Ribosome Rocks

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If we are to talk about molecules of life, the ribosome, made of ribosomal RNA (rRNA) and protein, is a key: it stands at the junction between information and action, i.e. between nucleic acids and proteins. It is an extraordinarily sophisticated machine that takes the information present in mRNA and uses it to build proteins, containing over 250K atoms, and measuring more than 200 in every direction. It consists of two units: the small ribosomal subunit (30S that performs the decoding of genetic information during translation and the large ribosomal subunit (50S) that forms the peptide bonds.[1]
This structure has been under biochemical and genetic research for the last forty years, but it is in the last two years that by means of crystallographic analysis, “the pace of progress has been breathtaking”[1]. To date, Ban and his group have solved the structure of the large subunit at 2.4Å[2], two groups have solved the structure of the small unit for two different organisms at 3Å and 3.3Å [3, 4] and the structure of the intact 70S ribosome complexed with tRNAs and mRNA has been solved at 5.5Å [5], not yet at atomic resolution.
Translation uses the genetic information in the messenger RNA (mRNA) to synthesize proteins. Transfer RNAs (tRNAs) are charged with an amino acid and brought to the ribosome, where they are paired with the corresponding trinucleotide codon in mRNA. The amino acid is attached to the nascent

[1]Svedberg=Sedimentation unit. The small ribosomal unt is 30S in bacteria and archea, 40S in eukaryotes. The large ribosomal unit is 50S in bacteria and archea, 60S in eukaryotes.

polypeptide and the ribosome moves on to the next codon. The cycle is repeated to produce a full-length protein [6].
There are three tRNA binding sites on the ribosome: the A (or aminoacyl) site, which bind s the tRNA with the new amino acid to be added; the P (or peptidyl) site, which holds the tRNA with the nascent polypeptide chain; and the E (or exit) site which binds the deacylated tRNA before its ejection from the ribosome.The 70S map proves that crystallography can be done on whole 70S ribosomes in defined functional states, a fact of the utmost importance for future investigations [7].
The new details confirm suspicions that ribosomes' RNA molecules, and not their proteins, participate directly in the formation of peptides and is the reason why it is said that the ribosome is a ribozyme. This confirms the theory of the RNA world that existed before proteins.
So right now we have the pieces of a big engine, and a good idea of how it works, but not the details of its inside. We have a large system that functions over time passing through a number of steps, and the accomplishment of each step allows the happening of the next one. Understanding the parts of the system will lead us to understand the system, but one has to be very careful in addressing the system: the parts do not sum to the whole.
This is a very exciting time for the study of ribosome. A physicist could make many contributions in the study of this amazing system, and I will address these later. Revealing the works of the ribosome will favor enormously the design of protein machines, nanotechnology and drug design. The studies on ribosome have already started to give insight on how some antibiotics work, disabling the active site of bacterial ribosome [8, 9].
In refering to the ribosome, Carl Woese says: "This most complex, beautiful, and fascinating of cellular mechanisms, the translation apparatus, is also the most important." I will address in this paper an example of the insight a physicist could give to the ribosome problem, this approach will be one proposed by Woese thirty years ago, before any of the experimental methods available today were even imagined. He suggested a model for protein synthesis which depended upon conformational changes in tRNA and allosteric transitions in place of translocation. In his paper [10] he proposes translation as a reciprocating ratchet mechanism in which the tRNA function both as readers and movers of the mRNA.
Last year, a model was proposed by Frank and collaborators [11] using a technique called three-dimensional cryo-electron microscopy confirmed that

there is a two-step mechanism in translocation[2]. Cryo-electron microscopy is one of the few techniques capable of visualizing large, dynamic molecules. In preparing for cryo-EM, researchers first immerse the ribosomes in water solution and then abruptly freeze them in supercold liquid ethane. The rapid freezing imprisons the ribosomes in ice, thus preserving their native structure. Using an electron microscope with a low-intensity beam to avoid damaging the molecules, they obtained images of the captive ribosomes and used computerized image analysis to produce detailed, 3-Dimensional map of ribosome motion from the otherwise low-contrast, noisy images produced by the electron microscope.

