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The ribosome is the molecular motor responsible for the protein synthesis within all cells. Ribosome motions along the messenger RNA (mRNA) to read the genetic code are asynchronous and occur along multiple kinetic paths. Consequently, observation and manipulation at the single macromolecule level is desirable to unravel the complex dynamics involved. In this communication, we present the study of translation kinetics of single ribosomes via the direct observation of fluorescent amino-acid incorporations.

In order to study the kinetics of amino-acid incorporation inside the growing protein by the ribosome, we use a home-made total internal reflection single-molecule fluorescence microscope (TIRFM). The mRNA-ribosome complex is attached to a polyethylene glycol modified glass coverslip surface by a streptavidin-biotin linkage. The ribosome is labelled with a quantum dot (QD) in order to localize it on the surface while a specific amino acid (lysine) is marked with Bodipy-FL [1]. This fluorescent dye is small enough to enter the ribosomal channel thus leaving intact ribosomal activity. The protein synthesis is observed in real time as the labelled amino acids are incorporated into the polypeptidic chain by the co-localization of QD and Bodipy-FL fluorescence signals.

We will discuss the future application of this technique to single-molecule observation of the translation process, proof reading or even protein folding.

Reference

[1] K. Perronet, P. Bouyer, N. Westbrook, N. Soler, D. Fourmy, and S. Yoshizawa. Journal of Luminescence, 127, 264, 2007.

2983-Pos Board B30

How Initiation Factor 2 Regulates the Fidelity of Translation Initiation

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During prokaryotic translation initiation, the small, 30S and large, 50S ribosomal subunits, along with formylmethionyl-transfer RNA (fMet-tRNA^{fMet}), assemble at an authentic AUG messenger RNA (mRNA) start codon. The fidelity of initiation is regulated by three initiation factors (IFs) which kinetically control individual steps along the reaction pathway. One such step is recruitment of the 50S subunit to a 30S initiation complex (30SIC), composed of the 30S subunit, mRNA, tRNA, and IFs 1, 2, and 3. Although the GTPase IF2 has been shown to be principally responsible for selectively accelerating the rate of 50S subunit joining to a correctly initiated 30SIC, the molecular mechanism underlying this catalytic activity remains unknown. In order to elucidate the mechanism through which IF2 selectively catalyzes 50S subunit joining, we have developed single-molecule Förster resonance energy transfer (smFRET) signals between fluorescently-labeled IF2 and two tRNAs, an initiator fMet-tRNA^{fMet} and an elongator Phe-tRNA^{Phe}. Using these IF2-tRNA smFRET signals, we have characterized the dynamics of IF2-tRNA interactions within correctly and incorrectly initiated 30SICs. Our data reveal that the residence lifetime of IF2 on the 30SIC (τ_{30SIC}), a parameter that directly controls IF2's ability to recruit the 50S subunit to the 30SIC, is highly regulated during initiation. We find that τ_{30SIC} is exquisitely sensitive to: (1) the presence of IF1 and IF3, (2) the GTP- *vs.* GDP- or nucleotide-free forms of IF2, (3) the presence of fMet-tRNA^{fMet} *vs.* Phe-tRNA^{Phe}, and (4) the presence of a correct AUG start codon. Thus, only in the presence of IF1, IF3, GTP, and a correctly formed codon-anticodon interaction between an authentic AUG start codon and an initiator fMet-tRNA^{fMet} is IF2 binding to the 30SIC significantly stabilized such that 50S subunit joining is efficiently catalyzed, ensuring the fidelity of this step of translation initiation.

