

The ribosome – a macromolecular machine par excellence

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The ribosome is the site in the cell where proteins are synthesized. Cryo-electron microscopy and X-ray crystallography have revealed the ribosome as a particle made of two subunits, each formed as an intricate mesh of RNAs and many proteins. Ligand-binding experiments followed by cryo-electron microscopy have helped to determine some of the key stages of interaction between the ribosome and the main ligand molecules.

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Seminal discoveries, made using biophysical methods (especially structural imaging), have gradually replaced the concept of the cell as a ‘compartmented soup’ in which interactions between molecules take place within compartments more or less by chance and in a diffusion-controlled way, with the concept of the cell as a highly organized compendium of macromolecular machines. The metaphor of the macromolecular machine gained wide acceptance after Bruce Alberts’ influential article appeared in a special issue of *Cell* [1] as an introduction to a series of reviews focusing on various localized cell functions. The metaphor is used to express dynamic interactions among two or more parts, involving a high degree of coordination and precision.

The ribosome is a molecular machine par excellence. Roger Garrett [2], in an article accompanying and critically acclaiming the publication of two low-resolution X-ray maps of the ribosome last year (30S subunit [3]; 50S subunit [4]), writes ‘the ribosome, together with its accessories, is probably the most sophisticated machine ever made’. To appreciate this statement fully, one has to realize that the ribosome Garrett is talking about is from the most primitive organisms — bacteria. Although the near universality of the genetic code implies that the basic mechanism of protein synthesis is the same throughout all life forms, eukaryotic protein synthesis necessarily involves a higher degree of regulation than prokaryotic protein synthesis, entailing more interacting molecules and a greater complexity of the ribosome itself.

Protein synthesis is among the most serious businesses of life — it has to be done fast and in a highly accurate fashion. First, the requirement for speed derives from the necessity for organisms to respond in a timely fashion to changes in their environment. The rate at which proteins are made by the ribosome is set by their size, and by the time that it takes for the elementary step — elongation of the polypeptide chain by one amino acid — which is of the order of 60 milliseconds. Thus, with a typical length of 250 residues, a protein takes 15 seconds to make. Second, the requirement for accuracy derives from the fact that the accurate folding of a protein, which is essential for its proper function, and the integrity of functional centers that rely on the proper placement of particular amino acid residues, may be jeopardized by even a single point mutation. A well-known example is the catastrophic effect of a single mutation in the gene encoding hemoglobin; the mutation leads to the polymerization of hemoglobin, which is the molecular basis of sickle-cell anemia.

The astounding accuracy of the protein-synthetic machinery — an error rate of one in 1000–10,000 amino acids—comes at a price: the high complexity of its design, and the large number of interacting components comprising it. For this reason, ribosomes make up much of the cell's mass, and much of the cell's metabolism is devoted to making ribosomal proteins and RNAs. As evolution generally ensures that resources are not needlessly squandered, we must conclude that this high accuracy of making proteins and faithfulness to the genetic code are simply required to sustain life over many generations.

Principle of translation

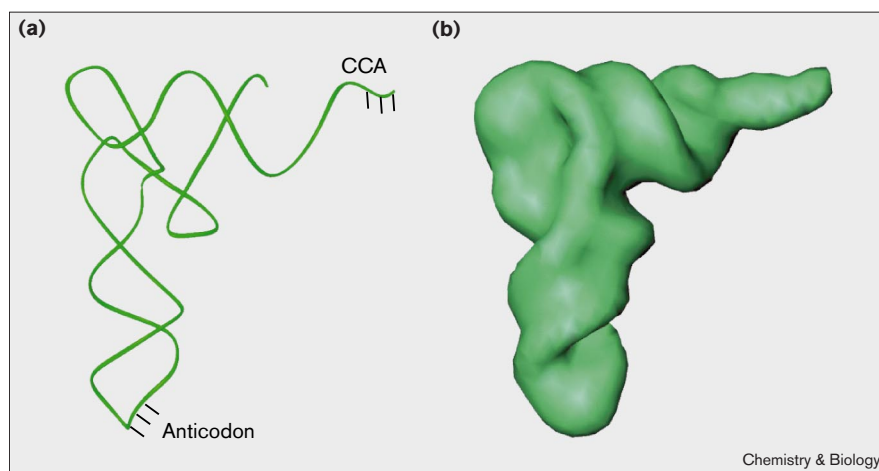
To understand the principle of ribosomal function requires examining the molecule that is at its very core, which enables the translation of the genetic code into a sequence of amino acids: the transfer RNA (tRNA; Figure 1). tRNA is a large, highly stable RNA molecule that is L-shaped, with the short arm ending in a single strand. Three nucleotides at the loop end of the long arm interact with, and recognize, the complementary three-nucleotide codon of the messenger RNA that carries the genetic information, and this loop is therefore called the 'anticodon loop'. On its other, single-stranded end, terminating in nucleotides with the bases CCA, the tRNA carries the cognate amino acid (i.e., the amino acid that corresponds to its anticodon, and thereby to the codon that the tRNA's anticodon is designed to recognize).

The principle of translation is that, through the mediation of the ribosome, the sequential, ordered arrangement of tRNAs along the mRNA strand carrying the genetic message induces the correct order for the corresponding amino acids to form the polypeptide. For this to happen, no more than two successive tRNAs are required to reside on the ribosome at any time. They must be positioned such that their two ends are both in close

proximity: the anticodon loops, which must make contact with two successive codons of the message, and the CCA ends, which must be close enough to allow transfer of the peptide bond, at the so-called peptidyltransferase center.

