Published in final edited form as:

J Mol Biol. 2016 February 27; 428(5 Pt A): 802–810. doi:10.1016/j.jmb.2015.12.003.

Ribosome Mechanics Informs about Mechanism

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Abstract

The essential aspects of the ribosome's mechanism can be extracted from coarse-grained simulations, including the ratchet motion, the movement together of critical bases at the decoding center, as well as movements of the peptide tunnel lining that assist in the expulsion of the synthesized peptide. Because of its large size, coarse-graining helps to simplify and to aid in the understanding of its mechanism. Results presented here utilize coarse-grained elastic network modeling to extract the dynamics, and both RNAs and proteins are coarse-grained. We review our previous results, showing the well-known ratchet motions and the motions in the peptide tunnel and in the mRNA tunnel. The motions of the lining of the peptide tunnel appear to assist in the expulsion of the growing peptide chain, and clamps at the ends of the mRNA tunnel with three proteins, ensure that the mRNA is held tightly during decoding and essential for the helicase activity at the entrance. The entry clamp may also assist in base recognition to ensure proper selection of the incoming tRNA. The overall precision with which the ribosome operates as a machine is remarkable.

Graphical Abstract

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Keywords

ribosome mechanism; dynamics simulations; RNA mechanics; mRNA

Introduction

Translation of the DNA genetic information into protein products is the essential process carried out by the ribosome. The ribosome is a remarkable intricate ribonucleo-protein machine, which interacts with a set of diverse substrates and co-factors during the process of translation. Understanding the ribosome's dynamics is essential for comprehending its mechanism. Much effort has gone into identifying the structural components of the ribosome in its different forms, the structures of the ribosomes of diverse organisms, and identifying where drugs bind. All of these studies lead to a viewpoint that the ribosome is a highly robust *molecular machine* with many moving parts. Indeed, the number of distinct functional ribosomal conformations (states) required during translation is unknown and may remain incompletely characterized by experiments despite the ribosome's importance for the viability of cells. Computations can play a role by discovering additional states.

There have been many attempts to develop more detailed understanding of the mechanisms of the ribosome's function. Cryo-electron microscopy (cryo-EM) has provided much of our understanding of the steps involved in protein synthesis [1–6]. From an analysis of 3-D cryo-EM snapshots of the intact ribosome in its various functional states, Frank and Agrawal [3] reported ratchet-like rotations of the 30S subunit relative to the 50S subunit during translocation, which closely resembles the motions observed in our dynamics simulations (Fig. 1) [7]. Many details have been understood regarding the sequential steps during translocation, but all details have not yet been fully developed [8, 9]. Other methods such as NMR and FRET are providing additional details [10–18]. Experimental structural determination can provide the structures of multiple states of ribosomal protein and RNA components, corresponding to many different conditions with different ligands bound, immobilized in various ways, in different environments, etc., but structure determination of all possible structural states to map out all details of the ribosome's mechanism is overall a daunting task. Computations can play a critical important in supplementing the available structural and functional information with simulations providing further details of the ribosome's dynamics. A complete understanding of the mechanism will only be fully realized if we collect information about its dynamics from a variety of different

computational simulations. The motions of the ribosome are being sampled successfully for extremely long times with atomic molecular dynamics simulations by the group of Sanbonmatsu. Such simulations do not always, however, yield simple descriptions of the mechanism. Different aspects of the dynamics of the ribosome and its constituents have been investigated using various computational approaches such as full-atomic molecular dynamics [19–27], coarse-grained molecular dynamics [28], normal mode analysis using a coarse-grained potential [7, 18, 29–32], elastic network models [33, 34] and combining experimental observations with computational methods [35].

We focus here on the mechanical behavior of the ribosome in a coarse-grained approach. Our use of simple elastic network models is an appropriate and informative tool for this purpose that can provide simple results to permit the comprehension of many mechanistic details of translation and protein elongation. More thorough investigations of a variety of models with various components included or excluded is important for understanding ribosomal functioning. Such simulations can complement and enhance what can be learned experimentally.