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Coupling of Ribosomal L1 Stalk and tRNA Dynamics during Translation Elongation

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Translation elongation necessarily requires large-scale movements of transfer RNAs (tRNAs) through the ribosome. While it is likely that these highly-coordinated tRNA movements are directed by conformational changes of the translating ribosome, data directly correlating ribosome and tRNA conformational dynamics are lacking. Using single-molecule ribosome-ribosome and ribosome-tRNA Förster resonance energy transfer (smFRET) signals, we have characterized the intrinsic conformational dynamics of the ribosomal L1 stalk as well as the coupling of L1 stalk and tRNA dynamics during translation elongation. We find that in post-translocation ribosomal complexes, the L1 stalk can occupy either an opened or closed conformation, with the probability and rate of transitions between these two states dependent on the occupancy and identity of the deacylated tRNA in the classical E site. Upon delivery of aminoacyl-tRNA into the ribosomal A site and peptide bond formation, however, a direct interaction between the closed L1 stalk and the newly deacylated tRNA residing in the hybrid P/E tRNA binding configuration is established spontaneously; this event involves coupled movements of the L1 stalk and the ribosome-bound tRNAs, as well as ratcheting of the ribosome. Our data reveal that the entire pre-translocation complex fluctuates between two global conformations: global state 1 (GS1) characterized by an open L1 stalk, classically-bound tRNAs and a non-ratcheted ribosome and global state 2 (GS2), characterized by a closed L1 stalk, hybrid-bound tRNAs, and a ratcheted ribosome. Binding of the ribosomal translocase, elongation factor G (EF-G), shifts the GS1/GS2 equilibrium towards GS2, promoting the intermolecular L1 stalk-tRNA interaction. Pre-steady state smFRET experiments reveal that the L1 stalk-tRNA interaction persists throughout the translocation reaction, suggesting that the L1 stalk allosterically collaborates with EF-G in order to direct tRNA movements during translocation.

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Dynamic Mode Switching and Loosely Coupled Conformational Events Observed in Single Ribosomes

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During protein synthesis transfer RNA (tRNA) and messenger RNA (mRNA) translocate through the ribosome in a rapid and stepwise fashion, a process that is catalyzed by the GTPase elongation factor G (EF-G). Single-molecule fluorescence resonance energy transfer (smFRET) measurements show that the ribosome complex can spontaneously adopt a key translocation intermediate in the absence of nucleotide hydrolysis. By obtaining smFRET data from multiple structural perspectives, we observe that tRNA and EF-G restructure the ribosome energy landscape, increasing the rate at which this intermediate is achieved. In particular, EF-G binding induces a fast dynamic mode of the L1 stalk, which facilitates rapid and reversible formation of the translocation intermediate. Bulk measurements indicate that the extent of conformational coupling is important in determining the rate of translocation. When the extent of coupling is increased, a faster rate of translocation is achieved. The rate of translocation is decreased when the conformational changes are further decoupled, such as by the addition of the antibiotic viomycin. Thus, the extent of coupling of conformational processes on the ribosome may present a new mechanism for the regulation of gene expression.

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An Allosteric Pathway Revealed in the Ribosome Binding Stress Factor BipA

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BipA is a highly conserved prokaryotic GTPase that functions as a master regulator of stress and virulence processes in bacteria. It is a member of the translational factor family of GTPases along with EF-G, IF-2 and LepA. Structural and biochemical data suggest that ribosome binding specificity for each member of this family lies in an effector domain. As with other bacterial GTPases, the ribosome binding and GTPase activities of this protein are tightly coupled. However, the mechanism by which this occurs is still unknown. A series of experiments have been designed to probe structural features of the protein to see if we can pinpoint specific areas of BipA, perhaps even individual residues, which are important to its association with the ribosome. Included in the list are the C-terminal effector domain of the protein, which is distinct to the BipA family of proteins, and amino acid residues in the switch I and II regions of the G domain. Using sucrose density gradients, we have shown that the C-terminal domain is required in order for BipA to bind to the ribosome. Moreover, deletion of this domain increases the GTP hydrolysis rates of the protein, likely through relief of inhibitory contacts. Additional evidence has revealed an allosteric connection between the conformationally flexible switch II region and the C-terminal domain of BipA. Site directed mutagenesis, sucrose gradients and malachite green assays are being used to elucidate the details of this coupling.

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Rare Codon Clustering: Implications for Protein Biogenesis

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Most amino acids are encoded by more than one codon. These synonymous codons are not used with equal frequency: in every organism, some codons are used more commonly, while others are more rare. Though the encoded protein sequence is identical, selective pressures favor more common codons for enhanced translation speed and fidelity. However, rare codons persist, presumably