The large (~80 Å) separation of the anticodon- and amino-acid-carrying ends of tRNA has its correspondence in a sharp division of labor between the two ribosomal subunits. One, the small (30S in the case of bacteria) subunit, is responsible for facilitating correct mRNA–tRNA interaction, for maintaining the reading frame, and for checking, monitoring and confirming codon–anticodon recognition. The other, large (50S) subunit catalyzes peptide-bond formation between the polypeptide chain already made and the newly supplied amino acid, organizes the advance of the whole subcomplex formed by mRNA and the tRNAs by the length of one codon ('translocation') with the help of a special protein factor, elongation factor G (EF-G), and assures the pre-folding and passage of the nascent chain through a special tunnel. From this description of the different roles of the two subunits it is immediately clear that they need to signal to each other the current status of their respective functional centers. (We will come back to the question of communication between the subunits at the end of this article.) For example, peptide-bond formation with the newly arriving amino acid (at the peptidyltransferase center, located on the large subunit) must be prohibited before it is known (at the codon–anticodon recognition site, or decoding site of the small subunit) that the newcomer tRNA is correctly matched to the current codon. During this so-called proof-reading phase, the amino acid of the newcomer tRNA is protected from a potentially disastrous, unwanted incorporation into the polypeptide chain by another elongation factor, EF-Tu, and it is only after receiving a go-ahead signal from the 30S subunit, following an as yet unknown mechanism, that EF-Tu will separate from its protégée.

Figure 1



The transfer-RNA molecule. (a) Ribbon diagram, showing the folding of the RNA strand. The positions of the three nucleotides forming the anticodon and of the CCA nucleotides at the end of the acceptor stem are indicated. (b) Surface representation of the molecule.

As in the case of EF-G, the action of EF-Tu requires energy released by GTP hydrolysis.

Much of this information has been known for years (see [5]), but the way the various components of the protein synthetic machinery interact in three dimensions remained a mystery until the advent of cryo-electron microscopy (cryo-EM) and reconstruction of macromolecules in the form of single particles (see [6]). The picture that emerged as the result of these studies, beginning with articles that appeared in 1995 [7,8], will be sketched out in the following section. Yet another step forward, toward a full understanding of translation, is presently being made using X-ray crystallography to examine ribosomes from thermophilic and halophilic bacteria [3,4,9,10].

Cryo-EM of single particles has the advantage that it is fast, requiring no crystallization, and that it can show molecular interactions unrestrained by crystal packing. Its disadvantage, compared with X-ray crystallography, is that atomic resolution is difficult to achieve. As in other areas of study, for instance the binding of Fab fragments to viruses (e.g. [11]), the potential that lies in combining the two techniques is now increasingly recognized [12].

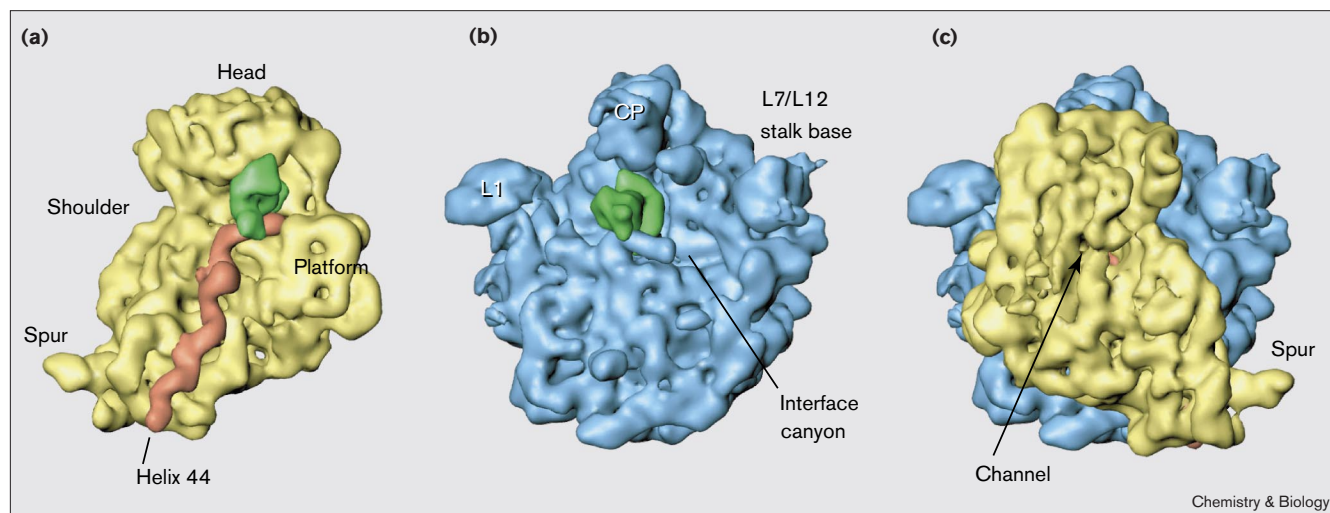
Make-up and topology

The bacterial ribosome is formed by an intricate mesh of three RNAs (16S for the small subunit; 5S and 23S for the large subunit) and ~50 different proteins (for *Escherichia coli*, 21 in the small subunit and 33 in the large subunit), with the RNA contributing the lion's share (64%) of the total volume. Cryo-EM of the *E. coli* ribosome, now at 11.5 Å resolution [13], shows the small

subunit (Figure 2a) to have a roughly trapezoidal shape when viewed frontally, from the interface side, and to be of uniformly narrow width when viewed sideways. 'Head' and 'body' are readily distinguished. From the body, two major lobes sprout upward, identified as the 'platform' and the 'shoulder'. The cleft between the platform and the head is known to be the site of the decoding center (i.e. the place where mRNA, A- and P-site tRNAs and a region of 16S RNA interact in a highly coordinated way). A channel formed between the head and shoulder, which leads into the cleft, is the likely conduit for the incoming mRNA [7,14,15]. At the bottom of the subunit, a thin (~20 Å) element protrudes that is formed by RNA, a landmark of unknown function that has been termed the 'spur'. Running vertically, along the entire length of the subunit body on the interface side, is a helix of the 16S RNA, identified as helix 44 [3]. Judging from its position, connected to the decoding center and in contact with several bridges originating from the large subunit, this helix may have a key role in the decoding and translocation steps, and in coordinating the actions of the two subunits.

