The Ribosome – a Restless Molecular Machine

by Joachim Frank

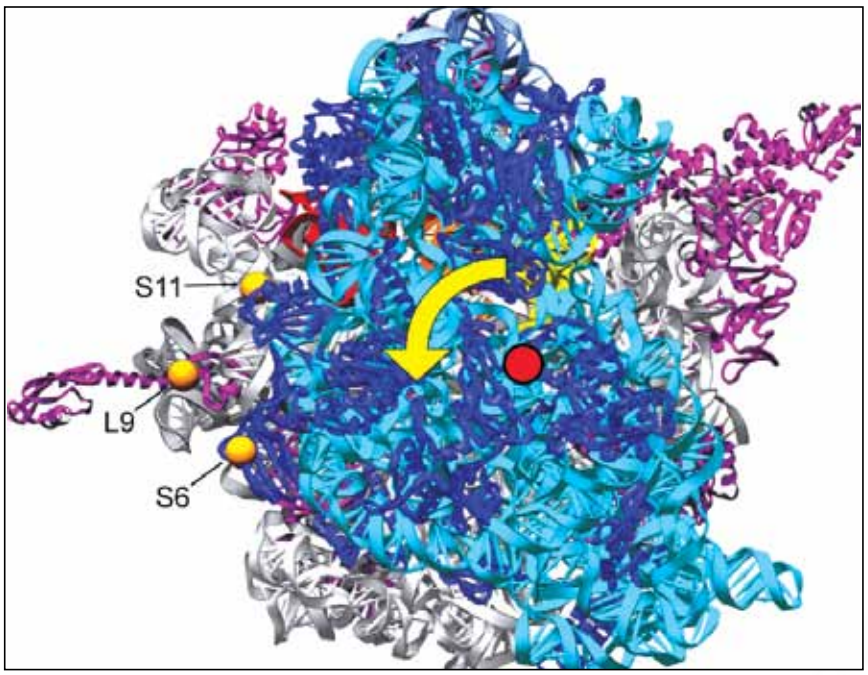
Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biophysics,
and Department of Biological Sciences, Columbia University,
New York, NY 10032

The year 2000, well known as the year of the Y2K scare, as the threshold to the new Millennium, and to specialists as the **annus mirabilis** in the long quest of X-ray crystallography for the structure of the ribosome (Ban et al., 2000; Schluenzen et al., 2000; Wimberly et al., 2000). This has a fourth special significance to me because of our discovery of the ratchet-like motion of the ribosome (Frank and Agrawal, 2000). Rajendra Agrawal and I found, by comparing cryo-electron microscopy density maps of the **Escherichia coli** ribosome with and without elongation factor G bound, that the two subunits rotate with respect to each other, back and forth, once for every amino acid that is added to a growing polypeptide chain. Thus, a good-sized protein of 200 residues takes 200 back-and-forth rotations to make, about 15 per second, consuming on average a total of 13 seconds in the cell. We hypothesized that this motion was part and parcel of the complex mechanism required to move mRNA and the tRNAs relative to the ribosome, in a process called translocation. In fact, such a ratchet-like relative motion of the two subunits had been much earlier proposed by Alexander Spirin (1968), well before the means of visualization by electron microscopy were available.

