than ~50 nM due to high background signals. The zero-mode waveguide (ZMW) is a nanophotonic structure consisting of a hole in a metal film on a transparent substrate. In conjunction with laser-excited fluorescence, they provide observation volumes on the order of zeptoliters, three to four orders of magnitude smaller than far-field excitation volumes, allowing fluorescence detection in the μM range. Here, we apply ZMWs and novel detection instrumentation developed by Pacific Biosciences and demonstrate direct observation of multiple rounds of binding and release of tRNAs at high concentrations on various mRNA templates during protein synthesis.

The applicability of ZMWs to translation was confirmed by recapitulation of prior single molecule experiments. We delivered ternary complex of EF-Tu(GTP) with Phe-Cy5-tRNA^{Phe} into the A site of ribosomal initiation complexes with fMet-tRNA^{fMet} with Cy3 in P site immobilized on ZMW surface. FRET observation and tRNA arrival time measured in ZMW at any states was consistent with the prior results obtained by TIRF. Next we monitored the elongation cycle through multiple arrival events of dye-labeled tRNAs onto mRNA-programmed ribosomes. Using a fMet-Phe-Phe-Phe mRNA template, we observed multiple arrivals (up to 3) of Cy5-labeled phe-tRNA^{Phe} in the presence of EF-G; event number and duration depend as expected on EF-G concentration. We demonstrated multicolor observation of three tRNAs using Cy2-labeled Lys-tRNA^{Lys} with Cy2 enables to observe three color elongation cycles for fMet-Phe-Lys-Phe or fMet-(Phe-Lys)x6 mRNA template with green, red and blue excitations. These experiments show that we can observe translation in real time from the perspective of tRNA ligands, and will reveal aspects of translation such as time-dependent tRNA occupancy of the ribosome.

1361-Pos

Following Movement of the L1 Stalk between Three Functional States in Single Ribosomes

Dmitri N. Ermolenko[1], Peter V. Cornish[2], David W. Staple[1], Lee Hoang[1], Robyn P. Hickerson[1], Taekjip Ha[2], Harry F. Noller[1].
[1]Department of Molecular, Cell and Developmental Biology and Center for Molecular Biology of RNA, University of California, Santa Cruz, CA, USA, [2]Department of Physics, University of Illinois, Urbana-Champaign, IL, USA.

The L1 stalk is a mobile domain of the large ribosomal subunit E site that interacts with the elbow of deacylated tRNA during protein synthesis. Using single-molecule FRET, we follow the real-time dynamics of the L1 stalk and observe its movement relative to the body of the large subunit between at least three distinct conformational states: open, half-closed and fully-closed. Pre-translocation ribosomes undergo spontaneous fluctuations between the open and fully closed states. In contrast, post-translocation ribosomes containing peptidyl-tRNA and deacylated tRNA in the classical P/P and E/E states, respectively, are fixed in the half-closed conformation. In vacant ribosomes, the L1 stalk is observed either in the fully closed or fully open conformation. Several lines of evidence show that the L1 stalk can move independently of intersubunit rotation. Our findings support a model in which the mobility of the L1 stalk facilitates binding, movement and release of deacylated tRNA by remodeling the structure of the 50S subunit E site between three distinct conformations, corresponding to the E/E classical, P/E hybrid and vacant states.

1362-Pos

Side-Chain Reactivity of a Nascent Peptide in the Ribosomal Exit Tunnel

Jianli Lu[1], Zhengmao Hua[2], William R. Kobertz[2], **Carol Deutsch**[1].
[1]Department of Physiology, University of Pennsylvania, Philadelphia, PA, USA, [2]Department of Biochemistry and Molecular Pharmacology, UMASS Medical School, Worcester, MA, USA.

Nascent peptides begin to fold in the ribosomal exit tunnel. This is not a unilateral act by the peptide. The tunnel, ~100Å in length and 10-20 Å in width, collaborates and is an active participant in translation. The precise mechanisms for this teamwork are unknown. To probe these peptide-tunnel interactions, we have engineered different side-chains adjacent to a cysteine in a molecular tape measure positioned at various locations inside the tunnel. In each case, we measured the kinetics of cysteine modification with a series of reagents of increasing size: trimethyl-, triethyl-, tripropyl-, and tributyl-ammonium maleimides. Three conclusions may be drawn. First, for a given side-chain and a given reagent, the modification rates decrease in going deeper into the tunnel from the exit port to the peptidyltransferase center (PTC). Second, the ratio of modification rate constants for trimethyl to tributyl reagent for an identical reporter cysteine increases monotonically with increasing distance into the tunnel from the exit port. Third, the tunnel near the exit port is relatively insensitive to the choice of adjacent side-chain, whereas a site deeper in the tunnel exhibits side-chain dependent reactivities. As a given amino acid moves along the tunnel during peptide elongation, its interactions with the tunnel are both site-specific and tuned to the unique primary sequence of each nascent peptide. [Supported by NIH grant GM 52302].