The large subunit (Figure 2b) has an approximately hemispherical shape, facing the small subunit with its flat side. The characteristic crown view, which is seen when the subunit lies on its flat side and was already noted in early electron microscopic studies [16], is due to three protuberances: the L7/L12 stalk along with its base, the central protuberance and the L1 stalk. The L7/L12 stalk, which is formed by one of the L7/L12 protein dimers, is often only weakly expressed or entirely absent in the cryo-EM map because of its high

Figure 2



Cryo-EM reconstruction of *E. coli* ribosome at 11.5 Å resolution. (a) Small subunit; (b) large subunit; (c) assembled ribosome. Reproduced with permission from [13].

flexibility, but its bilobed base (Figure 2b) is a reliable landmark of distinct shape. The central protuberance is formed, in major part, by the 5S RNA. In contrast to the L7/L12 stalk, the L1 stalk is always well defined, resembling a mushroom with a globular hat that is formed by the L1 protein, and a stem formed by double-stranded 23S RNA. Closer analysis of the flat side of the large subunit reveals a canyon (the interface canyon) that runs across the width of the subunit and is bordered by a ridge. At its bottom, halfway across, the canyon opens into a tunnel that penetrates the subunit and exits on its solvent-side back [7,17]. Such a tunnel was already observed in earlier, low-resolution reconstructions of prokaryotic [18] and eukaryotic [19] ribosomes from two-dimensional ribosome arrays. This tunnel probably serves as the conduit for the exiting polypeptide chain. Three observations argue in favor of this hypothesis. First, the initiator P-site tRNA was seen to point with its CCA end into the tunnel mouth [20], just as would be expected if the attached polypeptide chain were going into the tunnel. Second, the Sec61 channel, known to conduct the nascent polypeptide chain through the membrane of the endoplasmic reticulum in eukaryotes, attaches itself to the ribosome such that its central pore aligns perfectly with the tunnel exit [21]. Third, a reconstruction of *E. coli* polysomes revealed extra mass along a segment of the tunnel [14], which could represent the average of the density resulting from polypeptides, perhaps partially folded, at different stages of synthesis.

To see how the ribosome (Figure 2c) is assembled from its two subunits, one has to imagine the small subunit (Figure 2a) being flipped over and placed onto the large subunit (Figures 2b,3). To show the way the tRNA interacts with the ribosome, it is best to revert to an earlier conceptualization that was based on a 25 Å resolution reconstruction (Figure 4) and has since been experimentally confirmed [22,23]. In the assembled ribosome,

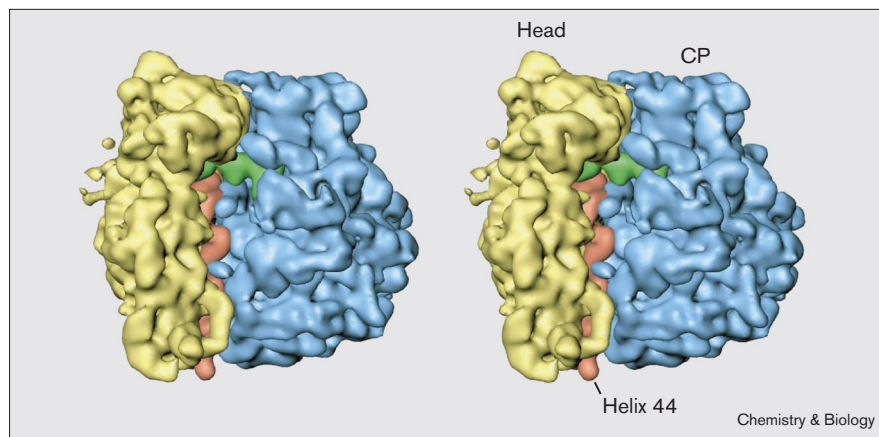
the two subunits face each other in such a way that a space of complex shape is created, the intersubunit space (Figure 4a,b). Its precise topology is maintained by many bridges (see below) that criss-cross a major part of the intersubunit space but leave out a corridor in the upper half, along which the binding of tRNA takes place. The cross-section of the corridor has the approximate shape of tRNA, like a lock fitted to a key [24]. Basically, as the tRNA is incorporated into the A (or aminoacyl) site, and progresses to the P (peptidyl) site and eventually to the E (exit) site, it moves along this corridor, virtually riding on the ridge of the interface canyon. Its movement goes from the L7/L12 stalk side (i.e., from the right in Figure 4c,d) toward the L1 stalk side of the ribosome (the left in Figure 4c,d). Simultaneously, the mRNA moves in the same direction in a semi-circle, threading into the intersubunit space through the entrance channel and exiting it through a gap between the head and the platform.

On the L7/L12 side, the EF-Tu•GTP•tRNA ternary complex and EF-G attach alternately, one delivering a new tRNA, the other inducing translocation of the tRNA from the A site to the P site, respectively. The interaction of these factors with the ribosome involves multiple binding sites on both subunits, and requires energy from GTP hydrolysis.