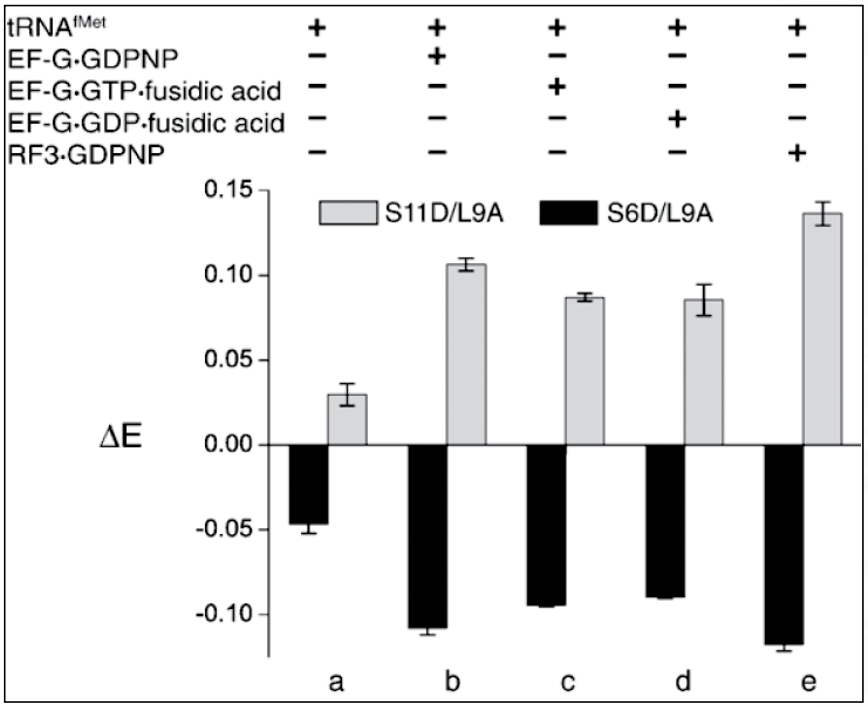
We could infer from sequence comparisons and other earlier work that eukaryotic and bacterial ribosomes must be quite similar in structure and functional principles, so it was not too much of a flight of imagination to assume that the same motion would be employed during the protein synthesis of multicellular organisms, as well, which include plants, animals, and thus, humans. The realization that each of the thousands of ribosomes in each cell of each living being on this planet the exponent keeps compounding as I'm writing this down!-performs this motion incessantly was a mesmerizing insight. I do remember driving through Vermont on the way from Albany to the Gordon Conference in New Hampshire, taking one of the rural undivided highways flanked by the unspoiled green of early summer, when I was suddenly overcome with this mind-boggling idea: that every leaf of every tree housed millions of these restless molecular machines. (It was fortuitous that I was alone in my car since I might have sounded exceedingly stupid in my euphoria). As I was driving on, the idea became even more obsessive: some of the waving motions of the leaves might not be due to the wind, but to some global effect of a sporadic but massive vector addition? Might the rotation of the earth have been affected in a subtle way, adding or subtracting a second over the span of the past 3 ½ billion years that the ribosome has been around?

Meanwhile a decade has gone by; we have learned more about the ribosome's incessant motion, and are grappling to put it into the conceptual framework of molecular machines. First, from what I stated before, it was not a surprise to find that eukaryotic ribosomes are busy rotating back and forth in a similar way (Spahn et al., 2004). Next, an experimental corroboration of the motion in the bacterial ribosome came from fluorescence experiments by Harry Noller's group: an acceptor **B** attached to the large subunit, and two nearby donors **A,C** attached on either side of **B** to the small subunit are expected to report anticorrelated distance changes upon EF-G binding: when **A–B** is large, **B–C** should be small, and vice-versa. This behavior could indeed be observed in the bulk FRET experiment in which EF-G was added to a sample with translating ribosomes (Ermolenko et al., 2007) (Fig.1). Single-molecule FRET experiments allowed signals to be picked up from single ribosomes, and these not only confirmed the results of the bulk study, but also showed that the motion is a stochastic process (Cornish et al., 2008).