The observations from the experiments throw a sequence of events going on in the ribosome (Figure 1) that correspond to two relative rotations of the subunits in opposite directions, making the system "rock", as illustrated in figure 2:
1. The elongation factor triggers a rotation of the 30S with respect to the 50S subunit by an angle of about $6(\pm 0.7)$ degrees, resulting on a maximum displacement, on the periphery of the ribosome, of $19(\pm 3)$Å. This is accompanied by a widening of a channel where the mRNA goes inside the ribosome. The position of the tRNAs relative to the subunits does not change, but are left with a skewed orientation.
2. GTP hydrolysis triggers conformational changes inside the ribosome that result in three events:
a. Translocation of the mRNA/(tRNA)2 complex with respect to 30S (requires mRNA to be free to move);
b. Release of the elongation factor G;
c. Reverse rotation towards the original positions of the subunits (a movement is arrested at an intermediate position due to the presence of fusidic acid).
The results thrown by this investigation have caused a lot of excitement between groups working with ribosome as it confirms several hypothesis such as: ribosome can not work without movement; there is a mechanism that

[2]When the peptide bond of the current tRNA has been formed in teh 50S subunit, the elongation factor G binds to the ribosome and triggers the translocation of the peptidyl tRNA from the aminoacyl site to the peptidyl site, and promotes the movement of RNA by exactly three bases (one codon). Conserving the so-called reading frame is most important for tranlation, since a change would result in a totally differnt polypeptide, without the correct structure nor function.

holds the mRNA and frees it for advancing in the reading frame and yet make the reading stable, and others.
From a physical point of view, I believe these results can be utilized to model this two state system. During translation, it might be that inside the ribosome, proteins change conformation aside from position, and we also know there are chemical reactions going on. Furthermore, the precise movements of the sites will not be available until the whole system can be resolved to atomic resolution. The ribosome is a huge system with complex force fields. It is not known how the stress is distributed in the ribosome, but we know that the chemical reactions contribute to changes in the energy landscape.
It might be possible to introduce a model explaining this two state system based on the ratchet mechanism it suggests. A similar approach has been introduced by Magnasco[12] and by Shibata[13] for protein motors and chemical engines, respectively, as an extension of the ratchet engine used by Feymann in his Lectures[14]. Thermal fluctuation is believed to play an important part in function of proteins.
The model would be proposed in the form of a linear ratchet potential, as shown in figure 3, where if we intend to get pass the ratchet, there will be a minimum force to overcome the barriers, this will be the preferred direction, whilst the force to move in the opposite direction is much larger, and this movement is not likely (rewinding the mRNA). A complication to the model noted by Shibata[13] is to include the chemical energy transduction in molecular systems, a task not easy to accomplish.
As for the future of the overall research on the ribosome, the participating teams formed by biologists and biochemists will probably make directed mutations on specific sites of the structures to observe the effects of protein production, or will not divert from their quest to make even better crystals, some other very interesting physical approaches can be made towards understanding the mechanics of the ribosome.
As for the physicists, the high resolution structures of the subunits could help to start building molecular dynamics multi scale methods on the works of the ribosome. This is done by studying the interaction potential of a given part of the ribosome and use this calculation as an input for the potential in the model of another part of the system.
Once the whole structure is solved at atomic resolution, and with powerful computational tools, a simulation of the whole system could be done by calculating the Hamiltonian of the system. The experimental groups themselves are now asking for the modeling capabilities of physics: "What we have at

present are a few snapshots, and ultimately what we would like is a movie of the ribosome in action". -Noller

The idea of understanding the way this most important complex works, is by itself very exciting, and according to the rate of the experiments, this is not far away and will be the subject of cutting edge research for the next decade.

