Peripheral membrane proteins of the Bin/amphiphysin/Rvs (BAR) and FerCIP4 homology-BAR (F-BAR) family participate in cellular membrane trafficking and have been shown to generate membrane tubules. The degree of membrane bending appears to be encoded in the structure and immanent curvature of the particular protein domains, with BAR and F-BAR domains inducing high- and low-curvature tubules, respectively. In addition, oligomerization and the formation of ordered arrays influences tubule stabilization. Here, the F-BAR domain-containing protein Pacsin was found to possess a unique activity, creating small tubules and tubule constrictions, in addition to the wide tubules characteristic for this subfamily. Based on crystal structures of the F-BAR domain of Pacsin and mutagenesis studies, vesiculation could be linked to the presence of unique structural features distinguishing it from other F-BAR proteins. Tubulation was suppressed in the context of the full-length protein, suggesting that Pacsin is autoinhibited in solution. The regulated deformation of membranes and promotion of tubule constrictions by Pacsin suggests a more versatile function of these proteins in vesiculation and endocytosis beyond their role as scaffold proteins.

2592-Pos

Membrane Properties Influence the Membrane Deformation Activity Mediated by BAR Domain Proteins

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The ability of cells to maintain and change the shapes of their membranes is vital for many cellular processes. Peripheral membrane proteins in the BAR (Bin/amphiphysin/Rvs) superfamily have been identified as membrane remodelers involved in cellular trafficking. Their membrane deformation abilities can be attributed to their intrinsically curved molecular shape. In addition, formation of oligomers in ordered arrays also aid in stabilization of curved membranes. However, changes in membrane topology also depend on mechanical properties of the bilayer, which in turn are influenced by factors such as temperature and lipid compositions. Using negative stain electron microscopy, we show that by altering these not-so-subtle membrane properties, we observe differences in the membrane deformation activities of several BAR domain proteins. Our results provide a systematic and unbiased approach towards understanding the general mechanisms underlying membrane deformation mediated by BAR superfamily proteins, and in particular the role of membrane properties in this process.

2593-Pos

Dimeric Endophilin Stimulates Self-Assembly and GTPase Activity of Dynamin

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Endophilin, which participates in membrane vesiculation during receptor mediated endocytosis, is a 40-kDa SH3 domain-containing protein that binds to the proline/arginine-rich domain of dynamin, a 100kDa GTPase essential for endocytic membrane scission. The N-terminal BAR domain of endophilin contains an amphipathic helix, which has been shown to penetrate the hydrophobic core of the membrane bilayer and initiate membrane bending which is subsequently stabilized by the remainder of the protein. When BAR domains dimerize, they present a concave, positively-charged surface that could interact with, and thereby deform, membranes containing negatively charged lipids. Since the oligomerization state of endophilin is important for its biological function we studied its dimer-monomer equilibrium using analytical ultracentrifugation and fluorescence polarization/anisotropy, which yielded Kd values of ~5 micromolar and 15 micromolar, respectively. We also demonstrated that endophilin significantly enhances the self-assembly of dynamins 1 and 2 and that this enhancement is proportional to the fraction of dimeric endophilin present. Moreover, there is a close correlation between the concentrations of endophilin that promote dynamin self-assembly and those that stimulate dynamin GTPase activity. Finally, we used two-photon FCS to study the interaction of EGFP-endophilin with polymerizing dynamin.
This work was supported by National Institutes of Health grant RO1GM076665 (DMJ).

2594-Pos

Variability of Dynamin and Clathrin Dynamics in Clathrin Mediated Endocytosis

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Clathrin mediated endocytosis (CME) is a pathway which internalizes receptors from the cell surface. The scission of clathrin coated vesicles from the plasma membrane requires dynamin. However, there are multiple models of dynamin mechanism without a consensus on the exact nature of its role. Total internal reflection fluorescence (TIRF) microscopy allows the visualization of fluorescently tagged proteins during individual endocytic events. TIRF provides better sensitivity than other techniques, however the analysis of this data remains challenging due to several factors including a low signal-to-noise ratio and an abundance of clathrin on the membrane. To overcome this problem, it is common to impose rules on the data and group intensity traces from individual clathrin spots, aligning them to a common event. We have examined the basis of these criteria, and in the experiments presented here we employed a very broad selection criteria. Using TIRF we imaged dynamin and clathrin in Cos7 cells, and characterized many individual vesicles. We observe a variety of dynamic behaviors at the cell membrane, including major differences in the time of loss of clathrin and dynamin fluorescence in individual traces. We found that grouping and aligning to a common event masked relevant differences and dynamics of the molecules with respect to each other. The time differences between clathrin and dynamin leaving the plasma membrane are not tightly correlated; these different behaviors could represent different sub-populations of membrane events, or heterogeneity within a single class of event. Our data indicates that another marker for endocytic events must be used. This will be especially important in the search for the mechanism of dynamin, to ensure that conclusions drawn from in vivo imaging studies pertain to a genuine biological action.

