

simulation determined that the inserted H segment is unlikely to climb the TR barrier in parallel with the peptide synthesis chemical step and that the nascent chain should first overcome the chemical barriers and move into the ribosome-TR gap region before the insertion into the TR tunnel. Furthermore, the simulations indicate that the coupled TR-chemistry free energy profile accounts for the biphasic force. Apparently, although the overall elongation/insertion process can be depicted as a tug-of-war between the forces of the TR and the ribosome, it is actually a reflection of the combined free-energy landscape. Most importantly, the present study helps to relate the experimental observation of the biphasic force to crucial information about the elusive path and barriers of the TR insertion process.

2487-Pos Board B179

Structure of the Mammalian Ribosomal 43S Preinitiation Complex Bound to the Scanning Factor DHX29

Yaser Hashem¹, Amedee des Georges¹, Vidya Dhote², Robert Langlois¹, Hstau Y. Liao¹, Robert A. Grassucci¹, Christopher U.T. Hellen², Tatyana V. Pestova², Joachim Frank¹.

¹Columbia University, New York, NY, USA, ²SUNY Downstate Medical Center, Brooklyn, NY, USA.

Eukaryotic translation initiation begins with assembly of a 43S preinitiation complex. First, Met-tRNA_i^{Met}, eukaryotic initiation factor 2 (eIF2) and GTP form a ternary complex (TC). The TC, eIF3, eIF1 and eIF1A cooperatively bind to the 40S subunit yielding the 43S preinitiation complex, which is ready to attach to mRNA and start scanning to the initiation codon. Scanning on structured mRNAs additionally requires DHX29, a DEXH-box protein that also binds directly to the 40S subunit. Here, we present a cryo-electron microscopy structure of the mammalian DHX29-bound 43S complex at 11.6Å resolution. It reveals that eIF2 interacts with the 40S subunit via its α -subunit and supports Met-tRNA_i^{Met} in a novel P/I orientation (eP/I). The structural core of eIF3 resides on the back of the 40S subunit establishing two principal points of contact, whereas DHX29 binds around helix 16. The structure provides insights into eukaryote-specific aspects of translation, including the mechanism of action of DHX29.

Reference:

Hashem, Y. *et al.* Structure of the mammalian ribosomal 43S preinitiation complex bound to the scanning factor DHX29. *Cell* 153, 1108-1119 (2013).

2488-Pos Board B180

Identification of Ribosome Biogenesis Factors Regulated by Nucleolar AAA-ATPase NVL2

Masami Nagahama, Nobuhiro Hiraishi, Yo-ichi Ishida.

Dept. of Biochemistry, Meiji Pharmaceutical University, Tokyo, Japan.

We previously reported that nucleolar AAA-ATPase NVL2 participates in 60S ribosome biogenesis and that its interaction with an RNA helicase MTR4/DOB1 may participate in this process. In general, AAA family ATPases act on a variety of intracellular complexes to facilitate their dissociation in an ATP dependent manner. Since MTR4/DOB1 is involved in the processing of rRNA precursors by cooperating with RNA exosome, a 3'-5' exonuclease complex, NVL2 may regulate these RNA-processing machineries. In this study, by using a proteomic analysis, we have screened for proteins, whose association with the complex are regulated by the NVL2 ATPase activity.

First, HEK293 cells that can inducibly overexpress wild-type or mutated NVL2 were further transfected with an expression plasmid for FLAG-tagged MTR4/DOB1 to establish double-stable cell lines. Then MTR4/DOB1-associated proteins were purified from the cell extracts by coimmunoprecipitation using anti-FLAG immunoaffinity beads followed by MALDI-TOF MS protein identification. We were able to identify the known components of the exosome complex such as RRP6, RRP4, RRP40, RRP42, and RRP43. We next compared the protein components of these complexes among the cells expressing wild-type and mutated NVL2 by fluorescent two-dimensional differential gel electrophoresis (2D-DIGE) analysis. As a result, increased intensities of eleven protein spots were specifically detected by expressing an ATP hydrolysis mutant of NVL2 (E365Q/E682Q). These proteins might be potential targets of NVL2, which are dissociated from the MTR4/DOB1-containing complexes during ribosome biogenesis in conjunction with NVL2 ATP hydrolysis.

2489-Pos Board B181

The Cryo-EM Structure of the Ribosome Bound to BipA

Danny N. Ho¹, Ala Shaqra², Wen Li³, Megan A. deLivron², Gil Dionne², Ning Gao⁴, Victoria L. Robinson², Joachim Frank^{3,5}.

