in the cytoplasmic side of the transmembrane domain of integrin α- and β-subunits named as the inner membrane clasp, a hydrophobic packing of a few transmembrane residues on extracellular side between α- and β-subunits that is termed as the outer membrane clasp, and the key interaction group of βA domain and βTD domain. Molecular details of this key interaction group as well as events that lead to detachment of βTD and βA domains have remained ambiguous.
Full-length structure of integrin αIIbβ3 embedded in a patch of lipid bilayer was used to simulate its interactions with three soluble RGD ligands as well as talin, using a molecular dynamics software. We showed that talin's interaction with the membrane-proximal and membrane-distal regions of integrin cytoplasmic-transmembrane domains significantly loosens the inner membrane clasp as well as an additional salt-bridge (R734-E1006), which facilitates integrin activation through the separation of integrin's α- and β-subunits. Also, it is shown that interaction of the Arg of the RGD sequence may activate integrin via disrupting the key interaction group between Lys346 on βA domain and Ser663/Ser664 on the βTD. Interestingly, we observed the full dissociation of βA and βTd domains when this interaction group was disrupted and was eventually dissociated as a result of a competition between the Arg of the RGD peptide with Ser664. Consequently, we proposed a mechanistic scenario as a potential mechanism for outside-in activation of integrin αIIbβ3 by soluble RGD ligand that reconciles the switchblade and dead-bolt models for integrin activation.

1209-Plat
Single Molecule Imaging of Human Epidermal Growth Factor Receptors
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Biophysical Society Meeting 2014.
The human epidermal growth factor receptor (HER/ErbB) family of receptor tyrosine kinases encompasses transmembrane signaling proteins important for cellular growth, differentiation, and survival. The four members of this family (EGFR, HER2, HER4 and the HER3 pseudokinase receptor) are able to bind to numerous different growth factor ligands, leading to their homo and hetero-oligomerization and subsequent activation. This combinatorial potential gives rise to a diverse signaling output, which is strongly affected by misregulation of any one receptor. For example, mutations in EGFR and HER2 overexpression are primary mechanisms driving lung and breast cancer, respectively. Under these conditions, heterodimerization of these receptors with the HER3 pseudokinase confers resistance to tumors treated with EGFR and HER2-targeted therapeutics. The molecular basis for these heterodimeric interactions and their regulation by growth factors remains poorly understood. HER receptor heterodimers have never been observed directly, and their existence remain inferred from the analysis of downstream signaling. To understand the scope and specificity of heterodimeric interactions between the HER3 pseudokinase receptor and its active HER homologs, we seek to directly investigate the underlying receptor activation mechanisms using high resolution fluorescence microscopy. We quantify the extent of heterodimeric interactions in response to ligand binding using both live cell single molecule tracking and stochastic optical reconstruction microscopy (STORM). These techniques allow us to probe the timescales of receptor interactions as a function of ligand binding, the dependence on receptor density at the membrane surface, and the specificity of heterodimer formation. This work aims to contribute to the fundamental understanding of the activation mechanism of HER receptors and to help advance development of new therapeutics targeting aberrant cross-talk among HER receptors in human disease.

Platform: Structure and Dynamics of RNA in Biology

1210-Plat
Deciphering Ribosomal Frameshifting Dynamics
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Ribosomes programmed by specific messenger RNA (mRNA) sequence elements can switch translation reading frames and synthesize different polypeptides from a single template. The *Escherichia coli dnaX* mRNA encodes two DNA polymerase III subunits, τ and γ, synthesized from 0-frame and probabilistic −1-slip across the slippery sequence: AAAAAAG. When further enhanced by structural barriers situated around the slippery sequence-an internal Shine-Dalgarno sequence and a stable hairpin stem loop, an 80% (= γ/(γ+τ)) frameshift efficiency is attained. Here, we attempt to determine the frameshift timing within one translation cycle by following a single ribosome translating a frameshift-promoting mRNA held on optical tweezers. In parallel, by mass spectrometry (MS), we survey the synthesized polypeptides to resolve where on the mRNA the ribosome has slipped.
From the mass spectra of polypeptides terminated at the −1-frame stop codon, we learned that the ribosome −1-slips from more than one codon position around the slippery sequence. Some −1-frameshifted polypeptides were found to bear an extra amino acid, or to lack one, indicating that slipping sizes of −4 and +2-nt also occurred. Similarly, distinctive large-scale fluctuating translocation dynamics were seen in our real-time single-ribosome translation trajectories. This reveals that a translocating ribosome can explore a broad range of frameshift pathways. Frequently adopted frameshift pathways, i.e. the more abundant frameshifted species resolved by MS, exhibit a preference for minimizing codon:anticodon base-pair mismatches on the ribosome after a slip. Mismatch-containing ribosomes can be prematurely terminated by release factors, resulting in release of incomplete peptides. Indeed, we observed higher yields of incomplete peptides that are terminated at frameshift sites where significant mismatches were encountered. These species coincide with the prematurely stalled ribosomes recorded in the translation trajectories. Collectively what emerges from our results is a versatile ribosomal frameshifting scheme during mRNA translocation, facilitating branching of frameshift pathways.