2595-Pos

Dephosphorylation of Dynamin1 is a Ca^{2+} Sensor that Triggers Clathrin-Independent Vesicle Recycling Processes in Pancreatic Beta Cells

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By tagging fluorphores to different endocytic proteins such as clathrin and dynamin, clathrin-mediated receptor internalization process has been visualized in a number of non-excitable cells *in vivo* using total internal reflection fluorescence microscopy. In contrast, systematic examination of the tempo-spatial relationship between different exocytotic and endocytic proteins has not been done in excitable cells. The clathrin-dependent receptor endocytosis in non-excitable cells has a long life time. In contrast, the clathrin-dependent vesicle recycling process is faster and subjected to further acceleration by increase in cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$), as proved by our previous data in pancreatic beta cells and also by recently papers on Calyx neurons. Moreover, elevated $[Ca^{2+}]_i$ triggers and accelerates a type of clathrin-independent but dynamin-dependent endocytosis in beta cells . As dynamin1 is a neuronal specific subtype of dynamin that can be dephorsphorylated at S774 and S778 positions upon stimulation-induced Ca^{2+} influx , we test whether it acts as a signal molecule to sensor increase in $[Ca^{2+}]_i$ and act to trigger vesicle recycling processes in our insulin-secreting cells. By mutating the S774 and S778 into A and E, we can mimic dephorsphorylated and phorsphorylated status of dynamin1. We show that dephorsphorylated dynamin1 has a statistically shortened life time as compared to the life times of dynamin1 in cells expressed wide-type dynamin1 or dynamin1 S774ES778E mutant. Moreover, dynamin1 S774AS778A is more likely to be recruited to the vesicle fusion sites, which represent clathrin-independent but dynamin-dependent endoytic events in beta cells. In the end, the fast capacitance decay evoked by homogenous elevation in $[Ca^{2+}]_i$ induced by flash-photolysis is selective inhibited by expressing either dynamin1 mutants in INS-1 cells, further reinforce the important role of dynamin1 phorsphorylation-dephorphosphorylation cycle in clathrin-independent vesicle retrieval process.

2596-Pos

Analysis of Clathrin Self-Assembly by Infrared Spectroscopy

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The clathrin protein self-assembles into a lattice that coats intracellular vesicles involved in sorting and transport of membrane-associated proteins. Inside a cell, clathrin self-assembly is initiated by interaction with adaptor proteins, but the basic self-assembly reaction can be recapitulated in vitro with recombinant fragments of clathrin that represent the C-terminal third of the

clathrin heavy chain. This self-assembling fragment is the central portion or Hub of the three-legged clathrin triskelion. Previous studies have suggested that assembly interactions between Hub legs display micromolar affinity and likely involve hydrophobic interactions, though assembly is modulated by a pH-sensitive salt bridge. The studies to be reported investigated whether mid-infrared spectroscopy of Hubs in solutions and also in a controlled humidity environment can be used to establish additional features of Hub self-assembly and potentially as a dynamic monitor of clathrin assembly. Comparison of spectra generated from assembled and disassembled clathrin revealed that hydration plays a role in assembly and that several absorption bands (1117 and 1220 wavenumbers) were present in assembled hubs that were absent in unassembled hubs. Such spectra were obtained on a Bruker 66v/S. Spectra generated during assembly suggested that a decrease in random coil and an increase in alpha helical content occur during Hub assembly, indicative of increased thermodynamic stability achieved during lattice formation. (Preliminary Raman data was also obtained from assembled Hubs.) These results demonstrate that analysis of Hub behavior in the infrared can be informative about the dynamics of clathrin self-assembly and suggest infrared spectroscopy as a novel approach to understanding the molecular details of clathrin-coated vesicle formation.

2597-Pos

Internalization of Two Distinct Receptors in Response to Occupation with a Bivalent Ligand Incorporating a Single Stimulus for Internalization

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Treatment of patients with CCK2 receptor antagonists potentiates pain relief induced by mu-opioid (MOP) agonists. In attempt to enhance this effect with a single bivalent ligand, we connected pharmacophores of non-peptidyl CCK2 receptor antagonist and MOP receptor agonist with a spacer. Spacer length of 16-21 atoms was consistent with simultaneous binding to both receptors, however provided no advantage in biological activity from that of two individual ligands (J Med Chem, 2009). Here, we extend this to examine effects of ligand tethering on receptor regulation. We prepared a series of CHO cell lines stably expressing yellow fluorescent protein (YFP)-tagged single receptor constructs or both of these receptors tagged with half of YFP (YN attached to one receptor and YC attached to the other). The YFP halves were not fluorescent until brought into spatial approximation to reconstitute YFP. These receptors bound their specific ligands effectively. The MOP agonist signaled normally and resulted in MOP receptor internalization. The CCK2 receptor antagonist did not stimulate receptor internalization. In the dual receptor-expressing cell line, bivalent ligands capable of binding both receptors simultaneously effected YFP fluorescence at the cell surface, and this signal internalized in a time- and temperature-dependent manner. Bivalent ligands with spacer arms too short to occupy both receptors simultaneously did not result in such a signal. Thus, a bivalent ligand is able to stimulate the association of two non-spontaneously-dimerizing receptors on the cell surface, and both of these receptors are internalized in response to binding a ligand of one receptor that stimulates internalization. Tethering provides a mechanism for dragging other surface molecules into the endocytic pathway.

