View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

complexes assemble at or near membranes. We develop coarse-grained models and effective energy functions for simulating such large multi-protein complexes with low to intermediate binding affinities ($K_d > 1$ μM). The models are validated against structure and binding-affinity data for a broad range of binary protein complexes. Using replica exchange Monte Carlo simulation techniques, we apply our model to study the assembly, energetics, and dynamics of the complex between Vps27/Hse1 and membrane-tethered ubiquitin. The yeast Vps27/Hse1 complex and the homologous mammalian Hrs/STAM complex deliver ubiquitinated transmembrane proteins to the ESCRT endosomal protein-sorting pathway that is important in many biological processes. Vps27 and Hse1 contain several folded domains and flexible linkers. We find that the membrane-tethered ubiquitin binds preferentially to the UIM domains of Vps27. However, the simulations also show that ubiquitin interacts with other domains. The observed multiple specific and non-specific ubiquitin-Vps27 interactions greatly enhance the overall binding affinity. In the complex, the structure of Vps27/Hse1 is highly dynamic and flexible, reflecting the ability of Vps27/Hse1 to bind to a diverse set of ubiquitinated protein targets. The models developed here can easily incorporate additional experimental information (e.g., from fluorescence, scattering, electron microscopy), and hold promise for simulations of other large multi-protein complexes.

534-Pos Board B413
Modulation of Membrane Mechanical Properties by Sar1, a Vesicle Trafficking Protein
Raghuveer Parthasarathy.
University of Oregon, Eugene, OR, USA.
The trafficking of cargo in cells involves dramatic changes in membrane shape and topology. Though trafficking is widely studied and the identities and biochemical interactions of the responsible proteins are well mapped, remarkably little is known about the mechanics involved. We focus on Sar1, the key regulator of the coat protein complex II (COPII) family that ferries newly synthesized proteins from the ER to the Golgi. Sar1 is the only member of the COPII coat that interacts directly with the ER lipid bilayer membrane. It has an amphipathic N-terminal helix; when Sar1 is GTP-bound, the helix is exposed and the hydrophobic hemi-cylinder can insert into the bilayer. To investigate whether Sar1 has a physical role beyond merely localizing the other COPII proteins, we directly measure the force involved in membrane deformation as a function of its presence or absence, using optical traps and membrane-bound microspheres to pull tethers from lipid membranes. The lipid composition and large available surface area mimic the composition and geometry of the ER. Measurements of tether forces and radii allow extraction of the membrane bending modulus, the material parameter that dictates the energy required for deformation. We find that the bending modulus measured in the presence of Sar1 with a non-hydrolyzable GTP analogue, at concentrations sufficient for dense membrane coverage, is half that measured without Sar1 or with Sar1-GDP. These results reveal a paradigm-altering insight into COPII trafficking: Sar1 actively alters the material properties of the membranes it binds to, lowering the energetic cost of curvature generation.

535-Pos Board B414
Blocking helix formation without blocking organellar localization in Plasmodium falciparum
John R. Gallagher[1], Sean T. Prigge[2].
[1]Johns Hopkins School of Medicine, Baltimore, MD, USA, [2]Johns Hopkins School of Public Health, Baltimore, MD, USA.
Transit peptide (TP) recognition in mitochondria and chloroplast localization is well described and requires a receptor to recognize the TP bound as an amphipathic alpha helix. This functional interaction leads to organellar import of the payload protein. *Plasmodium falciparum* (*Pf*), the causative agent of malaria, contains an organelle called the apicoplast. The apicoplast is evolutionarily related to the chloroplast, is essential to the metabolism of *Pf*, and contains numerous putative drug targets. As in chloroplasts, nuclear-encoded apicoplast proteins must be post-translationally targeted to the apicoplast. In contrast to chloroplast localization, molecular details of TP recognition in *Pf* are currently unknown. To assess if apicoplast TPs must form helical intermediates for proper organellar localization, we have examined the TP of *Pf* acyl carrier protein by circular dichroism (CD), nuclear magnetic resonance (NMR), and epifluorescent microscopy of mutant TP-GFP fusions. CD and NMR of acyl carrier protein with its TP in solution are consistent with the presence of a small population of helix in the TP. However, structure-disrupting proline mutations are correctly targeted to the apicoplast when observed *in vivo*. This observation contradicts the theory that apicoplast TP recognition occurs via a mechanism similar to chloroplast TPs, and instead suggests that the dominant population of disordered TP may be the active form and that *Pf* has evolved a distinct solution to the problem of organellar targeting.