The elongation cycle

Elongation cycle is the term used for the cyclic process, involving interactions of the ribosome with tRNA and elongation factors, during the course of which a new amino acid is added to the polypeptide chain and the mRNA is advanced by one codon. Although the molecular details of these interactions are as yet unknown, the sequence of main events has been inferred from biochemical experiments, chemical protection studies, and a variety of probing techniques that yield distances between

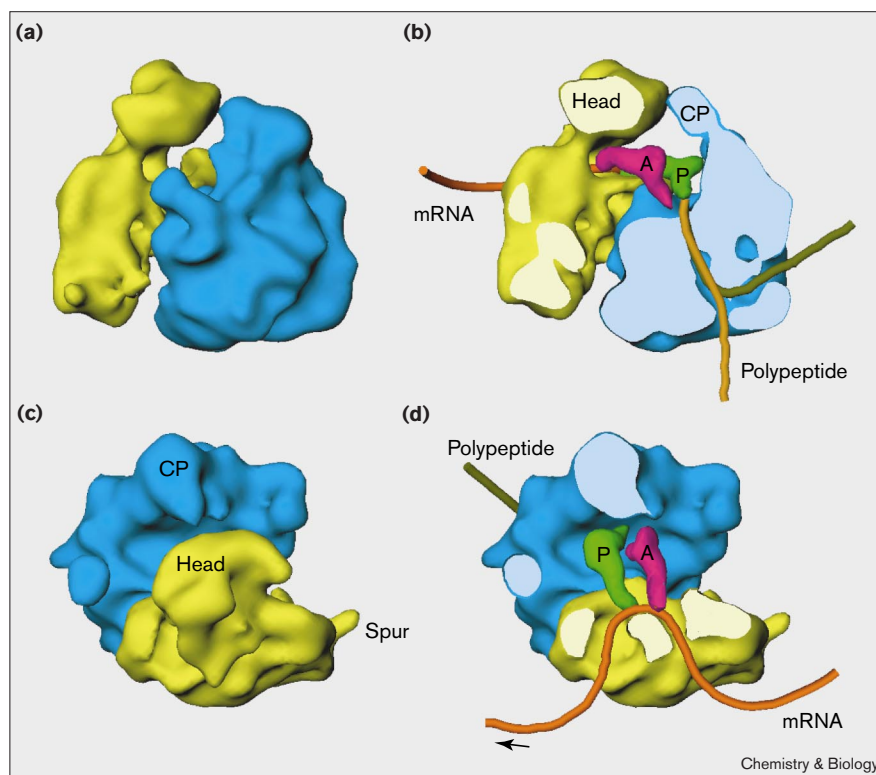
Figure 3



Stereo view of the cryo-EM reconstruction seen from the L7/L12 side, showing a view sideways into the intersubunit space. Reproduced with permission from [13].

Figure 4

Earlier cryo-EM reconstruction showing the *E. coli* ribosome at 25 Å resolution, along with suggested placements of tRNAs and schematic paths of mRNA and polypeptide chain. The large subunit is blue, the small subunit is yellow. (a) Side view from the L7/L12 side; (b) side view of ribosome cut by a plane intersecting the tunnel through the large subunit. The observed bifurcation of the tunnel suggested the existence of two exit paths of the polypeptide, which are both indicated. (c) Top view; (d) top view of ribosome cut by a plane intersecting the channel through the small-subunit neck. Reproduced from [42] with permission.



individual residues of protein and RNA components of the ribosome and its ligands [25]. Moreover, starting with the first localizations of tRNA [22,23] the use of cryo-EM in the past five years has yielded ‘snapshots’ of the ribosome and its bound ligands at various stages of the elongation cycle, resulting in the knowledge of the three-dimensional positions of these ligands (see [26]; Figure 5). By extrapolating between these positions, under the constraints that are imposed by the ribosome topology, a first attempt has been made to sketch out the dynamic sequence by animation [27].

Following the sequence sketched out in the cartoon (Figure 5), we start with the ribosome occupied by tRNAs in the P and E positions (iv), ready for incorporation of the next tRNA in the A site. This, incidentally, is the state that corresponds to the initiation of the translation, except that, in that case, the E site is not yet occupied: the ribosome carries a single initiator tRNA in the P site.

Next, in the step from panels (iv) to (i), ternary complexes with tRNAs cognate to all possible codons have a chance to interact with the new codon at the A site on the small subunit. When the correct ternary complex makes contact at this site, a sequence of events is triggered that results in GTP hydrolysis and accompanying conformational changes in the ribosome. The tRNA

occupying the E site moves to a transient position termed E2, where it clings to the protein L1.

