Toward a Cryo-Em Structure of the Ribosome Bound to BipA

ARTICLE *in* BIOPHYSICAL JOURNAL · JANUARY 2012

Impact Factor: 3.97 · DOI: 10.1016/j.bpj.2011.11.406

products from a single mRNA but also regulates product distribution as the frameshift efficiency is tuned. Although translation fidelity, e.g. mRNA miscoding, has been extensively studied, the detailed mechanism of frameshifting remains unsolved. For instance, where does the ribosome frameshift? At the first codon or second codon within the slippery sequence, or afterward?
Here we use optical tweezers to capture individual frameshifting events in real time at the single ribosome level. These studies are complemented by liquid-chromatography/mass spectrometry (LC/MS) assays that identify the incorporated amino acid residues among the frameshift products, revealing at which codon the ribosome slips.
We found that the ribosome can frameshift at any of the three codons around the slippery sequence, with the probability of a shift at each position biased by the location and stability of the downstream hairpin. The hairpin also affects the propensity of ribosome stalling at the slippery sequence and determines the overall frameshift efficiency.
While multiple frameshift sites are utilized, the primary location of −1 frameshifting is at the second slippery codon across the hairpin variants examined. By correlating our real-time translation traces with this knowledge of the dominant frameshifting pathway, we are working toward identifying the timing of frameshifting during one translation turnover cycle.
In addition, we observed an *in vitro* preference for translating certain dipeptide steps, such that full-length protein products were effectively synthesized once the types of dipeptide formed among amino acids are chosen properly. This preference indicates a strategy for optimizing synthetic peptide sequence for *in vitro* translation.

347-Pos Board B133
The Magnesium Dependence of Ribosome and tRNA Dynamics in Single Pre-Translocation Ribosomal Complexes
Madeleine K. Jensen[1], Jingyi Fei[1,2], Arianne C. Richard[1,3], Samuel H. Sternberg[1,4], Ruben L. Gonzalez, Jr.[1].
[1]Columbia University, New York, NY, USA, [2]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [3]National Institutes of Health, Bathesda, MD, USA, [4]University of California, Berkeley, CA, USA.
The structural dynamics of the cellular translational machinery are essential to the mechanism of protein synthesis. During the elongation stage of protein synthesis, for example, the translating ribosome oscillates between two functionally important global conformational states, referred to as GS1 and GS2, as it translocates along its messanger RNA (mRNA) template and sequentially incorporates aminoacylated transfer RNA (tRNA) substrates in the order specified by the mRNA. Structural transitions of the pre-translocation (PRE) ribosomal complex between GS1 and GS2 encompass extensive remodeling of ribosome-ribosome, ribosome-tRNA, and ribosome-mRNA interactions and regulate movement of the mRNA and its associated tRNAs through the ribosome by precisely one codon. Motivated by the crucial role that Mg^{2+} ions play in stabilizing RNA-RNA interactions and by the known Mg^{2+} dependence of the translocation reaction, we have used total internal reflection fluorescence microscopy to perform single-molecule fluorescence resonance energy transfer experiments on fluorescently labeled PRE complexes as a function of Mg^{2+} concentration. The data reveal how the thermodynamic stabilities of GS1 and GS2 and, consequently, the rates of GS1â†'GS2 and GS2â†'GS1 transitions in PRE complexes depend on the concentration of Mg^{2+} ions. Interpreting our data within the context provided by the atomic-resolution structures of GS1- and GS2-like ribosomal complexes and the well-characterized Mg^{2+} dependence of the translocation reaction rate will enable us to develop a comprehensive understanding of the way in which specific Mg^{2+} binding sites affect PRE complex conformational dynamics that are critical for the translocation step of translation elongation.

348-Pos Board B134
A Coarse-Grained Simulation Study of Ribosome and tRNA Dynamics during Translocation
Naoto Hori, Shoji Takada.
Kyoto University, Kyoto, Japan.
In the final step of the translation-elongation cycle, the complex of two tRNA and mRNA molecules need to be advanced by exactly one codon in the ribosome. To realize this movement, the two ribosomal subunits are thought to be largely rearranged by the so-called ratchet-like rotation. This dynamic behavior, known as translocation, is essential for the efficient and accurate translation. Although many experiments, including high-resolution structural studies, have been performed, the mechanism of this movement is still unclear.
Molecular dynamics simulation is a good tool to investigate such biological phenomena with atomic resolution. To overcome the limitations of system size and long-time scales, we have developed a coarse-grained (CG) simulation model of RNA and RNA-protein complexes. In this model, each nucleotide is represented as three CG beads and interactions are modeled with a structure-based potential, the parameters of which were determined by a fluctuation matching method to achieve higher accuracy.
Next we employed this CG model to study the dynamic behavior of a ribosome complex. In particular, we focused on the relationship among the inter-subunit motion, fluctuation of the tRNAs in the binding sites, and tRNA-mRNA movement. The inter-subunit rotation is reproduced well by using two reference structures which correspond to the unrotated and fully-rotated state of the ribosome. Some experiments have suggested that tRNAs form hybrid states during the translocation phase, and we were motivated to examine whether or not this occurs in our CG simulations.