536-Pos Board B415
Mapping of the Signal Peptide-binding Domain of Escherichia coli SecA Using Förster Resonance Energy Transfer
Sarah M. Auclair[1], Julia P. Moses[2], Monika Musial-Siwek[2], Debra A. Kendall[2], Ishita Mukerji[1], Donald B. Oliver[1].
[1]Wesleyan University, Middletown, CT, USA, [2]University of Connecticut, Storrs, CT, USA.
Identification of the signal peptide-binding domain within SecA ATPase is an important goal for understanding the molecular basis of SecA preprotein recognition as well as elucidating the chemo-mechanical cycle of this nanomotor during protein translocation. While recent studies have addressed this topic, the precise signal-peptide binding site on SecA remains controversial. The aim of the present study was to identify the SecA signal peptide-binding site using Förster Resonance Energy Transfer (FRET). FRET provides a more global view of the binding site and circumvents the common limitations of more genetic approaches where deletion and substitution mutagenesis can confound the correct interpretation of protein structure-function analysis. This study employs a collection of functional, monocysteine SecA mutants labeled with a donor fluorophore along with cysteine-containing, acceptor fluorophore-carrying PhoA signal peptides. Fluorescence anisotropy was utilized to determine equilibrium binding constants of 1.4 μM or 10.7 μM for the alkaline phosphatase signal peptide labeled at residue 22 or 2, respectively, for SecA, with a binding stoichiometry of one signal peptide bound per SecA protomer. Distance measurements determined for nine SecA mutants indicate that the signal peptide-binding domain encompasses a region proximal to residues 225–228, 371–375, 652–657, and 771–780 when mapped onto the recent NMR structure of SecA (Gelis, I., Bonvin, A., Keramisanou, D., Koukaki, M., Gouridis, G., Karamanou, S., Economou, A., and Kalodimos, C. (2007) Cell 131, 756–769). This places the signal peptide-binding domain within the heart of SecA, surrounded by and potentially responsive to domains important for binding nucleotide, mature portions of the preprotein, and the SecYEG channel component.
Funding provided by National Institute of Health

537-Pos Board B416
Evaluating Protein Interactions & Organelle Dynamics in Saccharomyces cerevisiae: Spatial Distribution of Molecular Chaperone/Co-Chaperones Evident at a Sub-organelle Level in the Endoplasmic Reticulum
Carissa L. Young, David Raden, Anne S. Robinson.
University of Delaware, Newark, DE, USA.
BiP/Kar2 is a member of the Hsp70 family of chaperones that resides in the endoplasmic reticulum (ER) of *S. cerevisiae*. Biochemical and genetics experiments have demonstrated BiP's association with selective co-chaperones in multiple critical processes of the cell including translocation of protein into the ER, protein folding/maturation, and ER-associated degradation (ERAD). BiP's relative high cellular concentration combined with the low resolution of traditional immunofluorescence techniques has hindered the determination of protein localization effects. We hypothesize that the spatial heterogeneity of chaperones is regulated by co-chaperones, and this heterogeneity serves as a means of dictating cellular functions. To generate physiologically-relevant data of protein dynamics in *S. cerevisiae*, variants of green fluorescent protein (GFP) coupled with advances in confocal light microscopy techniques have allowed us to track multiple fluorescently-tagged proteins *in vivo.*
Dual expression strains composed of fusion proteins, BiP and co-chaperone Sec63, reveal that a heterogeneous spatial distribution is evident at the sub-organelle level. Secondary confirmation of our results has been performed using immunofluorescence techniques in multiple *S. cerevisiae* strains. Deconvolution of fixed cell images has allowed us to reconstruct and quantify the co-localization of BiP and Sec63 in three dimensions. We have captured the spatio-temporal effects of protein dynamics in live cells by monitoring ER membrane and luminal proteins, in addition to the nuclear pore complex; confirmed that the ER is continuous through Fluorescence Loss in Photobleaching (FLiP) experiments; and captured rapid diffusion of ER resident proteins. Integration of experimental data and computational design enables us to develop stochastic models of biological systems that accurately reflect spatiotemporal effects of molecular chaperone/co-chaperone interactions.

538-Pos Board B417
Microtubule Network is Necessary to Direct and Maintain The Apical Localization of Slo1 Channels in Epithelial Cells
Enrique Alejandro Sanchez-Pastor, Jimmy W. Ou, Abderrahmane Alioua, Pallob Kundu, Enrico Stefani, Ligia Toro.
University of California, Los Angeles, Los Angeles, CA, USA.
The cytoskeleton plays a key role in different cellular processes such as cell motility, muscle contraction, mitosis and maintenance of cell shape. In polarized cells, microtubules are involved mainly in the apical targeting of proteins.