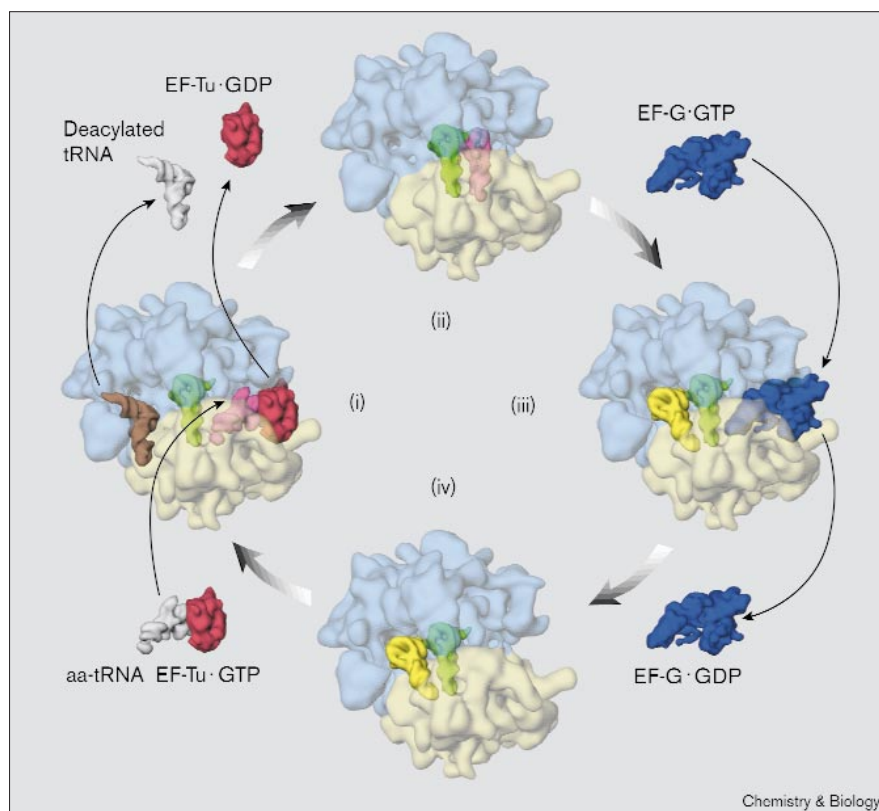
In the next step, from panels (i) to (ii), EF-Tu is removed, and the newly arrived tRNA is fully incorporated into the A site of the 50S subunit. This is followed immediately by the transfer of the peptidyl moiety from the P-site tRNA to the amino acid of the A-site tRNA. The stage of the elongation cycle depicted in panel (ii) shows the ribosome in its so-called pre-translocational state, in which both A and P sites are occupied by tRNAs. Translocation to the post-translocational state, shown in panel (iv), requires the interaction of EF-G with the ribosome (shown in the transition from (ii) to (iii)), GTP hydrolysis, and subsequent release of EF-G (transition from (iii) to (iv)), which brings the ribosome back into the post-translocational state.

Translocation

Within the elongation cycle, translocation, which is catalyzed by the interaction between EF-G and the ribosome, is one of the processes of pivotal importance. Although translocation is known to occur spontaneously even in the absence of EF-G, the rate is so low that it has no practical importance for the organism.

The main difficulty in studying EF-G interaction with the ribosome is that the binding is quite short-lived — EF-G

Figure 5



Four key stages of the elongation cycle, showing the positions of tRNAs and elongation factors EF-G and EF-Tu as mapped by cryo-EM of ribosome–ligand binding complexes. The ribosome is shown from the top, sliced open to reveal the intersubunit space. For an explanation of the stages depicted in panels (i–iv), see the text. Reproduced with permission from [35].

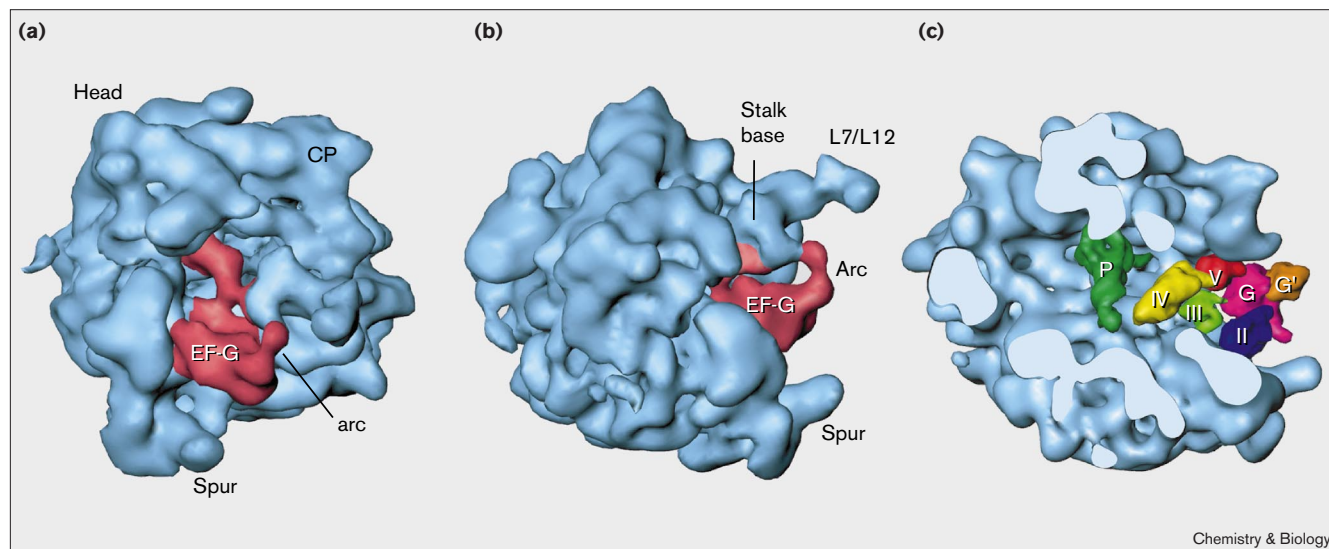
is released soon after GTP hydrolysis. An understanding of the translocation process therefore requires the study of the kinetics of this process (see [28]), but such analysis must be complemented by structural studies toward the ultimate goal of achieving an understanding of the constitutive molecular interactions in three dimensions. To this end, cryo-EM has been used in conjunction with antibiotics that are known to stop (fusidic acid [29,30]) or slow down (thiostrepton [31]) translocation at different stages. Another cryo-EM snapshot of a specific binding position has been obtained by using a nonhydrolyzable GTP analog [30]. Although the fusidic-acid experiment shows EF-G binding in the post-translocational state, both the thiostrepton and the GTP analog experiments should present different binding stages of the pre-state.