Exploring the dynamics of biological systems is key for understanding the ribosome's functional mechanisms. Computational techniques that utilize full-atomic empirical potentials, such as molecular dynamics simulations, are commonly used to describe harmonic and anharmonic motions of proteins and their complexes [36–39]. The low-frequency motions involving large portions of proteins, typically motions of whole domains, are often related to their biological functions [40], such as the transitions in proteins between open and closed conformations [41–50] and the ratchet-like motion of the ribosome during protein synthesis [3]. But, it is easier to comprehend these domain motions with coarse-grained models, even for systems that are much smaller than the ribosome. For extremely large systems like the ribosome, full atomic simulations are not only difficult to perform, because of the huge demand for computational resources, but also difficult to interpret in terms of identifying the important steps in mechanisms. In addition with atomic MD there are also difficulties in providing sufficiently long trajectories for a thorough investigation of its global motions, again because of heavy demands on computational resources. The coarse-grained models permit simpler more direct interpretations with a greater certainty of the complete consideration of all possible important motions.

Here we present a review of what we have learned to date from our own ribosome simulations, but also include some new results showing the details of how the ribosome clamps down on the entering mRNA, required for its helicase activity to unwind the mRNA [51] as well as to form a binding site that can sense the bases being prepared for presentation to the anti-codon of the tRNA. There are now many different structures of the ribosome and its components available in the Protein Data Bank (PDB) [52]. All results shown here are for the *T. thermophilus* structure, which was the first complete structure to be determined (pdb id::4V42 that replaces the original 1GIX and 1GIY files). Like other bacterial ribosomes it is comprised of two large subunits, the 30S (blue in Fig. 1) and the 50S (red in Fig. 1), which rotate relative to one another in its dominant ratchet motion, for each step of the synthesis.

Elastic Network Models

Elastic network models, namely the Gaussian network model (**GNM**) [53] for scalar descriptions of motions and the anisotropic network model (**ANM**) [54] used here to enable interpretation of the directions of motion. The starting structure is assumed to be the lowest in energy and the energy to be Gaussian in form, increasing with the square of the displacements from the starting structure:

$$\begin{aligned}
 U(t) &= (\gamma/2) \text{tr} [\Delta R(t)^T \Gamma \Delta R(t)] \\
 \langle \Delta R_i \bullet \Delta R_j \rangle &= (1/Z_N) \int (\Delta R_i \bullet \Delta R_j) \exp(-U/kT) d\Delta R \quad (1) \\
 &= (3kT/\gamma) [\Gamma^{-1}]_{ij}
 \end{aligned}$$

where U is the energy, γ is the spring constant and R_i is the displacement of the point i in the structure.

The contributions to the motions can be decomposed into normal modes, described by the eigenvalues and eigenvectors of this matrix Γ^{-1} . This is an efficient method to determine the harmonic motions of a structure around its starting structure. It accounts for the geometric constraints of the structure and yields a mechanical model. The overwhelming lesson from many applications of these methods is that it is the shape of the structure that determines the motions [55–57].

Usually elastic network models are applied to coarse-grained structures, and the results show a system's mechanical motions by employing a uniform potential (all springs taken to be identical) for the interacting node pairs in the system [58–60]. The coarse-graining is generally performed as one-point-per-amino acid for proteins, but lower resolution models, i.e. including several residues per node, have also proven satisfactory for determining the lower-frequency collective modes [57, 61] [62], as long as the overall shape of the molecule is preserved. The building block approach [63] and the minimalist network model [64] are other alternative efficient coarse-grained normal mode approaches.

It is straightforward to compute the correlations of the changes between any two points in the structure R_i and R_j by inverting the Kirchhoff matrix that is defined by placing a one in the matrix for points in the structure that are close to one another, using a cutoff distance for this purpose, and 0's elsewhere in the matrix, with the diagonal taken to be the negative sum of the other elements in a given row. This is the Kirchhoff matrix of contacts Γ , and usually a spring is placed between nearby points in the structure as defined by a cutoff distance (See Refs [7, 54] for further details of the method). In this case where we are coarse-graining by using C^α atoms for the proteins and two atoms for each nucleotide, the P and O4* atoms, we use a cutoff distance of 15Å. The dimensions of the square matrices to be diagonalized are 16,266 for the 30S subunit and 29,238 for the 70S subunit. Alternative ways of the coarse-graining have been investigated, and these give extremely minor differences. This insensitivity to details is one of the major advantages of these methods.

