configuration is a potential energy minimum. Using these models, we are able to simulate spontaneous tRNA translocation events and identify robust aspects of the dynamics. We find that detailed steric interactions are a dominant contributor to tRNA translocation dynamics. These results provide a framework for understanding the interplay between structure and dynamics, and suggest strategies to experimentally modulate the physical-chemical features that govern ribosome function.

1962-Pos Board B99
Single-Molecule Profiling of Ribosome Recoding Phenomena
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Messenger RNA (mRNA) sequence is central to translational control, with special sequences and secondary structures regulating translational dynamics. Shine-Dalgarno sequences, mRNA hairpins and pseudoknots, as well as nascent peptide-ribosome interactions, are known to pause or stall the ribosome. These stimulatory elements may lead to kinetic branchpoints during elongation and induce recoding events, wherein the ribosome is shunted into alternative pathways that result in either changes in reading frame or the bypassing of a region of the mRNA. Here, we present single-molecule fluorescence methods with zero-mode waveguides (ZMWs) to profile directly the translational rates of thousands of single ribosomes with codon resolution, illuminating the underlying dynamic mechanisms of recoding events. We investigated two recoding events: the −1 frameshifting in the dnaX gene and the ribosome bypassing of a 50 nucleotide untranslated region in gene60 of T4 phage. We observed multiple pathways induced by the stochastic interaction of the ribosome with the stimulatory elements; the ribosomes that undergo recoding in both frameshifting and bypassing are characterized by a pause in the rotated state. Such paused states allow unusual events in elongation and may be a central feature of translational control.

1963-Pos Board B100
Ribosome Assisted GTP Hydrolysis by EF-Tu - Mechanism and the Role of Asp21
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The elongation factor Tu (EF-Tu) is a member of the translational GTPase superfamily, whose GTPase activity is stimulated by the ribosome. Recently we elucidated the GTPase mechanism of EF-Tu using computer simulations (*Ram Prasad et al. PNAS USA, 110, 20509, (2013)*) and concluded that His84 of switch II region acts mainly in an indirect way (i. e., it neither acts as a general base nor stabilizes the TS in a major way). Additionally, we also concluded that although the proton transfer step occurs through an additional water molecule it does not constitutes the rate-limiting barrier. These computational predictions are further confirmed by a recent mutational and biochemical study (*Maracci et al. PNAS USA, doi:10.1073/pnas.1412676111, (2014)*). This work found that a mutation of Asp21 in the P loop also hampers the GTPase activity of EF-Tu. This observation suggested that the catalytic effect is modulated by the nature of amino acid side chain — thus we obtain a support to our proposal of allosteric control by the preorganization of the p-loop. However, in order to identify conclusively the origin of the observed mutational effects, and thereby the actual role of D21 on the GTPase activity of EF-Tu, it is essential to move to a quantitative structure function analysis rather than mere qualitative arguments. Thus, we conducted a computational study aimed at examining and quantifying the molecular origin of the catalytic effect of Asp21 on the GTPase activity of EF-Tu.

1964-Pos Board B101
Using Hydroxyl Radical Footprinting to Observe Ribosome Assembly Intermediates in vivo
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The assembly of the E. coli ribosome small subunit has been widely studied and characterized in vitro. Despite this, ribosome biogenesis in living cells remains poorly understood. This is a very complex process in which an rRNA is transcribed, folded, cleaved, and modified, while also binding with 20 different proteins. Very little is known about how the tertiary structure of the ribosomal RNA changes during assembly. There are a number of structure-probing methods that can be used to study rRNA in vivo, but virtually all of them lack the time resolution necessary to study a process like ribosome synthesis, which is completed within a few minutes.
Hydroxyl radical footprinting can be used to probe in vivo rRNA structure. The hydroxyl radicals which probe the rRNA can be produced in milliseconds using synchrotron X-rays. With this technique it is possible to examine ribosome assembly with meaningful time resolution. The hydroxyl radicals cleave the RNA backbone in solvent accessible regions, giving cleavage patterns that reflect regions of flexibility and rigidity within an RNA.
For the purpose of examining ribosome assembly, it is nascent ribosomes that are of interest, not pre-existing ribosomes that are already assembled. Therefore, the nascent ribosomes must be isolated from the background of pre-existing ribosomes. It has been shown that cells can take up labeled nucleosides that have been added to their growth media and incorporate them into nascent RNA transcripts. These can then be isolated using affinity methods. Once the nascent, assembling rRNA has been isolated, it can be analyzed by primer extension. The reverse transcriptase terminates at the cleavage sites. The cDNA fragments are then able to be analyzed by either slab gel, capillary electrophoresis, or high-throughput sequencing methods.

1965-Pos Board B102
Exploring the Mechanism of Dhh1-Mediated Translational Repression
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The yeast protein Dhh1, along with its orthologs in higher eukaryotes, have long been implicated in the regulation of protein expression by activation of mRNA decapping and subsequent degradation. More recent studies have argued that repression of protein production by Dhh1 occurs via a cap-independent mechanism.
Through a combination of in vitro and in vivo studies using reporter assays and protein tethering, we show that translational repression by Dhh1 occurs concurrently with the formation of mRNA species ''over-loaded'' with ribosomes - or polyribosomes. Through these studies, we are further able to establish a minimal functional unit of Dhh1 - comprising only of the two central RecA domains - which is capable of engendering general translational repression.
We are currently complementing these studies with high-throughput ribosome profiling analysis to ascertain the nature of Dhh1-mediated translational control across the genome with nucleotide resolution. These experiments will allow us both to look at endogenous genes affected by deletion or overexpression of Dhh1 and to look at reporter constructs again in the presence of tethered Dhh1 protein.

1966-Pos Board B103
Extra-Coding Characteristics of hERG mRNA are Essential for Channel Function
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The KCNH2 gene encodes the hERG protein, the alpha subunit of the rapid delayed rectifying potassium channel. This potassium channel plays an essential role in cardiac repolarization, and malfunction of this channel caused by mutation leads to Long QT Syndrome, type 2 (LQT2). LQT2 causes increased repolarization time in the heart, leading to ventricular arrhythmias, syncope and sudden death. There are over 600 documented mutations in KCNH2 associated with Long QT syndrome, with more mutations being reported regularly. These mutations occur throughout the length of the gene, without definitive mutational hotspots.
While much investigation has been done to characterize the impact that these mutations have on the hERG channel function, little investigation has been focused on what causes the hERG channel to be intolerant to mutation. Our hypothesis is that ''extra-coding'' characteristics on the mRNA level, such as GC content, rare codon usage and mRNA structure play a critical role in determining correct protein synthesis for the hERG channel, and that mutational changes that disrupt these characteristics lead to Long QT Syndrome. To investigate this hypothesis first, hERG SNPs will be analyzed to both identify trends in disease-causing SNPs, and to find differences between disease-causing and benign SNPs such as changes in local GC content or disruption of codon usage frequency. Secondly, using a codon-modified hERG mRNA with decreased rare codon usage, GC content and CpG islands when compared to native hERG mRNA, the role of mRNA structure and ribosomal movement in determining secondary and tertiary protein structure will be investigated.