1211-Plat
Unraveling the Mystery of Ribosome Induced RNA Unfolding
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During translation, the ribosome encounters various structures in the mRNA from simple hairpins to more complex tertiary RNA folds. These structures must be unwound for accurate decoding and translation to proceed. To probe the unwinding activity of the ribosome, we prepared dye labeled RNA transcripts and dye labeled ribosomes to directly report on the folded/unfolded states of the RNA at various stages along the translational elongation cycle. Consistent with previous studies, we found that the ribosome was sufficient to resolve RNA structures in the absence of any additional factors. Surprisingly, significant unwinding was observed in the absence of translocation or motion of the ribosome along the mRNA and can be attributed to dynamic motions within and between the two ribosomal subunits. This suggests that thermal energy resident within these ribosomal motions is sufficient to lower the energetic barrier of unfolding. The extent of unwinding was observed to depend on the relative stability of the RNA structure used and the number of tRNAs present in the ribosome. Differences between unfolding of these RNA structures in the presence and absence of the ribosome will be discussed.

1212-Plat
Single-Molecule Profiling of Ribosome Translational Phenomena
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Translation elongation is a heterogeneous process, involving multiple compositional factors stochastically binding to the ribosome to direct protein synthesis, which in turn regulates the conformation of the ribosome. The rate of translation is often regulated by the underlying messenger RNA (mRNA) sequence and structure. Here, we use single-molecule fluorescence resonant energy transfer (FRET) and colocalization with zero-mode waveguides (ZMWs) to correlate directly ribosome conformations and compositions of thousands of ribosomes simultaneously during multiple rounds of elongation. This allows us to profile global translational rates while delineating mechanistic details of the dynamics with codon resolution. We first studied translation of a canonical mRNA with uniform translation rates to establish the tight interplay between compositional factors and conformational dynamics of the ribosome during elongation. We then determined how mRNA sequences and structures, such as hairpins and possible ribosome-mRNA base pairing, as expressed in the the dnaX −1 frameshifting sequence, perturb the basal elongation process. Our results show how mRNAs can modulate and uncouple ribosomal conformational and compositional dynamics.

1213-Plat
The Ribosome Uses Cooperative Conformational Changes to Maximize the Efficiency of Protein Synthesis
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Execution of individual steps in the multi-step functional cycles of large, multi-component molecular machines such as the ribosome almost universally

requires remodeling of multiple, spatially distant structural components of the machine. In order to function efficiently, therefore, molecular machines likely must allosterically coordinate numerous, seemingly independent conformational rearrangements. Due to the significant technical challenges associated with characterizing their structural dynamics, however, the questions of whether and how large molecular machines coordinate such dynamics so as to maximize the efficiency with which they perform their biological functions remain exceptionally challenging to answer. Using a combination of structural and phylogenetic analyses, molecular genetics, single-molecule fluorescence resonance energy transfer, and in vitro biochemical assays, here we demonstrate that the ribosome uses cooperative conformational changes to maximize the efficiency with which it translocates and ejects its transfer RNA adaptors during protein synthesis. Interpretation of our data within the context provided by atomic-resolution ribosome structures and phylogenetic analyses of ribosomal RNA and ribosomal protein sequences leads us to propose a structure-based model for the observed cooperativity. Our results demonstrate that large, multi-component, molecular machines such as the ribosome can use networks of cooperative conformational changes to facilitate mechanical processes that would otherwise limit their catalytic rates.

1214-Plat
Rotational Motions of Domains in Elongation Factor G Detected by Single-Molecule Polarized Fluorescence Microscopy
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During the elongation cycle of protein synthesis, translocation of tRNAs and mRNA is catalyzed by the GTPase elongation factor G (EF-G) with high precision and speed. Conversion of the GTP to the GDP form of EF-G is considered essential for translocation, but the structural dynamics on the ribosome have not been reported. We used single molecule polarized total internal reflection fluorescence (polTIRF) microscopy to characterize tilting and rotational fluctuations within specific domains of EF-G. When EF-G binds to the ribosomal pre-translocation (PRE) complex, domains I and IV of EF-G undergo small rotations (10-15°) in conjunction with translocation, whereas domain III shows a much greater angular change, averaging 50°. Viomycin (Vio), which prevents translocation, reduces the rotational motions of domain III to 10-15° but has virtually no effect on the other domains. Spectinomycin also reduces domain III motions but less strongly than Vio. EF-G binding to ribosomal initiation complexes lacking A-site tRNA gives a similar pattern of domain rotations, but with shorter dwell times. In this case, the large rotation of domain III is barely inhibited by Vio. Irrespective of completion of translocation or presence of A-site tRNA, the initial 10-15° rotations of EF-G domains I, III and IV in the ribosome/EF-G complex indicate that the EF-G initially shifts the minimum of the free energy profile in the direction of translocation, suggesting that EF-G generates a force on the ribosome and/or the mRNA and tRNAs. Near the end of translocation, domain III completes its rotation either to push the mRNA and tRNAs (a working stroke) or to prevent reversal of translocation driven by thermal fluctuations (a ratchet). Supported by NIH grant GM080376 to YEG and BSC and AHA fellowship 12POST8910014 to CC.