From a simulation of the entire ribosome the computed correlations between the different parts of the ribosome structure can be derived in order to decipher its mechanism (see Table 1). The eigenvectors describe the direction of motion of each of the individual points in the

structure. The present results include the collective results for vectors of the first 100 modes of motion of the ribosome. The correlation is a cosine function, and so a value of one corresponds to a perfect correlation in the direction of motion of the two points, and zero means either no correlation or a tangential motion. The dominance of the ratchet motion is clear in the strong anti-correlation between the 30S and 50S subunits. All of the smaller separate components – the tRNAs and mRNA move in a positively correlated way. The tRNAs have nearly a zero correlation with the 30S and 50S subunits, while the mRNA is strongly correlated with the 30S and anti-correlated with the 50S subunits.

The Ratchet Motion Observed in a Complex with Three tRNAs and an mRNA

Overall the dominant motion that is observed is the ratchet motion between the ribosome's large and small subunits shown in Fig. 1. In the simulations the first 10 normal modes that represent the most collective motions of the ribosome show the ratchet-like rotation of the two subunits, the head rotation of the small subunit and various types of anti-correlated motions between the large stalks. The ratchet-like rotation together with different counter motions of the L1 and L7/L12 stalks were observed in several different normal modes. The large 50S subunit's parts are structurally interwoven with its proteins, and it has twice as many protein contacts with rRNA as the proteins in the small subunit do. The exceptions to this are the L1 and L7/L12 stalks that are extended without rRNA contacts and which have been difficult to visualize experimentally. The small subunit exhibits significant internal domain flexibility [65], with the large subunit being more rigid. The overall ratchet motion is well reproduced by our elastic network models [7], and is observed whether the tRNAs and mRNA are present, or not [29]. It is the nature of the ribosome structure itself that determines its ability to undergo the ratchet motion, and this ability does not depend upon the presence of the tRNAs or mRNA.

Many Other Complex Motions, Notably at the Decoding Center

The modeling of the ribosome structure in the complex having three tRNAs and mRNA shown here has demonstrated the computational efficiency of the Elastic Network Models. In addition to the ratchet motion, many other details are observed that are described below. Our investigation of the motions at the decoding region reveal many important details of the ribosome codon reading apparatus [29]. For example Fig. 2 shows the greater mobility of the anti-codon stem loop (ASL) of the A-tRNA in comparison with the motions of the P-tRNA and the mRNA. The A-site of the mRNA is more mobile when compared to motions at the P-site, which agrees well with the experimental B-factors, and also with the general ability of the A site to accommodate a diverse set of proteins and RNAs and the requirement for the P site to be held more rigidly to ensure fidelity by the strong recognition between codon and anti-codon. In this case we included in the simulation model a high-resolution atomic region [31, 62] for the codon and the anticodon parts of the mRNA and tRNAs at the A- and P-sites and the decoding center A1492, A1493 of the 16S rRNA in the small subunit. The ratchet-like rotation and the counter motions of the L1 and L7/L12 stalks were observed simultaneously with atoms at the decoding center and the codon/anticodon region moving. The missing proteins on the L7/L12 stalk were added to the crystal structure to create

density for that region of the elastic network. The extended protein L9 on the large subunit was deleted in the structure to prevent its extreme mobility, which would dominate the computed lowest frequency motions. About $\frac{3}{4}$ of the motions from the completely coarse-grained model are retained in this mixed coarse-grained model, so although this is an approximation it still provides the most important details. For the cumulative first 10 modes the mean-square fluctuations were calculated for different levels of mixed coarse-graining. The third slowest normal mode of this mixed coarse-grained ribosome model corresponds most closely to the ratchet-like motion of the subunits as observed in cryo-EM. The side chains of A1492, A1493 and the A-tRNA exhibit a larger mobility in the slow modes compared to the P-tRNA and mRNA. Analyses showed that G34 of the A-tRNA and U43 of the mRNA have smaller correlations with the decoding center [29]. The advantage of including these parts as atoms lies in observing significant details of the motions that are most closely related to its functional activities. For example, the bases A1492 and A1493 are significantly more mobile than their backbone, which could not be observed with a coarse-grained model.

