complex binds to the ribosome, it induces (fixes) the *pre-locked transition state*.
3. GTP hydrolysis leads to the release of EF-Tu:GDP from the ribosome and, as a consequence, to the setting of the *locked state* where amino acid end of the Aa-tRNA is fixed in the *a* site of PTC, side by side with the ester group of the Pept-tRNA in the *p* site of PTC.
4. The close proximity of aminoacyl group of Aa-tRNA to the ester group of Pept-tRNA in the locked state ribosome allows the reaction between them: transpeptidation takes place.
5. The resulting change in chemical situation at PTC (appearance of $–CCA_{OH}$ and –O-CO-C^{α}-NH-CO instead of –O-CO- C^{α}–) results in local shifts of the products, to *e* and *p* sites, respectively. Now the ribosome is in the metastable 'hybrid state' (A/*p*, P/*e* state) [61] and is ready for translocation (pre-translocation state).
6. Binding of EF-G with GTP to the pre-translocation state ribosome induces the *pre-unlocked transition state*, and precise contacts of the two tRNA residues with their A and P sites become disrupted.
7. GTP hydrolysis results in the reduction of the affinity of EF-G for the ribosome, the release of EF-G with GDP and the setting of an *unlocked state* of the ribosome (unlocked state II). The unlocked state allows intraribosomal movements of the tRNA residues, and the tRNA residue of the elongated Pept-tRNA displaces the deacylated tRNA and becomes fixed in the high affinity P site. The translocation has been completed. The deacylated tRNA may dissociate from the post-translocation state ribosome (from its *e* site) either spontaneously, or under the influence of the next step of Aa-tRNA binding.

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