349-Pos Board B135
Contacts Between Ribosome Parts Refined by Molecular Dynamics Simulations
Christian Blau[1], Lars V. Bock[1], Gunnar F. Schroeder[2], Niels Fischer[1], Holger Stark[1], Andrea C. Vaiana[1], Helmut Grubmuller[1].
[1]Max Planck Insititut fuer biophysikalische Chemie Goettingen, Goettingen, Germany, [2]Forschungszentrum Juelich, Juelich, Germany.
Biomachines such as the ribosome undergo substantial conformational changes during their work cycle. During ribosomal translocation, in particular, a broad variety of functional contact patches dynamically form and rupture, the detailed characterization of which is still lacking. Here we use extended atomistic simulations of the whole solvated ribosome, starting from 13 distinct translocational substates, to obtain a comprehensive picture of these contact patches. To that aim, we developed a novel analysis tool, which is broadly applicable to large flexible parts of simulated biomolecules, and enabled us to quantitatively extract contact occupancies and changes for all available conformational states.
For the 13 translocational sub-states of the ribosome, molecular dynamics simulations yielded extended all-atom trajectories. From these trajectories the frequency of all possible inter-atomic contacts between the 30S and 50S subunits was determined. As the search for atom contacts scales with the number of particles and simulation length, a fast, hierarchical algorithm based on kd-tree branch exclusion was developed and applied. Subsequently, contacting atom pairs were filtered according to contact frequency and then assigned to residues. From this information a graph was constructed whose edges connect contacting residues. The regions identified form this analysis provided a rigorous, intuitive, and comprehensive picture of ribosomal contact patches during transloaction, and explained how the ribosome maintains its fine-tuned intersubunit affinity despite drastic conformational changes.

350-Pos Board B136
Toward a Cryo-Em Structure of the Ribosome Bound to BipA
Danny N. Ho[1], Megan A. deLivron[2], Ning Gao[3], Victoria L. Robinson[2], Joachim Frank[1,4].
[1]Columbia University, New York, NY, USA, [2]University of Connecticut, Storrs, CT, USA, [3]Tsinghua University, Beijing, China, [4]Howard 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 proteins such as LepA, the ribosomal backtranslocase, 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 ribosomes to BipA *in vitro* enhances its GTPase activity.
Throughout these various studies, the full function, targets, and mechanism of BipA remains elusive. We present progress toward obtaining a Cryo-EM structure of the *S. enterica typhimurium* 70S ribosome bound to

BipA-GMPPNP. Our current model, 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 flexible domains and its bound form is different from the free-floating state, resembling properties of EF-G. This flexibility may aid in additional ribosomal contacts that enhance its GTPase activities.

351-Pos Board B137

Modelling Programmed Ribosomal Frameshifting with Elastic Network Model and Linear Response Theory

Kai-Chun Chang[1], Jin-Der Wen[1], Lee-Wei Yang[2].
[1]Institute of Molecular and Cellular Biology, National Taiwan University, Taipei, Taiwan, [2]Institute of Bioinformatics and Structural Biology, National Tsing-Hua University, Hsinchu, Taiwan.

The *dnaX* gene in *Escherichia coli* encodes comparable amounts of τ and γ subunits of DNA polymerase III by programmed ribosomal frameshifting (PRF). An early termination codon in the -1 frame of *dnaX* produces the γ subunit, which is a truncated form of the τ subunit. PRF is also involved in protein expression in many viruses, such as the Gag-Pol polyprotein in HIV. Thus, PRF provides an intrinsic regulating pathway at the translation level from the same mRNA.

In the *dnaX* mRNA, an upstream internal Shine-Dalgarno (SD) sequence, which lies within the open reading frame and forms base-pairing with the 3'-end of 16S rRNA, together with a downstream stem-loop structure promote PRF on the slippery site. However, the detailed mechanisms for ribosome stalling and backtracking during PRF remain to be elucidated. Here, we combine coarse-grained elastic network model (ENM) and linear response theory to model the intrinsic and perturbed dynamics of the ribosome; the latter results from the formation of SD-helix and the forces exerted by stem-loop hindrance during translation. We hypothesize that these opposing forces for ribosome progression, albeit from different RNA elements, induce similar conformational changes for the ribosome, possibly in an allosteric fashion. These changes in the ribosome are expected to promote the observed PRF efficiency. Ultimately, we wish to provide the mechanochemical bases of PRF through computational modelling and validations by single-molecule force measurements.

352-Pos Board B138

Site-Specific Labeling of Saccharomyces Cerevisiae Ribosomes

Anmol Grover, Marcel P. Bruchez.
Carnegie Mellon University, Pittsburgh, PA, USA.

The eukaryotic ribosome has more structural and functional complexity than lower organisms, yet has been the target of fewer biophysical studies. This is due to the difficulties with site specific labeling of ribosomes in eukaryotic cells and lysates. But the recent atomic resolution yeast ribosome structure and cryo-EM studies have resulted in renewed interest in the biophysical mechanisms of eukaryotic translation. We have explored the use of γ-PNA that can potentially bind to solvent exposed and phylogenetically variable sites in functional yeast ribosomal subunits: 60S and 40S. This method of labeling can be further exploited to label other sites on the yeast ribosomes and potentially a wide range of native eukaryotic ribosomes.