The Ribosome as an mRNA Helicase

Residues Arg131, Arg132, and Lys135 on S3, and also Arg47 and Arg50 on S4, which are known to have helicase activity [51], are close to the mRNA and move in coordination with the mRNA in the cumulative ten slowest motions. During the global motion, S3 also moves into close proximity with the mRNA in such a way that its side chains Glu161, Gln162 and Arg164, as well as that of Arg49 of S4, are near enough to the mRNA to interact with it. These residues and the new contacts formed in the alternative conformations may have an important role for the helicase activity of the ribosome [29].

Protein S5 Acts to Orient the mRNA for Translation Fidelity

Mean-square distance fluctuations between S5 residues and the nucleotide A27 show that the entire S5 ribosomal protein remains closer to the mRNA than the S3 and S4 proteins. This suggests that the tight positional alignment of S5 with mRNA (Fig. 3) is functionally important to orient the strand for correct frame reading at the A-site, confirming previous work [29]. But, the opening and closing of the other two proteins around the mRNA can be investigated because in the dynamics simulations these interactions are less persistent.

Proteins S3, S4, and S5 Also Act as a Gate for the mRNA

In a new result, we have seen how the three proteins S3, S4, and S5 appear to control the entry of the mRNA. In Fig. 4 it can be seen that protein S3 has the largest mobility. In one normal mode where it is moving, the motions alternately close with these three proteins move to clamp around the mRNA where it enters the ribosome, with a similar group of three proteins acting as a clamp where the mRNA exits from the ribosome (See Fig. 5). This motion is coordinated with the head swivel motion of the 30S subunit. One of the motions observed alternately has the 5' end open with the 3' end closed or vice versa. The mRNA at the entrance to the tunnel for the mRNA is then constrained by proteins S3, S4, and S5, which constrain the mRNA in a clamp-like fashion. S3 motions are largely responsible for this closing as can be seen in Fig. 5. During the closing of the entry to the mRNA tunnel the

shape of the opening changes. It becomes narrower on one side, suggesting the possibility that it might even be sensing the base of the incoming mRNA. This sensing of the mRNA bases could be structurally transmitted to coordinate the selection of the tRNA to ensure that the anticodon of the tRNA matches this codon of the mRNA. The allostery in the structure permits the mRNA sensing to be transmitted to the entry of the tRNA to ensure matching between the codon and anti-codon. This is a new finding and requires some further investigation of the allostery within the ribosome. This is an example of how specific ribosome motions could lead to structures that sense specific parts and relay the information over distances, in this case to ensure proofreading of the incoming tRNA anti-codon. At the exit site of the mRNA channel the ribosomal proteins S7, S11 and S18 are involved in constraining the mRNA. This closure may relate to the decoding itself to sid in holding the mRNA rigidly. The mRNA interacts with the 3' end of the 16S RNA forming the Shine-Dalgarno complex for the initiation step; and the 3' end of the 16S RNA may act as a 'hook' to reel in the mRNA to facilitate its exit [29]. It is likely that this alternation between 2 states - open entry-closed exit and closed entry-open exit plays an important role overall in ensuring the fidelity for decoding. The ribosome structure appears to exert strong control and directs mRNA entry, translocation and exit dynamics.

tRNA Dynamics within the Ribosome

The ribosome undergoes large motions to effect the translocation of the tRNAs and mRNA. These experimentally observed motions during translocation are inherently controlled by the ribosomal shape so that the ratchet motion pushes these internal components ahead along a nearly linear pathway. Normal mode analysis further reveals the mobility of the A- and P-tRNAs increasing in the absence of an E-tRNA. In addition, the dynamics of the E-tRNA is affected significantly by the absence of the ribosomal protein L1. In the simulations, the L1 arm distorts and appears to attach to the E-tRNA and likely assists in removing the E-tRNA from the ribosome.