2598-Pos

Vesicular Monoamine and Glutamate Transporters Select Distinct Synaptic Vesicle Recycling Pathways

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Monoamine neurotransmitters including dopamine, norepinephrine and serotonin are involved in a number of vital functions, including the control of movement, attention, motivation, emotional state, learning, and memory. The role of dopamine in reward requires the release of more dopamine in response to reward-relevant burst firing than to the single action potentials of background pacemaking activity. We have developed a new reporter, VMAT2-pHluorin to follow vesicular recycling required for dopamine release. We used the vesicular monoamine transporter VMAT2 and the vesicular glutamate transporter VGLUT1 to compare the localization and recycling of synaptic vesicles that store monoamines and glutamate, and observed several surprising differences. First, VMAT2 segregates partially from VGLUT1 in the dopaminergic synapses, but not in glutamatergic neurons. Second, post- stimulus endocytosis is slower for VMAT2 than VGLUT1 in both cell populations. During the stimulus, however, the endocytosis of VMAT2 accelerates dramatically in dopamine neurons, indicating a novel mechanism to sustain high rates of release. Furthermore, we find that in both cell types, a substantially smaller proportion of VMAT2 than VGLUT1 is available for evoked release. VMAT2 also shows considerably more dispersion along the processes after exocytosis than VGLUT1. Even when expressed in the same cell type, the vesicular transporters select distinct pathways for the recycling of synaptic vesicles that release dopamine and glutamate.

2599-Pos

Uptake by Human Astrocytes of Lipid Vesicles Modeling the Lipid Composition of Myelin

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The myelin sheath is composed of around 80% lipids including cholesterol, phospholipids, sphingomyelin, cerebroside sulfate and cerebrosides, and in smaller proportions, ceramides and glycerophosphatides. During pathological processes inside the central nervous system (CNS), the damage of this axonal insulation may expose the surrounding glial cells to lipid aggregates that result from this demyelination process. Indeed, microglia, considered to function as the local macrophages, can phagocyte myelin and cell detritus. Human astrocytes are another key example of glial cells where uptake of myelin debris may take place. Astrocytes are key regulators of several neuronal protective mechanisms, but they are also involved in the pathogenesis of certain autoimmune and inflammatory CNS diseases. Aiming to probe the behavior of human astrocytes exposed to different myelin lipids, we monitor the dynamics of lipid vesicle uptake by culture cells, and explore how varying specific myelin lipid components regulate the uptake kinetics and cell viability. A human astrocyte cell line obtained from a glioblastoma is used for all the experiments. Cells are exposed to NBD-PE or calcein labeled 50 nm small unilamellar vesicles (SUVs) of various lipid compositions reflecting various combinations of the myelin lipid components. Vesicle uptake is then monitored through fluorescence spectroscopy at different time points. Significant uptake is observed within 30 minutes, reaching saturation levels around 2 hours. These results are corroborated through flow cytometry, where astrocyte fluorescence stabilizes at around 2 hours. Additionally, we observe the presence of a smaller population of scatter cells thatshowed higher liposome uptake. Finally, by using fluorescence/DIC microscopy, liposomes are found to spread in the astrocyte cytoplasm after 4 hours of incubations. Interestingly, crowding of liposomes around the nucleus is observed after 12 hours of incubation, suggesting a sorting mechanism to be determined.

2600-Pos

Atomistic and Continuum Modeling of Cellular Uptake of Nanotubes and Viruses

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Most viruses and bioparticles endocytosed by cells have characteristic sizes in the range of tens to hundreds of nanometers. Recent experimental observations have shown that nanoparticles such as carbon nanotubes (CNT) can enter animal cells. The process of viruses and nanoparticles entering and leaving animal cells is thought to be mediated by the binding interaction between ligand molecules on the viral capid and their receptor molecules on the cell membrane. Here we conduct coarse grained molecular dynamics and theoretical studies of the intrinsic interaction mechanisms of nanoparticles and viruses of different shapes and sizes with a lipid bilayer [1-3]. Theoretical models are proposed to explain the observed size and shape effects in various entry mechanisms.

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