But the real surprise came from the observation by Ermolenko et al. (2007) and in single-molecule FRET observations (Cornish et al., 2008; Fei et al., 2008; Kim et al., 2007) that for the intersubunit motion and associated movements of the L1 stalk and tRNAs to happen, no EF-G is required at all, as long as the concentration of magnesium ions is in the range of physiological concentrations. As all surprises in Science, this one offered a new insight. After accommodation of a new aminoacyl-tRNA into the A site and subsequent transfer of the peptide bond to the amino acid on the A site, the ribosome — it now appears — is constantly oscillating between the normal state (termed Macrostate I) with the tRNAs in the classic A/A, P/P positions and an intersubunit-



A



B

Fig. 1. Corroboration of the intersubunit rotation using FRET. (A) An acceptor is placed on protein L9 on the large subunit, and donors are placed on the small subunit in flanking positions on proteins S11 and S6. Acceptor and donors are indicated by yellow spheres. (B) Distance changes reported by changes in FRET efficiency for S11-L9 and S6-L9, upon binding of various ligands to the ribosome, are anticorrelated, and confirm counter-clockwise rotation of the small subunit (yellow arrow in (a); red circle marks rotation center) upon binding of EF-G and RF3. (From Ermolenko et al., 2007; reproduced with permission by Elsevier. Ltd.)

rotated state (Macrostate II) in which the tRNAs are allowed to slip into the A/P, P/E hybrid positions. These random oscillations of the pre-translocational ribosome are evidently driven by Brownian motion, and the conformation of the ribosome required for the binding of EF-G is being constantly visited even in a sample devoid of EF-G. The role of EF-G in binding to the ribosome, then, is to "nail down" the ribosomal conformation that productively leads to the next steps, namely GTPase activation, GTP hydrolysis and Pi release, accompanied by conformational changes in EF-G and the decoding center that unleash the final steps of translocation (Ratje et al., 2010; Taylor et al., 2007).

Two cryo-EM studies, on the footsteps of the FRET results, produced a beautiful confirmation of the existence of a heterogeneous mixture in a factor-free pre-translocational sample (Agirrezabala et al., 2008; Julian et al., 2008). Classification techniques had to be used to extract the two subpopulations, resulting in three-dimensional density maps of ribosomes bound with tRNAs in conformations either characterized as {Macrostate I, A/A, P/P} or {Macrostate II, A/P, P/E} (Fig. 2). More recent studies conducted with large datasets and refined methods of classification indicate that the passage from one state to the other goes through numerous intermediates (Agirrezabala et al., 2011; Connell et al., 2008; Fischer et al., 2010; Frank, 2010; Fu et al., 2011).

The new perspectives provided by the complementary experimental studies, mainly using smFRET and cryo-EM, have been articulated in recent reviews (Frank and Gonzalez, 2010; Munro et al., 2009). Although the collection of relevant experimental data is far from complete, the conceptual framework for analyzing the rich troves of information coming from the two experimental techniques and X-ray crystallography, as well as exploratory molecular dynamics simulations (Gumbart et al., 2011), has been set.