Collective Dynamics of the Ribosomal Peptide Tunnel

The collective dynamics of the polypeptide exit tunnel have also been investigated. The low frequency fluctuations show three distinct regions in the tunnel - the entrance, the neck and the exit (See Fig. 6 where the tunnel lining is shown in a mesh representation). The linings of each of these regions have distinctly different motions. Generally the lining of the entrance region moves in the exit direction to assist in the removal of the peptide, with the neck itself having significantly more complex motions. The exit region has significantly larger motions rotating with the exit vector as the rotation axis. Notably the universally conserved extensions of ribosomal proteins L4 and L22 located at the narrowest entrance region of tunnel generally undergo opening and closing motions, which may have an important role in the nascent polypeptide gating mechanism to ensure the rigidity of the reaction center while closed; this motion corresponds closely to the peristaltic motions that Joachim Frank has reported at this site [30].

The Intrinsic Motions of the tRNAs Relate Closely to Their Motions within the Ribosome

Next we report on investigations of the tRNAs by themselves. We [66] showed that the motions apparent from multiple tRNA structures closely resemble those observed in the elastic network simulations. The first three most important normal mode motions of the tRNA are shown in Fig. 7 [67]. Each structure is shown moving along its conformational change pathway. The first of these shows the acceptor arm with large motions, and this may be required to facilitate the amino acid accommodation when the tRNA enters the ribosome to find its appropriate binding position. The second mode involves a bending of the structure that is similar to the motions observed when the ratchet motion takes place and forms Noller's hybrid state [68, 69]. The third mode of motion involves the anticodon stem loop unwinding and extending and this can clearly play a role in facilitating finding the proper match between codon and anticodon. We also found that the motions of the tRNA at all three sites A, P, and E are nearly the same, after their rigid body motions were removed [70]. The tRNA elbows interact with regions of the 50S subunit and undergo large conformational changes; *whereas the anticodon stem loops move in concert with the 30S*. The tRNA bending during the ratchet motion is essential and is intrinsic to the tRNA structure as observed in Fig. 7B. These bent tRNA structural intermediates appear to be the same as Noller's hybrid states [68, 69].

The first two motions of the independent tRNAs mentioned above are likely to be involved when the tRNA first enters the ribosome to ensure proper accommodation of the acceptor arm and the anticodon of the tRNA. After the tRNA is fully bound in the ribosome, these parts can no longer move, and in fact these parts of the tRNA are observed to be the most rigidly held parts of the tRNAs when they are inside the ribosome. Because the charged amino acid ends of the A- and P-tRNAs are bound near the peptidyl transferase center at the core of the 70S complex, they are the least fluctuating parts of the tRNAs. Likewise the anticodon is held very rigidly against the codon of the mRNA.

Conclusion

We have many observations from our simulations that relate directly to individual aspects of the ribosome's mechanisms. The ratchet motion is incredibly robust and is difficult to subvert, even after removal of all of the proteins for example [29]. This dominant motion ensures that the active components – the tRNAs and mRNA properly transit through the ribosome. Many of the results observed during our simulations conform to what was previously known about the ribosome and its motions, such as the hybrid bent state of the tRNAs and the peristaltic motion within the peptide tunnel. Other conformational transitions are observed in the simulations, which suggest new functions, such as the partial closing of the mRNA tunnel opening to sense the bases of the codon and transmit this information for the selection of the proper tRNA, and this remains to be verified. Some new specific interactions of functional importance have been discovered from the simulations, and these could be tested by mutagenesis. Overall the high level of precision of the allosteric interactions in the ribosome ensure high fidelity of translation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- The ribosome is a complex machine and coarse-grained simulations can yield details of its mechanism
- The well-known ratchet motion between the two large subunits is the most important motion, and this is observed in the simulations during which the t-RNAs form the hybrid state
- The mRNA is clamped into position by several different motions of components lining the mRNA tunnel
- The peptide tunnel lining has regions with distinctly different motions that assist in the exit of the nascent peptide
- Coarse-grained dynamics models provide a useful method for comprehending the molecular mechanisms of the largest molecular structures