These three-dimensional imaging experiments have gone a long way toward advancing our knowledge about the interactions of the individual domains of EF-G with the ribosome, and the likely role they play in the translocation event. The shape of EF-G, and the fact that it forms multiple contacts with the ribosome, invoke the image of an octopus attaching itself with tentacles to a large object (Figure 6). Five main contacts are seen in the experiment where fusidic acid is used, two involving the small subunit and three the large subunit. Most interesting is

the contact made by domain IV, which reaches deep into the cleft region of the small subunit, precisely where the anticodon end of the A-site tRNA has been localized in separate experiments.

The observed position of domain IV has immediate implications for the action of EF-G: the presence of domain IV precludes the simultaneous presence of the A-site tRNA at that location. This means that, upon binding, domain IV must either actively displace the A-site tRNA or block a retrograde movement of the tRNA from the P site back to the A site. Additional experiments are required to determine which of these two alternatives applies. A fitting of the X-ray structure of EF-G in its GDP state [32] into the density attributed to EF-G shows that domain IV (along with domains III and V connected to it) is rotated in the direction of the contact point in the cleft of the small subunit; it is therefore likely that translocation is accompanied, or effected by, a large conformational change in the EF-G molecule.

The fact that domains III, IV and V of EF-G appear to move as ‘one block’ is very interesting in the context of the molecular mimicry paradigm. This originates with the observation [33] that the X-ray structure of EF-G is very similar to that of the complex formed by EF-Tu, tRNA

Figure 6

Cryo-EM reconstruction of EF-G bound to the *E. coli* ribosome in the presence of fusidic acid. **(a)** Side view, showing EF-G clamping the two subunits, with domain IV reaching into the cleft of the small subunit; **(b)** oblique (close to top) view from the solvent side of the small subunit, which brings out the arc connection between EF-G and the stalk base; **(c)** view from the top, with the ribosome sliced open to give a free view of the intersubunit space. The X-ray structure of EF-G was fitted into the extra density seen in (a,b), and

is shown here pasted into the density map of the empty ribosome. The six domains (I-G, I-G', II, III, IV, V) are shown in different colors. Optimum fit was achieved by rotating the three interconnected 'tRNA mimics', domains II, IV, and V, clockwise. Also shown is the position of P-site tRNA (green) as observed in another study. (a) and (b) are reproduced with permission from [30]; (c) is reproduced with permission from a cover illustration accompanying [29].

and GTP ('ternary complex'), which delivers tRNA to the ribosomal A site. The similarity of these molecules was taken to suggest a close similarity in their binding interactions with the ribosome, a conjecture that was indeed experimentally confirmed subsequently by cryo-EM [29,34,35]. A detailed comparison between the structures of the two molecules indicates that, within EF-G, domains III, IV and V of EF-G jointly mimic the shape of tRNA, with domain IV playing the role of the anticodon arm.

As the joint movement of domains III, IV, and V is relative to domains I and II that are themselves rigidly connected, one could regard the contacts formed by the G domain — a subdomain of domain I — with the large subunit and domain II with the small subunit as anchoring points of the molecule, against which the other domains are able to perform their work. The G domain contact is with the α -sarcin-ricin loop, a highly conserved RNA segment in the stalk base that is known to be involved in the GTP hydrolysis (see [36,37]). The tip of domain V interacts with a groove of the 50S subunit, in the region of the stalk base. Finally, an additional arc-shaped connection exists between the G' domain (another subdomain of domain I) and the stalk base, most likely involving protein L11 [13].

The existence of multiple contacts between EF-G and the ribosome and the observation of conformational changes

affecting both EF-G and the ribosome itself pose considerable challenges in attempts to deduce the nature and sequence of the molecular events underlying translocation. Many more experiments have to be conducted and critically evaluated in the context of kinetic studies. Some new clues have emerged in studies with a nonhydrolyzable analog of GTP [30] and with thiostrepton [31].

Conformational changes and communication between the subunits

A change in the relative positions of the subunits during the elongation cycle has long been postulated; for instance, a ratchet-like model of translocation posits that the small subunit rotates back and forth, thereby advancing the mRNA to the next codon [38,39]. Although there is no evidence thus far for this particular mechanism of action, there is growing evidence for conformational changes in many parts of the ribosome between different states of the elongation cycle. In one case, the local rearrangements caused by the switching of a bistable RNA helix (helix 27 of 16S RNA [40]) near the decoding center of the small subunit was shown to cause a wave of changes affecting the structure of the ribosome, reaching remote regions [41]. In another case, the binding of EF-G to the ribosome in the presence of fusidic acid was seen to be accompanied by a shift of the head, and a rotation of the spur, of the small subunit [30]. An interesting change in

the GTPase-associated center, when comparing the cryo-EM density map of the ribosome from *E. coli* with the X-ray structure of the large subunit from *Haloarcula marismortui* [4], has recently been found by Gabashvili *et al.* [13]: the α -sarcin-ricin loop appeared in changed positions relative to surrounding landmarks, rotated by 17°, a finding that may have functional significance.

As an increasing number of conformational changes affecting both subunits are discovered, the way the subunits communicate with each other becomes a focus of attention. In looking for a physical substrate for the postulated signaling process, one is immediately drawn to numerous connections visible between the subunits. These connections, or bridges, were first discovered in a cryo-EM map (Figure 4) at relatively modest (25 Å) resolution [7,42]. The number of bridges discovered in that map, six, has proliferated as the resolution increased to 11.5 Å (16 bridges; cryo-EM map of [13]; Figure 2a,b) and 7.8 Å (20 bridges; X-ray map of [9]). Judging from the helical appearance that is evident in both X-ray [9] and cryo-EM maps [13], most of these bridges are probably made of RNA strands, but for some the involvement of proteins is well documented [43].