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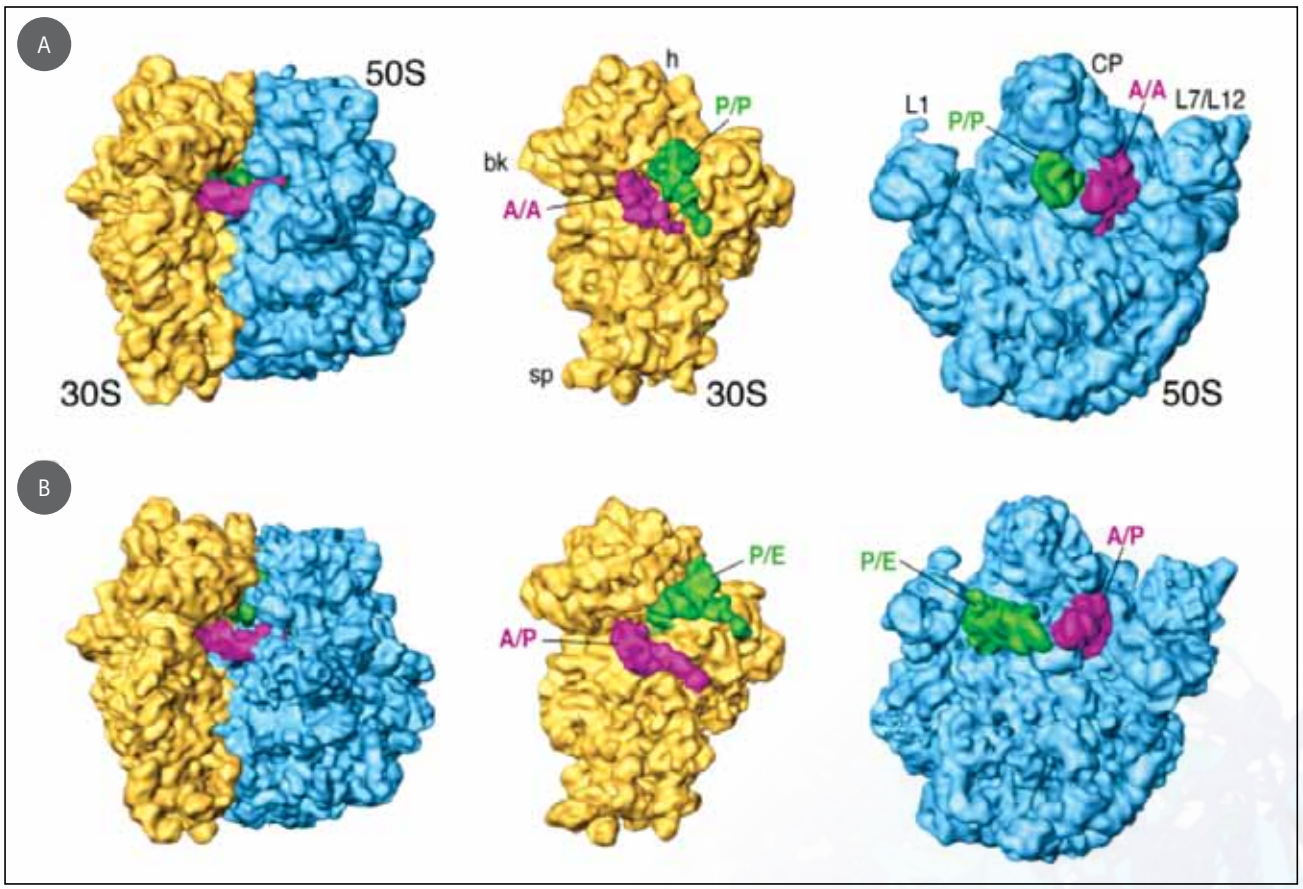

Fig. 2. Cryo-EM study of a pre-translocational ribosome complex in the absence of EF-G. The sample proves to be heterogeneous, with part of the molecules in the classic state {Macrostate I, A/A, P/P} (panel A) and part in the intersubunit-rotated, hybrid state {Macrostate II, A/P, P/E} (panel B). Annotations: 30S: small subunit; bk: beak; h: head; sp: spur; 50S: large subunit; L1: L1 stalk; CP: central protuberance; L7/L12: L7/L12 stalk; A/A, P/P, A/P, P/E: tRNA positions. (From Agerrizabala et al., 2008; reproduced with permission by Cell Press).

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About the Author

Dr. Joachim Frank is a Howard Hughes Medical Institute Investigator, Professor of Biochemistry and Molecular Biophysics, and Professor of Biological Sciences at Columbia University. He developed the single-particle reconstruction approach in cryo-electron microscopy, and has applied this technique to the study of the structure and dynamics of the ribosome. Dr. Frank has received many awards for his research, including the Elizabeth Robert Cole Award of the Biophysics Society. He is a member of the National Academy of Sciences and a Fellow of the American Academy of Arts and Sciences and of the American Academy of Microbiology. He is also a Distinguished Professor of the State University of New York at Albany.

Dr. Frank has published over 200 peer-reviewed articles, written numerous book chapters, and authored/edited six books including *Three-Dimensional Electron Microscopy of Macromolecular Assemblies* (Oxford University Press 2006) and *Molecular Machines in Biology* (Cambridge University Press 2011)