Figure 1. Three alternative conformations of the 70S ribosome for the slowest mode, which is a ratchet motion

The top row shows the ribosome viewed from the 30S side, and the bottom row is for the same motions, but with the 30S subunit removed. Top row shows the 30S subunit in blue and the 50S subunit in red. In the lower row, the 50S subunit is in red, the 3 tRNAs; in purple, and a short piece of mRNA just below them in blue [7].

Figure 2. Mobility at the A and P-sites for the mRNA, tRNAs and nucleotides A1492, A1493
 Shown are the relative magnitudes of the mean-square fluctuations averaged for the 10 slowest modes. Dark yellow designates the highest mobility and dark purple the lowest. Interestingly, the greater mobility at the A site is consistent with the A site's ability to accommodate different ligands [31].

Figure 3. The path of the mRNA (cyan) through the ribosome

The mRNA does not follow a straight path through the ribosome. It interacts strongly with ribosomal proteins S3 (brown), S4 (blue) and S5 (purple) at the 3' end, while its 5' end interacts with the 16S rRNA (green) at its 3' end. The three proteins S3, S4, and S5 form the mRNA entry clamp.

Figure 4. Opening and closing of the mRNA tunnel entrance comes primarily from motions of the S3 protein

Results are shown from mixed coarse-grained simulations of the ribosome, where all of the parts near and including the mRNA and the tRNAs are included as atoms. This shows part of the entry channel for the mRNA, with the three proteins, S3, S4, and S5 (in blue). The mRNA is shown in red, and the tRNA in green. The two extremes of motion shown demonstrate how proteins S3 and S4 move (compare the space bars in the two parts of the figure) to close around the entering mRNA. This closing might correspond a sensing of the incoming tRNA anticodon.

Figure 5. The fluctuations of the positions of the gating proteins at the entrance to the mRNA tunnel
are shown for residues on the S3, S4, S5 proteins. It is clear that S3 has the largest motions with S4 intermediate and S5 showing relatively small fluctuations [29].

Figure 6. Motions of the lining of the peptide tunnel

The peptide tunnel wall is shown as a green mesh. Some parts of nearby proteins are shown in purple. The growing polypeptide passes through the narrow gate at the entrance and emerges into the broader part of the tunnel. The intermediate parts of the tunnel lining move in the direction of the red exit arrow and the bottom parts near the exit rotate around the red axis [30].

Figure 7. The important intrinsic motions of the tRNA structure are shown in multiple overlays of the structures at different stages of its motion, deformed according to modes 1 (in A), 2 (in B), and 3 (in C). Part A shows a mode where the acceptor arm moves as required for amino acid accommodation in the ribosome. Part B shows a mode where the tRNA bends as required to form the hybrid state during the ribosome's ratchet motion, and part C shows an unwinding and extension of the anticodon stem-loop required for accommodation of the anticodon to bind to its cognate codon [67, 70].

Correlations of the motions of the different components of the ribosome, for the first 100 normal models from the elastic network model

Table 1

Correlations are expressed as dot products. Overall this shows the strong anti-correlation from the ratchet motion between the 30S and 50S subunits, the positive correlations among the tRNAs and mRNA, and the near zero correlations of the tRNAs with the 30S and 50S subunits, and the positive correlation of the mRNA with the 30S subunit. Strong positive correlations are shown in bold face [7].

	50S	A-tRNA	P-tRNA	E-tRNA	mRNA
30S	-0.99	-0.06	-0.10	-0.07	0.50
50S		-0.01	0.03	-0.01	-0.55
A-tRNA			0.77	0.17	0.42
P-tRNA				0.31	0.39
E-tRNA					0.19