1215-Plat
Refining Crystal Structures Against Cryo-EM Data using Molecular Dynamics Simulations to Obtain a Complete Atomistic Pathway of Transfer RNA Translocation
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The movement of tRNAs during translocation is accompanied by large conformational changes of the ribosome such as intersubunit rotations. Here, we present a method to cover conformational changes of the ribosome that occur on timescales not accessible to equilibrium Molecular Dynamics (MD) simulations through the combination of X-ray crystallography, cryo-EM data and MD simulations. Cryo-electron microscopy (Cryo-EM) provides medium/low-resolution density maps for many intermediate states of large molecular complexes. In contrast, X-ray crystallography provides high-resolution structures, usually limited to few states. To obtain pathways connecting the intermediate states of tRNA translocation, we start MD simulations from crystal structures with an additional biasing potential. This biasing potential, which maximizes the correlation between atomic model and cryo-EM map (Tama 2008), allows us to drive the ribosome from one intermediate state to another, covering the full translocation pathway. This method of cryo-EM driven MD was implemented in the high-throughput and highly parallel MD simulation package GROMACS.

1216-Plat
The Effect of Codon Translation Rates on Cotranslational Protein Folding Mechanisms of Arbitrary Complexity
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Bacterial cells use the naturally occurring variability in the rate at which different codons are translated to guide the folding of nascent proteins into ordered, biologically-active structures during their synthesis by the ribosome. Predicting how codon translation rates effect cotranslational protein folding mechanisms is therefore of fundamental biological interest. Here, we demonstrate that cotranslational folding mechanisms sampling an arbitrarily large number of states can be accurately modeled by treating this problem using the Markov chain formalism. This allows a general equation to be derived that describes the probability that a nascent protein is in any one of these conformational or thermodynamic states as a function of translation rates of individual codons in an mRNA molecules' open reading frame, which we show is accurate in modeling molecular dynamics simulations of cotranslational folding. Using this framework we demonstrate that there exists scenarios in which, contrary to conventional wisdom, fast-translating codons can actually increase the amount of cotranslational folding that occurs. This approach can be applied to the cotranslational folding of cytosolic and membrane proteins, and possibly the processing of nascent chains by auxiliary factors such as chaperones and enzymes.

1217-Plat
Protein Synthesis by Ribosomes: Mapping In Vitro onto In Vivo Rates
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All living cells rely on ribosomes, powerful nanomachines that synthesize proteins by translating the information encoded in mRNA molecules. Over the past two decades, the various substeps of the translation process have been studied in much detail using in vitro systems, but it is often questioned to what extend these results can be applied to real living cells. Although in vivo translation should proceed via essentially the same steps as in vitro, the average protein synthesis rate turns out to be much faster. This difference in synthesis rate has led to a long and controversial debate because - in contrast to in vitro systems - it has not been possible to study individual conformational transitions of the translating ribosome in vivo. Here, we address this long-lasting and unresolved puzzle and introduce a general computational scheme, which enables us to map in vitro onto in vivo rates. Using new experimental data on in vitro translation at 20°C and 37°C, we first derive a complete set of in vitro rates for the individual substeps of translation. We then use our scheme, together with available in vivo data, to determine the corresponding in vivo rates. As a result, we obtain a comprehensive description of in vitro and in vivo translation for various experimental and growth conditions. This description allows us to predict codon- and mRNA-specific translation rates, which can be used, e.g., to investigate translational pauses and ribosome traffic.

Symposium: Molecular Basis of Voltage Dependence

1218-Symp
Thermodynamic Analysis of Voltage-Sensing Mechanisms
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Voltage-gated ion channels (VGIC) form a large superfamily of ion channels and the activation of these channels underlie electrical and chemical signaling in a variety of cell types. Structure-function studies are widely used to deduce the energetic effects of a mutation by measuring macroscopic currents and fitting their voltage-dependence to a Boltzmann function. However, in absence of detailed kinetic models, this approach can introduce serious errors in free-energy estimates because of the inherent assumption that the channel activation is a two-state process. We recently developed analytical tools that allows us to calculate the free energies required for activation of voltage-dependent processes without any prior knowledge of the underlying gating scheme. Our method involves measurement of conjugate displacement associated with the force that drives the activation of these channels. In the case of voltage-gated ion channels, gating charge movement is the conjugate displacement and the force is voltage across the membrane. We show that by measuring the median voltage of charge transfer, VM, and the total gating charge per channel, we can calculate the chemical free energy difference between the resting and activated state of the channels. These free-energy measurements can be extended to other members of the VGIC superfamily to obtain a measure of interaction energies