1363-Pos

A Universal Zone in the Ribosomal Exit Tunnel for Helix Formation in Kv1.3

LiWei Tu, **Carol Deutsch**.
Department of Physiology, University of Pennsylvania, Philadelphia, PA, USA.

Crystal structures of Kv channels have given us a reasonably complete view of the structure of a mature Kv channel. However, details of its structure acquisition are missing. We now report on the biogenesis of secondary structure of the transmembrane segments and intervening linkers of Kv1.3. Using a combination of accessibility assays, both cysteine pegylation (Tu et al., 2007; Lu and Deutsch, 2005) and N-linked glycosylation (Mingarro et al., 2000), we derive the following principles of folding for nascent sequences within their native contexts. First, native helical transmembrane sequences initially form helices only within the distal 20Å of the ribosomal tunnel near the exit port. We refer to this region as the α-zone. Helix formation in transmembrane segments thus occurs vectorially from N- to C-terminus as each segment moves sequentially into the α-zone. Second, linker sequences also form compact structures inside the α-zone even in the absence of helix formation of their C-terminal flanking transmembrane segments. Third, helical structures, whether transmembrane or linker segments, formed in the tunnel retain their helicity in the translocon. These principles emerge from a diversity of native transmembrane and linker sequences that comprise Kv1.3 and may therefore be applicable to protein biogenesis in general.

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References: Tu et al., Biochemistry 46: 8075, 2007; Lu and Deutsch, Biochemistry 44: 8230, 2005; Mingarro et al., BMC Cell Biol. 1: 3, 2000.

1364-Pos

Single- Molecule Force Measurement for 30S- mRNA Interaction in Translation Initiation

Tomoaki Masuda[1], Ryo Iizuka[1], Takashi Funatsu[1], Sotaro Uemura[2].
[1]The University of Tokyo, Tokyo, Japan, [2]Stanford University, Stanford, CA, USA.

Bacterial ribosome is a molecular machine composed of 30S and 50S subunits that translates the genetic code in mRNA into an amino acid sequence through repetitive cycles of tRNA selection, peptide bond formation and translocation. Translation initiation is one of the essential processes in protein synthesis that involves the assembly of initiator fMet-tRNA^{fMet} and three initiation factors (IF1, IF2 and IF3) to 30S subunit with GTP hydrolysis by IF2 to form 70S-mRNA-fMet-tRNA^{fMet} complex efficiently and correctly. These processes are occurred with dynamic intersubunit rotation and repositioning of IF2 and initiator tRNA. We expect that GTP hydrolysis by IF2 plays a key role in 30S-mRNA interactions as well. To understand the dynamics of 30S-mRNA interactions, we performed rupture force measurement between 30S subunit and mRNA by optical tweezer assay.

The rupture force for 30S-mRNA complex in the absence of tRNAs and any IFs showed a single distribution with a peak at 5.7 pN. The addition of tRNA^{fMet} to the complex increased the rupture force to 15.2 pN, while the rupture force for 70S-mRNA-tRNA^{fMet} complex showed 16.5 pN. These results show that the binding of tRNA^{fMet} to the 30S-mRNA complex contributes to the initiation stability, which is greater than 50S binding.

Intriguing results were obtained in the presence of IF2. The rupture force for 30S-mRNA-fMet-tRNA^{fMet}-IF2(GTP) showed 16.1 pN, which is not significantly different from 15.2 pN for 30S-mRNA- tRNA^{fMet}. However, the binding of 50S subunit to this complex led to significant changes, the rupture force for 70S-mRNA-fMet-tRNA^{fMet}-IF2(GDPNP) and 70S-mRNA-fMet-tRNA^{fMet}-IF2(GDP) showing 22.1 pN and 20.5 pN, respectively. These results indicate that IF2 with GTP hydrolysis contributes the initiation stability in 30S-mRNA interactions, which enables efficient initiation of protein synthesis.

1365-Pos

Structural Analysis of Bound Molecules to Ribosome by EM-Fitting

Atsushi Matsumoto.
Japan Atomic Energy Agency, Kizugawa, Japan.

In the previous study, we systematically analyzed many three-dimensional electron microscopy (EM) density maps of 70S ribosome at various functional states available in the EM DataBank to reveal the global conformational differences between the 70S ribosome structures by our new flexible-fitting approach, in which the best-fitting atomic model for each EM map was built by deforming the PDB structure of the 70S ribosome using normal mode analysis of the elastic network model.