¹Biological Sciences, Columbia University, New York, NY, USA,

²Molecular and Cellular Biology, University of Connecticut, Storrs, CT,

USA, ³Biochemistry and Molecular Biophysics, Columbia University, New

York, NY, USA, ⁴School of Life Sciences, Tsinghua University, Beijing,

China, ⁵Howard Hughes Medical Institute, Chevy Chase, MD, USA.

BipA (also known as TypA or YihK) is a highly conserved translational GTPase that exhibits ribosomal binding. While it shares high homology with well-characterized elongation factors such as LepA, the ribosomal back-translocase, and EF-G, the translocase, BipA has distinct functions and mechanisms. Studies have found that BipA participates in a variety of processes such as the expression of pathogenicity islands in enteropathogenic *E. coli* (EPEC), defense against bactericidal peptides in *S. enterica*, low-temperature and low-pH response, and adaptation pathways under sudden stress conditions. With such high conservation and varied functions, BipA has been implicated as a global regulator of cellular processes and maintenance. It has also been shown that the addition of intact 70S ribosomes to BipA *in vitro* enhances its GTPase activity.

Throughout these various studies, the full function, targets, and mechanism of BipA has remained elusive. We present progress toward obtaining a high-resolution cryo-EM structure of the *S. enterica typhimurium* 70S ribosome bound to BipA-GMPPNP. Our current 9Å reconstruction, while not high enough in resolution to resolve residue-nucleotide interactions, confirms that BipA's binding site overlaps with that of EF-G. This is in accordance with biochemical assays that show competition between EF-G and BipA for binding. Additionally, the structural features of BipA in the reconstruction suggest that it has a flexible CTD and its bound form is different from the free-floating state, resembling properties of EF-G. Moreover, our structure suggests that the stabilization of BipA binding to the ribosome may be enhanced by the presence of an A-site tRNA. The flexibility of the CTD may aid in the formation of additional ribosomal contacts that enhance BipA's GTPase activities. Indeed, new biochemical data reinforce these observations and may suggest the existence of a novel mechanism of translational regulation.

2490-Pos Board B182

Quantifying the Spatial Organization of Bacterial Ribosomes using Three-Dimensional Super-Resolution Microscopy

Marissa Lee¹, Jared Schrader², Gene-Wei Li³, Jonathan Weissman³, Harley McAdams², Lucy Shapiro², W.E. Moerner¹.

¹Chemistry, Stanford University, Stanford, CA, USA, ²Developmental Biology, Stanford University School of Medicine, Stanford, CA, USA,

³Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA, USA.

Correctly localizing proteins is essential for many biological processes. Since bacteria lack organelles compartmentalizing transcription and translation, it is possible that the site of protein synthesis is organized around the location of the corresponding gene. This would provide a mechanism to create a high local concentration of a specific protein. Past work supports this hypothesis of complete spatial and temporal overlap between the positions of active ribosomes and the corresponding gene (model 1). However, other results support a conflicting model with separation (on the order of hundreds of nanometers) between the gene and its site of translation (model 2). The latter case also implies that transcription and translation do not occur simultaneously. The small size of bacteria prohibits using conventional fluorescence imaging to determine the positions of individual ribosomes. To overcome this, we apply the method of 3D super-resolution microscopy which reveals individual ribosome position with tens of nanometer precision. In order to distinguish between models 1 and 2, we combine 3D super-resolution microscopy with the translation level of individual genes mapped to cell position on a population of *Caulobacter crescentus* cells in the same developmental state. According to model 1, high spatial density of ribosomes correlates with the positions of highly translated genes on the chromosome. We quantify the level of correlation for individual cells and compare the correlation with simulated ribosome positions from model probability distributions. Combining high resolution single molecule information with translation profiling data reveals the underlying spatial heterogeneity of ribosome organization on a well-defined system.

2491-Pos Board B183

Elucidating Ribosomal Translocation with Internal Coordinate Flexible Fitting

Samuel C. Flores.

Cellular and Molecular Biology, Uppsala University, Uppsala, Sweden.

Determining conformational changes of large macromolecules is challenging experimentally and computationally. The ribosome has been observed crystallographically in several states but many others have been seen only by low-resolution methods including cryo-electron microscopy. Meanwhile the crucial dynamics between states remain out of reach of experimental structure determination methods. Most existing computational approaches model complexes at all-atom resolution, at very high cost, or use approximations which lose some of the most interesting dynamical details. I have developed Internal Coordinate Flexible Fitting (ICFF), a multiscale method that uses full atomic