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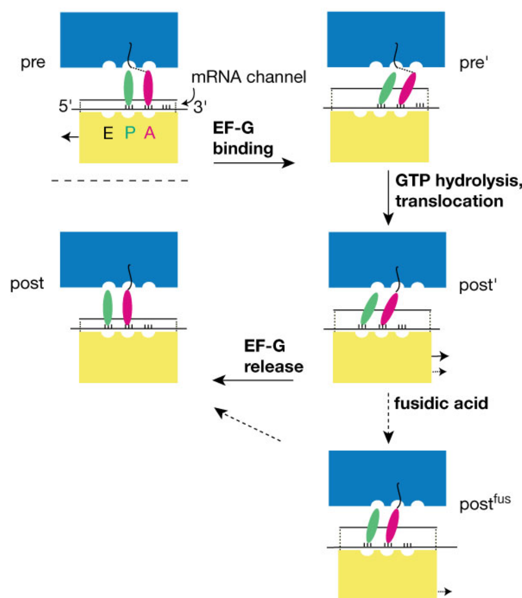

Figure 1: Diagram showing three steps of the translocation process. 'pre' state: tRNAs are in A (magenta) and P (green) sites on both 30S (yellow) and 50S (blue) subunits; the peptide bond has already been formed and the elongated peptidyl moiety is now on the A-site tRNA; the subunits are in the original orientations; both entrance and exit channels for mRNA, indicated by its 3' and 5' ends, are narrow. 'pre" state, following EF-G binding: 30S subunit has rotated with respect to the 50S subunit, without changing the positions of the tRNAs relative to either subunit; mRNA channel has widened enabling mRNA advance in the next step. 'post" state, following GTP hydrolysis (transient state, not experimentally observed): tRNA and associated mRNA have advanced from A and P sites to P and E sites (which might involve transitional, A/P and P/E hybrid states29); relative subunit orientations are unchanged. 'postfus' state, assumed when fusidic acid is present: small subunit has partially rotated back, complete reversal is blocked by the presence of EF-G/GDP which is not released. 'post' state, assumed after EF-G is released under normal conditions: small subunit is fully rotated back into original orientation; tRNAs are in P and E sites; mRNA channel is tight again, securing mRNA in its new position. Nature 406:318-322.

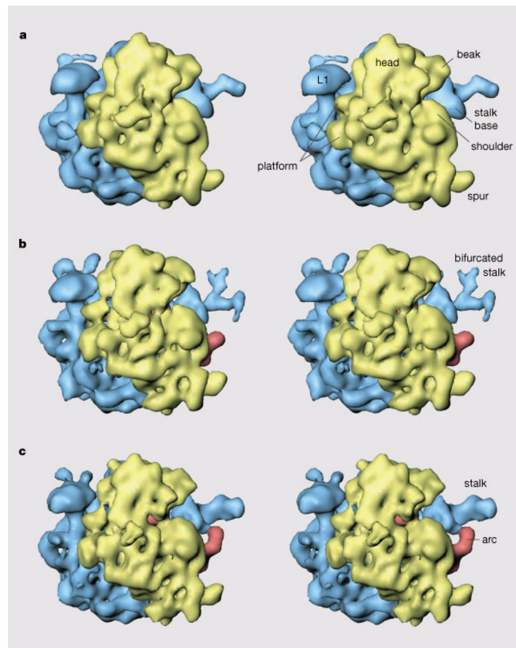

Figure 2: Stereo representation of the rotational movement of the 30S subunit (yellow) with respect to 50S subunit (blue) upon EF-G (red) binding. a, 70S/fMet-tRNAMetf complex; b, 70S/EF-G/GMPP(CH2)P complex; and c, 70S/EF-G/GDP/fusidic acid complex. The three-dimensional maps of the 70S complexes are shown from the 30S solvent side. The masses corresponding to EF-G in b and c are only partially seen in this view. The movement of the 30S subunit is anticlockwise from a to b and clockwise from b to c. Landmarks: L1, protein L1; arc, arc-like connection between EF-G and stalk base.Nature 406:318-322.