353-Pos Board B139

Molecular Architecture of a Late 40S Ribosome Assembly Intermediate

Dr. Harish Vashisth, Prof. Charles L. Brooks, III.
University of Michigan, Ann Arbor, MI, USA.

Ribosomes are self-assembling macromolecular machines which catalyze the synthesis of proteins in all cells, and are made up of two subunits that differ in size due to the presence of RNA and proteins in different ratio. Although significant structural details of the organization of mature ribosomes in prokaryotes and eukaryotes have been elucidated in the past decade, ribosome biogenesis and assembly processes are poorly understood. Ribosome assembly in eukaryotes requires at least 200 essential transiently associating assembly factor proteins that facilitate the RNA folding and recruitment of ribosomal proteins. We have determined the structure of a late cytoplasmic 40S ribosome assembly intermediate from Saccharomyces Cerevisiae with the help of Cryo-electron microscopy and multiscale molecular simulation techniques. We have collected maps of the immature 40S particles and its components at various resolutions, and apply molecular dynamics based flexible fitting (MDFF) to fit an initial atomic model in to the cryo-EM maps of immature 40S particles with minimal structural perturbation. We verify that the largest conformational change observed in RNA can be robustly generated with an independent technique called normal mode flexible fitting (NMFF). We find that the pre-40s particles in addition to ribosomal RNA and proteins also contain 7 types of assembly factors, the joint activity of which blocks the translation initiation pathway in immature 40S subunits.

Protein-Nucleic Acid Interactions I

354-Pos Board B140

Single-Molecule In Vitro and Live-Cell Studies of Allostery through DNA

Shasha Chong[1], Sangjin Kim[1], Erik Brostrmer[1], Dong Xing[2], Hao Ge[1,3], Xiaodong Su[2], Yujie Sun[2], X. Sunney Xie[1,2].
[1]Department of Chemistry & Chemical Biology, Harvard University, Cambridge, MA, USA, [2]Biodynamic Optical Imaging Center, Peking University, Beijing, China, [3]School of Mathematical Sciences & Centre for Computational Systems Biology, Fudan University, Shanghai, China.

Often in nature, a macromolecule undergoes conformational changes upon binding of a ligand in order to modify its affinity for another ligand at a distant binding site. This phenomenon, called ''allostery'', is a fundamental mechanism for dynamic regulation of macromolecular properties. Although allostery is well-documented in proteins, it is less recognized for DNA-protein interactions, in which DNA has been often considered a mere template providing recognition sequences for proteins. Here we investigate the allosteric interactions through DNA both in single molecule experiments in vitro and in live cells. In the in vitro experiments, we demonstrate that when two proteins specifically bind to DNA within tens of base pairs, the binding affinity of one protein is altered by the other. We prove that this is not due to protein-protein interactions but to allostery through DNA. As the distance between the two proteins is varied, this allosteric coupling oscillates between positive and negative cooperativity with a periodicity of ~10 base pairs, the helical pitch of the B-form DNA. The allostery through DNA is explained in terms of the free energy associated with the overall conformation of the ternary complex. We also demonstrate that such allostery affects gene expression in live E. coli cells, suggesting its physiological relevance.

355-Pos Board B141

Remote Control of DNA-Acting Enzymes by Molecular Boundary Conditions

Hua Bai[1], James Kath[1], Felix Zorgebiel[1], Nigel D.F. Grindley[2], John F. Marko[1].
[1]Northwestern University, Chicago, IL, USA, [2]Yale University, New Haven, CT, USA.

The rate of action of an enzyme at a point along a long DNA molecule is usually assumed to be determined by the local properties of that region of the nucleic acid chain such as its sequence, flexibility, and molecular stress. Here we show that enzymes that relax DNA torsional stress display rates which strongly depend on how the distant ends of the molecule are constrained: experiments with different size colloidal particles tethered to the end of 10 kb DNAs reveal enzyme rates that are inversely correlated with particle drag coefficient. This effect can be understood in terms of the coupling between molecule extension and local molecular stresses: the rate of bead thermal motion controls the rate at which transition states are visited in the middle of a long DNA. Torsional stress is not a requirement for this effect. Importantly, some enzymes show rates unaffected by bead size. Our results reveal a new mechanism through which variation in chromosome domain architecture, and more generally, large-scale architecture of large thermally fluctuating substrates, can control enzyme rates.

356-Pos Board B142

A Hybrid Approach to Understand the Organization of the Mycobacterial Partition Assembly

Barnali N. Chaudhuri.
Hauptman Woodward Institute, Buffalo, NY, USA.

The segrosome or the bacterial chromosome partition cassette (ParABS) is a molecular motor, which is composed of two proteins (ParB and ParA) and a set of centromere-like DNA sequences (*parS*). ParBs spread by self-associating on the *parS*-proximal chromosome to form the partition assembly, which is a nucleoprotein filament of unknown nature. The partition assembly