of BipA using amide hydrogen/deuterium exchange mass spectrometry (HDXMS). These data indicate that GTP- and ppGpp-binding lead to large scale conformational changes that are propagated throughout BipA, underscoring the idea that BipA is a metastable molecule where mutually exclusive association of GTP or ppGpp drive equilibria to alternate distinct conformations resulting differential ribosome binding. Molecular dynamics simulations together with covariance analysis are being used to explore the dynamic allostery between the GTPase and novel C-terminal domain.

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Single-Molecule Fluorescence Observations of Eukaryotic Translation

Nathalie Westbrook[1,2].
[1]Institut d'Optique, Palaiseau cedex, France, [2]CNRS, Paris, France.

For the two last decades, structural and single-molecule investigations of the prokaryotic ribosome have provided a better understanding of the highly dynamic nature of translation. Eukaryotic ribosome has received far less attention, most of the conclusions being extrapolated from prokaryotic data. Although both ribosomes share functional and structural features, eukaryotic ribosome contains many additional domains of unknown function. Applying single-molecule techniques to eukaryote ribosomes would clarify the function of these additional domains.

In order to study structural rearrangement of the ribosome during eukaryotic elongation, we bypass canonical initiation using an initiation factor free Internal Ribosome Entry Site (IRES) enabling elongation in the desired reading frame. Ribosomal subunits, purified from Rabbit Reticulocyte Lysate (RRL), are first bound to the IRES. Ribosome-mRNA complexes are then fixed on a microscope coverslip via the 5'end of the mRNA. All factors necessary for elongation are provided by injection of RRL. using single molecule Total Internal Reflection Fluorescence (TIRF) microscopy, we monitor elongation thanks to a fluorescently labelled oligonucleotide hybridized on the mRNA. The oligonucleotide is detached by the helicase activity of a translating ribosome. The evanescent wave generated by the TIRF apparatus allows solely the detection of molecules close to the surface. In this configuration, probe detachments induce the loss of fluorescence signals which are used to measure the distribution of translation speed. A thorough care is given to photobleaching during data analysis. Controls using antibiotics further show that the loss of fluorescence is a signature of a single ribosome activity.

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Deciphering the Nature of the 30S:BipA Ribosome Complex

Ala M. Shaqra.
University of Connecticut, Storrs, CT, USA.

BipA is a member of the translational family of GTPases and is required for bacterial survival under various adverse environmental conditions. It is the only protein that differentially binds to two ribosomal particles. Under normal growth conditions when GTP levels are high, BipA associates with 70S ribosomes. However, during stress or other unfavorable environmental changes, BipA binds to 30S ribosomes. This association is driven by increased levels of guanosine tetraphosphate (ppGpp), an alarmone responsible for adaptation to adverse growth conditions in bacteria. The significance of this differential association is not understood. We identified a single site substitution in BipA that functions to suppress the cold sensitivity of an *Escherichia coliinfA* (initiation factor 1, IF1) deletion strain suggesting a role for BipA in translation initiation events. Biochemical and biophysical measurements of the GTPase activity of this protein reveal that it binds ppGpp with similar affinity as wild type BipA, binds GTP weakly and only associates with the 30S ribosomal particle. Coupled with our crystallographic data, these results indicate that this protein behaves similarly to the ppGpp-bound form of wild type BipA. These data suggest that BipA acts as mediator between the cellular environment and the ribosome, possibly modulating the translation initiation events in response to cell stress.

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Free-Energy Landscape of Ribosome Translocation Analysed using MD Simulations and Cryo-EM Density Maps

Hisashi Ishida, Atsushi Matsumoto.
Japan Atomic Energy Agency, Kyoto, Japan.

Ribosome is one of the supra-biomolecules used in the process of translating genetic information for the synthesis of polypeptides. In the course of its synthesis, two tRNA molecules move with mRNA through ribosome, changing their positions at the A (animoacyl), P (peptidyl), and E (exit) sites. This process, called translocation of tRNAs, is catalyzed by the elongation factor G (EF-G) using energy of GTP hydrolysis. Recent results from pre-steady-state kinetic analysis and cryo-electron microscopy (cryo-EM) suggest that there is a dynamic multistep process during translocation, where large-scale structural rearrangements of both ribosome and EF-G occur. However, the dynamic mechanism of translocation is unclear at the atomic level.

