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Peripheral membrane proteins of the Bin/amphiphysin/Rvs (BAR) and Fer-CIP4 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

Shih Lin Goh, Qi Wang, Holger Sondermann.
Cornell University, Ithaca, NY, USA.

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

Justin A. Ross[1], Barbara Barylko[2], Lei Wang[2], Hunter Banks[2], Joseph P. Albanesi[2], David M. Jameson[1].
[1]University of Hawaii, Honolulu, HI, USA, [2]University of Texas Southwestern Medical Center, Dallas, TX, USA.

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

Alexa L. Mattheyses, Claire E. Atkinson, Sanford M. Simon.
The Rockefeller University, New York, NY, USA.

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

Wen Du[1], Lin Liu[2], Kuo Liang[3], Jingze Lu[1], Fei Li[3], Tao Xu[1,2], **Liangyi Chen**[1].
[1]Institution of Biophysics, Beijing, China, [2]Institute of Biophysics and Biochemistry, Huazhong University of Science and Technology, Wuhan, China, [3]Department of General Surgery, XuanWu Hospital, Capital Medical University, Beijing, China.

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

R. Loney[1], C. Hinojos[2], M. Lane[3], F.M. Brodsky[3], **J.W. Powell**[1].
[1]Reed College, Portland, OR, USA, [2]Bruker Optics, Billerica, MA, USA, [3]University of California, San Francisco, San Francisco, CA, USA.

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