In MDCK cells, Slo1 channels are mainly expressed at the apical surface. Similarly, transiently transfected MDCK cells target Slo1 channels to their apical surface. To study the role of the microtubules on apical localization of Slo1 channels, we used a viral construct of Slo1 attached to EGFP (Slo1-EGFP) to induce the expression of Slo1. After 72 hrs of infection, cells were either treated with vehicle (control) or with 33 μM nocodazole to disrupt the microtubules. Cells were fixed and immunolabeled with anti α-tubulin to visualize the microtubule network. Using high-resolution confocal microscopy, we found that in control cells the microtubules are localized towards the apical surface, as Slo1. After nocodazole treatment, the microtubule network is completely disrupted and Slo1-EGFP is observed in both apical and basolateral surfaces. To further understand the mechanisms of Slo1 targeting in MDCK cells, we blocked protein synthesis with 1 μg/ml cycloheximide simultaneously with microtubule disruption. In these cells, Slo1-EGFP expression, although still localized at both surfaces, was less prominent at the basolateral surface than without cycloheximide. This result indicates that a part of Slo1-EGFP localized at the basolateral surface after microtubule disruption is redirected from the apical surface and a part of these proteins are newly synthesized. Thus, our results show that in MDCK cells the microtubule network plays an important role in Slo1 apical localization and that normal Slo1 traffic is likely transcytotic first reaching the basolateral surface, then traveling to the apical surface via the microtubule network. Supported by NIH.

539-Pos Board B418

N-Terminal LQT2 Nonsense Mutations Cause a Dominant-Negative Destabilization of WT hERG Subunits

Elon C. Roti Roti, Gail A. Robertson.
University of Wisconsin-Madison, Madison, WI, USA.

Familial mutations in *hERG*, the *Human Ether-a-go-go Related Gene*, disrupt cardiac I_{Kr}, leading to Type 2 Long QT Syndrome and potentially fatal ventricular arrhythmias. Of the more than 200 mutations identified to date, eight are nonsense mutations in the hERG 1a amino terminus (NT). How these mutations cause disease is unknown. We found that constructs corresponding to six of these LQT2-linked mutations encoded stable, truncated NT fragments when expressed in HEK cells. We coexpressed these fragments with WT hERG 1a and 1b subunits to mimic heterozygous expression in LQT2 patients and assessed their effects on WT hERG trafficking and expression levels. Western blots revealed that the polypeptides significantly decreased WT 1a and 1b protein expression levels. We hypothesized N-N terminal interactions may mediate the observed reduction and indeed found levels of N-deleted hERG 1a subunit (N-del) were unaffected by the LQT2 polypeptides. Surprisingly, the LQT2 fragments promoted 1a N-del maturation. This indicates the polypeptides are not inherently misfolded and can rescue 1a N-del trafficking, possibly by interacting with downstream regions of the subunit. Reduction of WT protein levels by LQT2 polypeptides therefore requires an intact N-terminus. To determine if the LQT2 polypeptides destabilize WT subunits, we blocked protein synthesis using cycloheximide and tracked WT protein levels over time. Co-expression with LQT2 polypeptides caused a rapid loss of both immature and mature hERG 1a and 1b compared with control indicating accelerated degradation in the ER. In summary, this study demonstrates a novel disease mechanism in which LQT2-linked nonsense mutations in the hERG 1a N-terminus act in a dominant-negative manner by destabilizing ER-resident WT subunits.

Support: HL081780 (GAR) and AHA predoctoral fellowship and T32HL007936 (ECRR).

540-Pos Board B419

hERG Trafficking is Dependent on a Cytosolic Chaperone Network

Valerie Walker, Jason Young, Alvin Shrier.
McGill University, Montreal, QC, Canada.

The KCNH2 or human ether-a-go-go related gene (hERG1) encodes the Kv11.1 α-subunit of the hERG potassium channel that underlies the rapidly activating delayed rectifier current IKr. In the heart, mutations in KCNH2 cause a reduction in IKr resulting in the proarrhythmic type 2 long-QT syndrome (LQT2). While multiple factors can cause the loss of the functional phenotype, the dominant mechanism is a trafficking deficiency due to abnormalities in protein folding that result in endoplasmic reticulum (ER) retention. To identify chaperones or co-chaperones potentially involved in the folding and ER retention of hERG we performed a proteomics analysis to identify hERG-interacting proteins. In addition to Hsc70 and Hsp90, key members of the cytosolic chaperone system, we found the co-chaperones Dja1, Bag2, Hop, Tpr2, FKBP38, and the lumenal chaperone calnexin. We recently reported on the putative ER-resident hERG chaperone FKBP38. FKBP38 co-precipitates with hERG both *in vivo* and *in vitro*. Functionally, siRNA knockdown of FKBP38 reduces wild type (WT) hERG trafficking. *In vitro* experiments have also confirmed that translated hERG C-terminus (CT) and cyclic nucleotide binding domain (CNBD) bind to Hsc70 and Hsp90 as well as Dja1 and related family members Dja2 and Dja4. *In vivo* results indicate that overexpression of the Dja proteins result in differential reduction of hERG trafficking. This reduction in hERG trafficking appears to be related to the proteasomal degradation system as inhibiting the proteasome with lactacystin diminishes this effect and re-establishes hERG trafficking to control levels. Another protein involved in degradation, Chip, was also tested and preliminary work indicates that its overexpression leads to decreased hERG expression. Taken together, these data allow us to outline a model of chaperone-mediated hERG maturation and quality control.