It is clear that some of the bridges are needed to stabilize the association of the subunits in a defined configuration. An exact registration of the subunits is required because the main ligands, tRNA (singly or in association with EF-Tu and GTP in the ternary complex), EF-G and the release factors all have to interact with both subunits simultaneously. This means that the three-dimensional constellation of the binding sites must be very well defined and exactly reproducible at equivalent time points along the elongation cycle. For instance, EF-G, as we have seen, interacts with the ribosome at a total of at least five sites, which are scattered over a large region.

Other bridges, however, must have dynamic functions, working either by modulating or controlling the relative positions of the subunits, or by passing a signal in either direction regarding the status of a particular component 'encoded' in its conformational state.

Conclusions

Coming back to the beginning metaphor, it is not the machine at rest but the machine in action that we wish to understand. Very soon there will be the first atomic-resolution model depicting the ribosome in a single static form. The challenge then is to study the many possible dynamic modes of this system, correlating them to the known function, and using a host of three-dimensional cryo-EM experiments to determine which of the modes are realized. Time-resolved imaging using cryo-EM (see the spray freezing method [44]) will be particularly valuable in this respect.

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References

1. Alberts, B. (1998). The cell as a collection of protein machines: preparing the next generation of molecular biologists. *Cell* **92**, 291-294.
2. Garrett, R. (1999). Mechanics of the ribosome. *Nature* **400**, 811-812.
3. Clemons, W.M.Jr., May, J.L.C., Wimberly, B.T., McCutcheon, J.P., Capel, M. & Ramakrishnan, V. (1999). Structure of a bacterial 30S ribosomal subunit at 5.5 Å resolution. *Nature* **400**, 833-840.
4. Ban, N., Nissen, P., Hansen, J.C., Capel, M., Moore, P.B. & Steitz, T.A. (1999). Placement of protein and RNA structures into a 5Å-resolution map of the 50S ribosomal subunit. *Nature* **400**, 841-847.
5. Hill, W.E., Dahlberg, A., Garrett, R.A., Moore, P.B., Schlessinger, D. & Warner, J.R. (1990). *The Ribosome, Structure, Function, and Evolution*. American Society for Microbiology, Washington, DC.
6. Frank, J., Penczek, P., Agrawal, R.K., Grassucci, R.A. & Heagle A.B. (2000). Three-dimensional cryoelectron microscopy of ribosomes. *Methods Enzymol.* **317**, 276-291.
7. Frank, J., *et al.*, & Agrawal R.K. (1995). A model of protein synthesis based on a new cryo-electron microscopy reconstruction of the *E. coli* ribosome. *Nature* **376**, 441-444.
8. Stark, H., *et al.*, & van Heel M. (1995). The 70S *Escherichia coli* ribosome at 23 Å resolution: fitting the ribosomal RNA. *Structure* **3**, 815-821.
9. Cate, J.H., Yusupov, M.M., Yusupova, G.Zh., Earnest, T.N. & Noller H.F. (1999). X-ray crystal structures of 70S ribosome functional complexes. *Science* **285**, 2095-2104.
10. Schlünzen, F., *et al.*, & Yonath A. (1999). The identification of selected components in electron density maps of prokaryotic ribosomes at 7Å resolution. *J. Synchrotron Radiation* **6**, 928-941.
11. Che, Z., *et al.*, & Smith, T.J. (1998). Antibody-mediated neutralization of human rhinovirus 14 explored by means of cryoelectron microscopy and X-ray crystallography of virus-fab complexes. *J. Virol.* **72**, 4610-4622.
12. Davis, C. & White S. (2000). Electrons and X-rays gang up on the ribosome. *Structure* **8**, R41-R45.
13. Gabashvili, I.S., *et al.*, & Penczek, P. (2000). Solution structure of the *E. coli* ribosome at 11.5 Å resolution. *Cell* **100**, 51-63.
14. Frank, J., Penczek, P., Grassucci, R.A., Heagle, A., Spahn, C.M.T. & Agrawal, R.K. (2000). Cryo-electron microscopy of the translational apparatus: experimental evidence for the paths of mRNA, tRNA, and the polypeptide chain. In *The Ribosome: Structure, Function, Antibiotics, and Cellular Interactions*. (Garrett, R.A., Douthwaite, S.R., Liljas, A., Matheson, A.T., Moore, P.B. & Noller, H.F., eds) ASM Press, Washington, DC.
15. Müller, F. & Brimacombe, R. (1997). A new model for the three-dimensional folding of *Escherichia coli* 16S ribosomal RNA. I. Fitting the RNA to a 3D electron microscopic map at 20 Å. *J. Mol. Biol.* **271**, 524-544.
16. Lake, J.A. (1976). Ribosome structure determined by electron microscopy of *Escherichia coli* small subunits, large subunits and monomeric ribosomes. *J. Mol. Biol.* **105**, 131-159.
17. Ban, N., *et al.*, & Steitz, T.A. (1998). A 9 Å resolution X-ray crystallographic map of the large ribosomal subunit. *Cell* **93**, 1105-1115.
18. Yonath, A., Leonard, K.R. & Wittman, H.G. (1987). A tunnel in the large ribosomal subunit revealed by three-dimensional reconstruction. *Science* **23**, 813-816.
19. Milligan, R.A. & Unwin, P.N.T. (1986). Location of exit channel for nascent protein in 80S ribosome. *Nature* **319**, 693-695.
20. Malhotra, A., *et al.*, & Frank, J. (1998). *Escherichia coli* 70S ribosome at 15 Å resolution by cryo-electron microscopy: localization of fMet-tRNA^{fMet} and fitting of L1 protein. *J. Mol. Biol.* **280**, 103-115.
21. Beckmann, R., *et al.*, & Frank, J. (1997) Alignment of conduits for the nascent polypeptide chain in the ribosome-Sec61 complex. *Science* **278**, 2123-2126
22. Agrawal, R.K., *et al.*, & Frank, J. (1996). Direct visualization of A-, P-, and E-site transfer RNAs in the *Escherichia coli* ribosome. *Science* **271**, 1000-1002.
23. Stark, H., *et al.*, & van Heel M. (1997). Arrangement of tRNAs in pre- and posttranslocational ribosomes revealed by electron cryomicroscopy. *Cell* **88**, 19-28.

