that the phosphorylation of residue S2849 leads to the formation of an arginine claw that is absent in the non-phosphorylated protein. This finding at least partly elucidates the phenotypes stemming from several disease-linked human mutations in DP. We are currently determining if R2834H, a mutation that has been linked to arrhythmogenic right ventricular cardiomyopathy, disrupts the claw structure; we are also examining the effects of methylation of R2834, which has recently been shown to control the extent of phosphorylation. This work will illuminate the structural mechanisms by which DP adhesion is ultimately controlled.

1957-Pos Board B94

The Role of Higher-Order SPOP Oligomers for Localization to Cellular "Bodies" and Ubiquitination Activity

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Light microscopically detectable, non-membrane bound cellular ''bodies'' are large protein assemblies with liquid-like properties, but the biophysical basis of their formation is unclear. Weak, multivalent protein interactions can result in higher-order complexes and can enable the formation of cellular bodies. The inherent size heterogeneity of higher-order complexes renders them difficult to characterize biophysically. As a result, their size distributions remain largely unquantified, limiting molecular insight into their biological functions. We report a novel mechanism governing cellular body formation of the Speckle-type POZ protein (SPOP), which was recently identified as tumor suppressor, is a ubiquitin ligase substrate adaptor that localizes to nuclear puncta. We demonstrate that its cellular localization is dependent upon the ability of SPOP to form higher-order homo-oligomers through indefinite self-association, mediated by two distinct oligomerization domains. Furthermore, in vitro ubiquitination of substrates is enhanced through higher-order self-association of SPOP, suggesting that SPOP puncta are hotspots of substrate ubiquitination. One of SPOP's domains dimerizes with nanomolar affinity yielding stable SPOP dimers as ''building blocks'' for indefinite self-association, while the other domain dimerizes with micromolar affinity, rendering SPOP oligomers highly dynamic. Together, this results in isodesmic self-association, in which each addition of a dimer occurs with the same affinity, independent of the oligomer size. From this model, we describe the size distribution of SPOP oligomers, providing for the first time a quantitative analysis of protein assemblies participating in the formation of cellular bodies. Mutations within both oligomerization domains have been observed in a variety of cancers, supporting our conclusion that SPOP self-association is important for its biological function. We propose that dynamic, higher-order protein self-association is a general mechanism underlying the formation of cellular bodies, which may serve as switches to fine-tune signaling cascades.

Ribosomes and Translation

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Towards a Whole-Cell Model of Ribosome Biogenesis: Kinetic Modeling of SSU Assembly

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Ribosome biogenesis is a coordinated process involving the hierarchical association of 21 proteins to the 16S rRNA in the small subunit and 33 proteins to the 5S and 23S rRNAs in the large subunit. The process is further complicated by effects arising from the intracellular environment such as molecular crowders and the location of ribosomal operons within the cell. We report on our progress on the construction of a whole-cell model of ribosome biogenesis. Here we describe a detailed kinetic model accounting for the association of 18 of the 20 ribosomal proteins to the 16S rRNA to form the small subunit in vitro. Construction of the model is guided by the Nomura map of thermodynamic protein binding dependencies as well as kinetic cooperativity data. The complex chemical reaction network is simplified to 180 distinct assembly intermediates by removing infrequently used species. The 5′-central-3' binding order proposed in the literature is reproduced and an alternate assembly pathway, 5′-3′-central, is predicted which accounts for 30% of the total reaction flux. Biologically relevant assembly intermediates are identified and compared to intermediates observed using cryo-electron microscopy. Integration of this assembly model into an in vivo, spatially resolved whole-cell model of biogenesis accounting for the transcription and translation of ribosomal components using realistic cellular geometry will be discussed.

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A Structural Model of the Ribosome-Bound Protein Insertase YidC Reveals Lateral Translocation of the Nascent Chain

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The integration of membrane proteins into the cytoplasmic membrane of bacteria usually occurs co-translationally. The universally conserved YidC protein mediates this process either individually as a membrane protein insertase, or as a membrane protein chaperone in concert with the canonical protein-conducting channel, the SecY complex. However, little is known about the structural basis of YidCs interaction with ribosome, and its co-translational insertion activity. Here, we present a structural model of YidC based on evolutionary co-variation analysis, lipid versus protein exposure and molecular dynamics simulations. The model suggests a distinct arrangement of the conserved five transmembrane domains and an amphipathic helical hairpin between TM2 and TM3 on the cytoplasmic surface of the bilayer. The model was used for docking into a cryo-electron microscopy reconstruction of a translating YidC-ribosome complex carrying the YidC substrate FOc. This structure revealed how a single copy of YidC interacts with the ribosome at the ribosomal tunnel exit and suggests a site for membrane protein insertion at the YidC protein-lipid interphase. This site was confirmed by chemical crosslinking of FOc to TM3 of YidC. Together, these data suggest a mechanism for the co-translational mode of YidC-mediated membrane protein insertion.

1960-Pos Board B97

RNA Structural Modulation in the Heart of the Ribosome

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DEAD-box RNA helicase DbpA is one of the RNA maturation factors that *E. coli* employs during its ribosome assembly process. DbpA binds tightly and specifically to hairpin 92 of the 23S ribosomal RNA which is located in the peptidyl transferase center. Therefore, DbpA is implicated in RNA structural rearrangement in a ribosome region that is crucial for cell survival. When the helicase inactive R331A DbpA construct is expressed in *E. coli* cells, a 45S particle accumulates. This particle is a misassembled intermediate of the large ribosome subunit. It is not known if the 45S misassembled particle rearranges inside the cell and forms the active 50S large ribosome subunit, or if the resulting RNA structural misfolding is so severe that the 45S particle is designated by the cell for degradation. To understand the fate of the 45S particle in the cell, the ability of the 45S particle to form a native 50S subunit is tested by pulse chase. First, in the cell expressing R331A DbpA and lacking the wild type DbpA from their genome, RNA is labeled with [5,6-^{3}H] uridine for a specific amount of time, and then transcription of new RNA is stopped by the addition of rifampicin. Cell culture aliquots are obtained at a series of time points after stopping the transcription of new RNA, and ribosomal profile analyses are performed using sucrose gradient ultracentrifugation. The ribosome profile experiments demonstrate that the conversion of the 45S intermediate to the 50S large subunit particle does occur in the cells. The conversion rate of the 45S particle to the 50S particle is currently being measured.

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Simulating Ribosome Dynamics and tRNA Translocation

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With advances in structure determination and continued growth in high-performance computing (HPC), molecular dynamics (MD) simulations can now be employed to study large-scale conformational rearrangements in molecular machines, such as the ribosome. In the cell, proteins are synthesized by the joint action of the ribosome and transfer RNA (tRNA) molecules, enabling messenger RNA (mRNA) to be translated into peptides. In the elongation cycle of translation, tRNA molecules and the associated mRNA move between binding sites, a process known as tRNA translocation. During translocation, tRNA movement (~20-50 Å) is coupled to large-scale collective rotations in the ribosomal subunits. In order to better understand the physical relationship between these rotations and tRNA displacements, we use MD simulations that employ a simplified description of the energetics, which elucidate the role of sterics, and molecular flexibility during tRNA translocation. For the ribosome, we construct forcefields for which each experimentally-derived