We used all-atom molecular dynamics (MD) simulations to direct 70S ribosome complexed with EF-G at the post-translocational state (PDB code: 2WRJ) towards the translocational and pre-translocational states (EMDB code: EMD-1365 and EMD-1363) by fitting 70S ribosome into cryo-EM density maps. Additionally, the simulations were assisted by umbrella sampling simulations, in which biased potentials were imposed on the centers of masses of the protein molecules in the 70S ribosome, to relax the transitional conformations and to construct the free-energy landscape of the translocation. Multistep structural changes, such as a ratchet-like motion between the small and large ribosomal subunits, and a hinge-like motion of elongation factor G (EF-G) were observed during the translocation. The free-energy landscape shows that there are semi-stable states between two stable states at the pre- and post-translational states. It was shown that a loop of nucleic acids from the large ribosomal subunit, which is located near the P- and E-sites, plays an important role in the translocation of P-tRNA and E-tRNA.

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Study of Helicase Activity of the Ribosome using Single-Molecule FRET

Yi-Lan Chen, Kai-Chun Chang, Jin-Der Wen.
Institute of Molecular and Cellular Biology, Naitonal Taiwan University, Taipei, Taiwan.

RNA structures may become barriers to the ribosome during translation. In the meanwhile, this feature is adopted to regulate translation in some systems such as ribosomal frameshifting. Previous studies have shown that the ribosome itself can open RNA duplex structures. Recent experiments using optical tweezers further revealed that the ribosome opens RNA hairpin structures by using two active mechanisms. However, RNA structures under the action of ribosomes are still elusive. Therefore, we aim to use single-molecule FRET (Förster Resonance Energy Transfer) to directly observe the interaction between the ribosome and RNA structures. In this study, we use an RNA which begins with six unique codons. This RNA is paired with different DNA oligomers labeled with Cy3 or Cy5 dye on either or both of the ends. These hybrids have been measured, in the absence of ribosomes, to characterize its fluorescent and FRET aspects. Next, they will be translated by our purified *in vitro* translation system. Therefore, we could control each translocation step of the ribosome by adding one unique aminoacyl-tRNA at a time. With this design, we will be able to observe ribosomal helicase activity when the ribosome acts on the structures at various states.

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Single-Molecule Study on Structural Rearrangement of the 5'UTR of *rpsO* Messenger RNA

Yi-Ju Wu, Cheng-Han Wu, Jin-Der Wen.
Institute of Molecular and Cellular Biology, National Taiwan University, Taipei, Taiwan.

Translation initiation of mRNA can be regulated through different ways in the cell. One of the common mechanisms is to modulate the structural elements of mRNA. Escherichia coli ribosomal protein S15 (ecS15, encoded by the *rpsO* gene) regulates its own biosynthesis by interacting with the 5' untranslated region (5'-UTR) of its cognate mRNA. When ecS15 is synthesized in excess in the cell, the protein represses translation via binding to the 5'UTR of its mRNA and blocks the ribosome from accessing the initiation site. The 5'UTR region of *rpsO* mRNA can fold into either a pseudoknot or a double-hairpin structure, but only the former can bind the ribosome and ecS15. The pseudoknot and double-hairpin structures exist in equilibrium in solution. While the pseudoknot form has been dissected extensively in previous studies, the function of the double-hairpin is still unknown. In this study, we manage to characterize the structural dynamics of the 5'UTR of the *rpsO* mRNA by using optical tweezers. This technique allows us to observe conformational change of single RNA molecules in real time. Our preliminary results show that the double-hairpin structure can be rearranged to the pseudoknot conformation. We also observed some structures other than the pseudoknot and double-hairpin, and the structures have similar unfolding transition distances as the pseudoknot. According to our present data, we suggest that the double-hairpin structure may be a necessary and key intermediate in the folding pathway to the pseudoknot and that some pseudoknot-like structures can form from the 5'UTR of the *rpsO* mRNA, though their functions remain elusive.

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Rearrangements of Nascent Peptide Inside the Ribosomal Exit Tunnel

Jianli Lu, **Carol Deutsch**.
University of Pennsylvania, Philadelphia, PA, USA.

All proteins, from bacteria to man, are made in the ribosome and are elongated, one residue at a time, at the peptidyl transferase center (PTC). This growing peptide chain wends its way through the ribosomal tunnel to the exit port, ~ 100 angstroms from the PTC. Regulation of the movement of the peptide within the tunnel and allosteric communication along the tunnel during