24. Frank, J. & Agrawal, R.K. (1998). The movement of tRNA through the ribosome. *Biophys. J.* **74**, 589-594.
25. Wilson, K.S. & Noller, H.F. (1998). Molecular movement inside the translational engine. *Cell* **92**, 337-349.
26. Frank, J. (1998). The ribosome structure and functional ligand-binding experiments using cryo-electron microscopy. *J. Struct. Biol.* **124**, 142-150.
27. Frank, J., Heagle, A.B. & Agrawal, R.K. (1999). Animation of the dynamical events of the elongation cycle based on cryoelectron microscopy of functional complexes of the ribosome. *J. Struct. Biol.* **128**, 15-18.
28. Rodnina, M.V., Savelsbergh, A. & Wintermeyer, W. (1999). Dynamics of translation on the ribosome: molecular mechanics of translocation. *FEMS Microbiol. Rev.* **23**, 317-333.
29. Agrawal, R.K., Penczek, P., Grassucci, R.A. & Frank, J. (1998). Visualization of elongation factor G on the *Escherichia coli* ribosome: The mechanism of translocation. *Proc. Natl. Acad. Sci. USA* **95**, 6134-6138.
30. Agrawal, R.K., Heagle, A.B., Penczek, P., Grassucci, R.A. & Frank, J. (1999). EF-G dependent GTP hydrolysis induces translocation accompanied by large conformational changes in the 70S ribosome. *Nat. Struct. Biol.* **6**, 643-647.
31. Stark, H., Rodnina, M.V., Wieden, H.J., van Heel, M. & Wintermeyer W. (2000). Large-scale movement of elongation factor G and extensive conformational change of the ribosome during translocation. *Cell* **100**, 301-309.
32. Czworkowski, J., Wang, J., Steitz, T.A. & Moore P.B. (1994). The crystal structure of elongation factor G complexed with GDP, at 2.7 Å resolution. *EMBO J.* **13**, 3661-3668.
33. Nissen, P., *et al.*, & Nyborg, J. (1995). Crystal structure of the ternary complex of Phe-tRNA^{Phe}, EF-TU, and a GTP analog. *Science* **270**, 1464-1472.
34. Stark, H., Rodnina, M.V., Rinke-Appel, J., Brimacombe, R., Wintermeyer, W. & van Heel, M. (1997). Visualization of elongation factor Tu on the *Escherichia coli* ribosome. *Nature* **389**, 403-406.
35. Agrawal, R.K., Heagle, A.B. & Frank, J. (2000). Studies of elongation factor G-dependent tRNA translocation by three-dimensional cryo-electron microscopy. In *The Ribosome: Structure, Function, Antibiotics and Cellular Interactions*. (Garrett, R.A., Douthwaite, S.R., Liljas, A., Matheson, A.T., Moore, P.B., Noller, H.F., eds) ASM Press, Washington, DC.
36. Hausner, T.P., Atmadja, J. & Nierhaus, K.H. (1987). Evidence that the G2661 region of 23S rRNA is located at the ribosomal binding sites of both elongation factors. *Biochimie* **69**, 911-923.
37. Wool, I.G., Gluck, A. & Endo, Y. (1992). Ribotoxin recognition of ribosomal RNA and a proposal for the mechanism of translocation. *Trends Biochem. Sci.* **17**, 266-269.
38. Bretscher, M.S. (1968). Direct translation of a circular messenger DNA. *Nature* **218**, 675-677.
39. Spirin, A.S. (1969). A model of the functioning ribosome: locking and unlocking of the ribosome subparticles. *Cold Spring Harbor Symp. Quant. Biol.* **34**, 197-207.
40. Lodmell, J.S. & Dalberg, A.E. (1997). A conformational switch in *Escherichia coli* 16S ribosomal RNA during decoding of messenger RNA. *Science* **277**, 1262-1267.
41. Gabashvili, I.S., Agrawal, R.K., Grassucci, R., Squires, C.L., Dahlberg, A.E. & Frank, J. (1999). Major rearrangements in the 70S ribosomal 3D structure caused by a conformational switch in 16S ribosomal RNA. *EMBO J.* **18**, 6501-6507.
42. Frank, J., *et al.*, & Agrawal, R.K. (1995). A model of the translational apparatus based on a three-dimensional reconstruction of the *Escherichia coli* ribosome. *Biochem. Cell Biol.* **73**, 757-765.
43. Culver, G.M., Cate, J.H., Yusupova, G.Gh., Yusupov, M.M. & Noller, H.F. (1999). Identification of an RNA-protein bridge spanning the ribosomal subunit interface. *Science* **285**, 2133-2135.
44. Berriman, J.A. & Unwin, P.N.T. (1994). Analysis of transient structures by cryo-electron microscopy combined with rapid mixing of spray droplets. *Ultramicroscopy* **56**, 241-252.