In this study, we analyze the structures of smaller molecules bound to ribosome, such as tRNA and elongation factors, which are included in the EM density maps of the 70S ribosome, by building atomic models of these molecules in the bound state. The structures of these bound molecules may be different from those in the isolated state or the X-ray crystal structures. In this analysis, we need to spot these molecules in the EM density map, which is occupied mostly by the 70S ribosome. By fitting the atomic model of the 70S ribosome into the EM density map and eliminating the density of the regions overlapped with the atomic model, we are able to extract the regions for the bound molecules. Our results show that the best-fitting atomic model of the 70S ribosome built in our previous sturdy can extract the regions for the bound molecules more clearly than the original PDB structure.

1366-Pos

Spontaneous Vs. Allosteric Dissociation of E-Site tRNA During Polypeptide Elongation

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During ribosome-catalyzed polypeptide chain elongation, dissociation of the deacylated tRNA from the E-site has been proposed to be either spontaneous or triggered allosterically by binding of the next cognate ternary complex to the A-site. Using fluorescent labeled tRNAs, we have measured single molecule fluorescence intensities and single molecule FRET between adjacent tRNAs in the ribosome. From these measurements we have been able to determine tRNA occupancy in the ribosome, and thus whether E-site tRNA dissociates before or after A-site occupancy. In the former case there are a maximum of 2 simultaneously bound tRNAs, while the latter case results in transient binding of 3 tRNAs simultaneously. In a total internal reflection florescence microscope, ribosomes were attached to glass microscopes slides via a biotinylated mRNA coding for MRFFRFY.... (single letter amino acid sequence). When synthesis was initiated with $tRNA^{fMet}$ fully charged with formylated-Met, 60-70% of the ribosomes lost their E-site tRNA prior to ternary complex binding (2-tRNA pathway) at the 2nd and 3rd elongation cycles (R and F respectively). In contrast, for synthesis initiated with uncharged initiator $tRNA^{fMet}$, >90% of the ribosomes followed the 2-tRNA pathway in the 2nd cycle, but only ~15% in the 3rd cycle (~75% following the 3-tRNA route, 10% not categorized). In cycles 4 and 5, almost all ribosomes followed the 2-tRNA pathway. Thus, the length of the peptide chain and/or the specific amino acids bound to the P-site tRNA strongly influence the allostery of E-site tRNA dissociation. Such allostery is also sensitive to the presence or absence of polyamines, Mg^{2+} concentration, and the specific codons in positions 2 and 3. Supported by NIH R01grant GM080376 and NIST ATP grant 70NANB7H7011 through Anima Cell Metrology, Inc.

1367-Pos

Enhancement of Single Molecule Fluorescence Signals by Colloidal Silver Nanoparticles in Studies of Ribosome Dynamics

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Metal enhanced fluorescence (MEF), in which a surface plasmon near a noble metal alters the spectral properties of an organic fluorophore, increases fluorescence intensity without a concomitant increase in photobleaching rate. To improve recordings of single molecule fluorescence signals from individual ribosomes, we studied the emission of Cy3- and Cy5-labeled ribosomes and tRNAs attached near 50-80 nm silver colloidal particles on a glass microscope coverslip. The fluorescence of Cy3 and Cy5 labeled initiation complexes (ICs) near 50 nm silver particles was increased 4- and 5-fold, respectively, compared to labeled ICs in the absence of silver colloids. Photobleaching lifetime was not significantly accelerated, resulting in 4-5 fold enhancement of total photon emission before photobleaching. Fluorophores showing enhanced fluorescence were colocalized with the colloidal particles, as detected by light scattering. Other ribosomes or tRNAs that were farther away had intensities similar to those on plain glass. Aggregates of silver colloidal particles themselves produced wavelength-shifted luminescence similar to fluorescence, presumably due to resonance between nearby metal particles. With ribosomes bound to the glass substrate near the silver particles via a short mRNA, interaction between $tRNA^{Arg}$-Cy3 in the ribosomal P-site and fMet-Arg-Phe-$tRNA^{Phe}$-Cy5 in the A-site had FRET efficiency and dynamics similar to ribosomes farther away and on plain glass. Binding of Cy5-Arg-$tRNA^{Arg}$ to ICs labeled with Cy3 on the large subunit protein L11, in the absence of the translocase EF-G, produced FRET efficiency and dynamics characteristic of specific codon-dependent A-site binding. Addition of EF-G reduced FRET efficiency, as expected. These tests demonstrate that the colloidal silver nanoparticles increase fluorescence and total photon emission without compromising the biophysical activity of ribosomes.
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1368-Pos

Establishing a Fluorescence-Based Helicase Assay for Monitoring Eukaryotic Protein Synthesis Initiation

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Efficient mRNA recruitment to the human ribosome requires a region of single stranded RNA close to the cap structure. The eukaryotic initiation factor 4A (eIF4A) is a DEAD box helicase that is essential for unwinding any stable secondary structure that would inhibit this step. Although studied for many years, the majority of helicase assays involving eIF4A and its associated proteins have failed to rigorously analyze the kinetic events that occur during mRNA unwinding. To provide this kinetic understanding we have developed a continuous fluorescence-based assay to measure RNA duplex unwinding events. This assay utilizes an RNA oligonucleotide modified with cyanine 3 (Cy3) annealed to a complimentary strand modified with black hole quencher (BHQ). Separation of the RNA duplex region significantly enhances the Cy3 fluorescence, enabling us to measure RNA helicase activity in real time by fluorescence spectroscopy. Data will be presented to show how we are using this assay to determine the kinetic role of accessory factors on the helicase activity by eIF4A. Moreover, we show how this data combined with a continuous coupled ATPase assay is enabling us to determine the relationship of ATP hydrolysis to the unwinding of duplex RNA by this DEAD box helicase.
We will also present data employing RNA duplexes of different lengths, allowing us to understand how the processivity of eIF4A is influenced by other initiation components.
This study will provide us with the foundation to begin understanding the kinetic framework of mRNA recruitment to the human ribosome.

DNA, RNA Structure & Conformation I

1369-Pos

Structure and Mechanism of the glmS Ribozyme

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The self-cleaving glmS ribozyme is a mechanistically unique functional RNA among both riboswitches and RNA catalysts as its catalytic activity provides the basis of genetic regulation and depends upon glucosamine-6-phosphate (GlcN6P) as a coenzyme. A substantial body of biochemical and biophysical data relating the structure and function of the glmS ribozyme has been amassed, in our laboratory and others, in a relatively short period of time since its discovery. However, a precise and comprehensive mechanistic understanding of coenzyme function in glmS ribozyme self-cleavage has not been elaborated. Careful consideration of the available biochemical and biophysical data relating the structure and function of the glmS ribozyme necessitates that general acid and general base catalysis in a coenzyme-dependent active site mechanism of RNA cleavage are inherently interdependent. We propose a comprehensive mechanistic model wherein the coenzyme, GlcN6P, functions both as the initial general base catalyst and consequent general acid catalyst within a proton-relay thus fulfilling the apparent biochemical requirements for activity. This analysis in combination with other considerations regarding the effects of coenzyme binding on riboswitch structure and function suggests that the development of glmS ribozyme agonists as prospective antibiotic compounds must satisfy strict chemical requirements for binding and activity.

1370-Pos

Antibiotic Development by Investigation of the glmS Riboswitch

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Although bacterial infections have always been of significant interest to researchers and physicians, the drug-resistant bacterial strains that have recently developed are causing new concerns and are much more difficult to combat. Our current methods for treating bacterial infections include broad-spectrum