541-Pos Board B420

Characterizing Nicotine-Induced α4* nAChR Upregulation with Fluorescence Microscopy

Rigo Pantoja, Rahul Srinivasan, Fraser J. Moss, Sindhuja Kadambi, Princess I. Imoukhuede, **Henry A. Lester**.
Division of Biology, Caltech, Pasadena, CA, USA.

Nicotine addiction is the world's leading preventable cause of mortality. Smokers also have a much lower incidence of Parkinson's disease. A plausible cellular/molecular mechanism for some responses to chronic nicotine is selective chaperoning of acetylcholine receptor number and stoichiometry (S-CHARNS). To investigate S-CHARNS in a neuronlike environment, we are using single-molecule fluorescence microscopy to monitor localization and trafficking of α4(eGFP)β2 and α4(eGFP)β4 nAChRs expressed in neuroblastoma cells (N2a). As in previous investigations on native neurons and heterologous expression systems, we find large pool(s) of intracellular α4(eGFP)β2 receptors predominantly localized in the endoplasmic reticulum (ER). We also find α4(eGFP)* receptors on vesicles (mobile puncta with diffraction-limited profiles consistent with the point-spread function of our microscope). Time-lapse photobleaching analyses of docked or fused vesicles indicate that vesicles carry 1-2 α4(eGFP)β2 receptor(s). In contrast, cells expressing α4(eGFP)β4 display (a) fewer ER-localized α4(eGFP)* receptors, (b) 5-fold more α4(eGFP)* receptors/vesicle, and (c) ~10-fold more receptors at the plasma membrane (perhaps resulting from (a) and (b)). The β2 and β4 subunits differ most in the M3-M4 intracellular loop, which includes several motifs that may govern the distinct roles of β2 and β4 subunits in α4* receptors. Chronic exposure (24-48 h) to 0.1 μM nicotine results in increased α4(eGFP)β2 or α4(eGFP)β4 receptors at the plasma membrane. We observe many more α4(eGFP)β2 receptors in the filopodia in nicotine-treated cells compared to non-nicotine treated control cells. Thus high-resolution, quantitative imaging of S-CHARNS is providing data required to understand, and eventually to manipulate, changes due to chronic nicotine exposure.

R.P. and R.S. contributed equally to this work. Grants: NS11756, DA17279, Michael J. Fox, Philip Morris, Targacept. Fellowships: Ford and APA-DPN (RP). AHA postdoctoral (FJM).

542-Pos Board B421

Protein Kinase C Regulation Of K_{ATP} Channel Recycling

Paul T. Manna, Andrew J. Smith, Tarvinder K. Taneja, Sarah Fletcher, Jonathan D. Lippiat, Asipu Sivaprasadarao.
University of Leeds, Leeds, United Kingdom.

Pancreatic ATP sensitive potassium (K_{ATP}) channels play an important role in insulin secretion, linking the metabolic state of the beta cell to its excitability. Protein kinase C (PKC) has previously been shown to down regulate the cell surface density of K_{ATP} channels in cardiac, neuronal and recombinant cells, but there are no studies on pancreatic beta cells. Here we show that activation of PKC results in significant down regulation of K_{ATP} channel conductance in the model beta cell line, INS1e. To investigate the underlying mechanism, we expressed a Kir6.2 construct containing an extracellular HA epitope plus SUR1 in INS1e and HEK293 cells and examined the role of PKC in regulation of cell surface density and endosomal trafficking. We found that PKC activation with the phorbol ester drug PMA reduced the surface density of K_{ATP} channels by reducing recycling of endocytosed channels, but had no effect on the rate of endocytosis. Endocytosed channels entered the peripheral and perinuclear compartments when PKC was activated, but remained at a peripheral location when PKC was inhibited with chelerythrine. Since pancreatic beta-cells express 9 different isoforms of PKC, we asked which of these isoforms is responsible for the observed effect. Using the dominant negative approach, our results show that PKC epsilon regulates endosomal trafficking of K_{ATP} channels. We conclude that that activation of PKC epsilon leads to dramatic changes in the distribution of internalised K_{ATP} channels and that the decrease in channel surface density is due to inhibition of channel recycling. The results may have important consequences for the electrical excitability of the pancreatic beta cell and therefore the insulin secretory response.