Here, we transplanted pNSC-DA neurons into 6-hydroxydopamine-induced rat models of PD and monitored their behavior for 16 weeks. In striatum *in vivo*, we first determined that the grafted pNSC-DA neurons secreted DA(using HPLC with a 200-µm microdialysis probe at a sampling rate of 0.002 Hz); then we recorded DA release by electrochemical amperometry (using a 7-µm carbon-fiber electrode at a sampling rate of 1000 Hz), showing that the grafted pNSC-DA neurons significantly rescued DA release in slices (7%) and *in vivo* (16%). Furthermore, the transplanted cells survived for at least 16 weeks and dramatically rescued the apomorphine-induced asymmetric rotation behavior in PD rats. Thus, we draw the conclusion that transplanted pNSC-DA neurons can functionally integrate into the brain and partially relieve Parkinsonian symptoms in rats. Our work provides direct evidence for a therapeutic role of pNSC-DA neurons in treating Parkinsonian syndrome.

2652-Pos Board B344

FisB Mediated Membrane Fission During Sporulation in Bacillus Subtilis

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Membrane fission is a fundamental process required for endocytosis, membrane trafficking, enveloped virus budding, phagocytosis, cell division and sporulation. Despite the diversity of fission reactions, there are only two fission machineries known in eukaryotes (dynamin and ESCRT-III), and none in bacteria. We describe FisB, a protein that mediates membrane fission during sporulation in the rod-shaped bacterium B. subtilis. Upon starvation, B. subtilis divides asymmetrically, producing a large mother cell and a small forespore. The mother cell then engulfs the forespore. Like in phagocytosis or endocytosis, engulfment ends with a fission event that releases the forespore into the mother cell cytoplasm.

FisB, a 254 amino acid long protein, conserved among spore-forming bacteria, is expressed in the mother cell after asymmetric division. It possesses a short cytoplasmic N-terminus, one predicted transmembrane domain, and a large extracytoplasmic portion. Fluorescently tagged FisB forms dynamic foci in the mother cell membrane, mainly around the forespore. Foci are captured and immobilized at the pole where fission occurs shortly afterwards. In FisB knock-out cells, engulfment proceeds normally, but the fission step is severely impaired. Removing the sole dynamin analog does not have any effect, and B. subtilis does not have an ESCRT III homolog.

FisB reconstituted into artificial liposomes efficiently mediates membrane mixing only in the presence of cardiolipin (CL), whose levels increase from 1-2% during vegetative growth to 5-6% during sporulation. Binding of the extracytoplasmic domain to CL was confirmed directly in a flotation assay. Furthermore, CL is located to the poles, presumably into rafts that prefer negatively curved regions. Thus, we hypothesize that the dynamics of FisB and that of CL are intimately coupled.

2653-Pos Board B345

Fret Based Thermodynamics and Kinetics Investigation of Endophilin Dimerization

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Endophilin is a homodimeric endocytic accessory protein which functions as a membrane curvature sensor, and as a membrane curvature generator, and membrane binding of this proteinthe recruitment of additional endocytic accessory proteins. However, the mechanism by which endophilin dimerizes has remained unclear. In this study, we employ the Forster resonance energy transfer technique to characterize the thermodynamic and kinetic information of endophilin dimerization/dissociation in solution. Large temperature dependence of dimerization affinity and dimer dissociation kinetics are revealed for the endophilin N-BAR domain, reporting dimerization affinity ranges from low nanomolar to sub-nanomolar and an activation enthalpy of 66 kcal/mol for the dimer dissociation reaction. We discuss the contributions of various interfacial molecular interactions to the stability of endophilin N-BAR dimers. Furthermore, to elucidate the role of the SH3 domain in the dimerization of full-length endophilin, we monitor the dissociation kinetics of full-length endophilin A1 and its truncated isoforms. Mutants without H0 helix or SH3 domain show significantly faster dissociation kinetics relative to full-length endophilin A1. This observation indicates the presence of an intra-dimer, inter-monomer cross-interaction of H0 helix and SH3 domain from different subunits of the same homodimer. This interaction likely results in even higher dimerization affinity of full-length endophilin relative to the BAR domain.

2654-Pos Board B346

Endophilin Rapidly Bends Membranes to Promote Endocytosis

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Synaptic Vesicle (SV) endocytosis is responsible for generating synaptic vesicles at the presynaptic terminals. To sustain high-frequency brain activities, endocytosis must act rapidly to regenerate SVs, which prevents vesicles from depletion. Endophilin is a synaptic protein that plays critical role in promoting SV endocytosis. Endophilin contains a Bin-Amphiphysin-Rvs (BAR) domain that has been shown to bend flat membranes into highly curved tubules in vitro. Our recent findings demonstrate that the membrane-bending activity is required for endophilin's function in vivo. Here, we perform biophysical analyses to investigate molecular mechanisms for endophilin to generate membrane curvature. Our preliminary results show that the endophilin BAR domain is an active membrane bender instead of a passive sensor. These results will help us to understand how endophilin acts to support SV endocytosis in neurons.

2655-Pos Board B347

Endocytosis of Liposomes Containing Sulfo-Cerebrosides by an Astrocytomal Cell Line: Is it a Charge Mediated Process or Does it Involve a Specific Receptor?

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Sulfo-cerebrosides (SCB) are glycosphingolipids found in high concentrations in the central nervous system (CNS), particularly in myelin where they play an important structural role. In addition, these lipids play an important role in various biological processes such as establishing a connection between the neuronal axon and myelin, and regulating cell growth and neuronal plasticity. Moreover, recent in-vitro studies from our group have shown that astrocytes avidly endocyte liposomes containing SCB without inducing a pro-inflammatory response. This indicates that astrocytes are involved in the homeostasis of the SCB in the CNS. In this work, we explore the specificity of the process of endocytosis of liposomes containing SCB, using an astrocytoma cell line as a model. Our hypothesis is that this process is receptor-mediated and not due to an electrostatic interaction with the cell surface. We show that (PG enriched) negatively charged liposomes do enhance endocytosis, but at a significantly lower level compared to SCB. We also show that specific uptake can be inhibited by screening the surface with polyethylene glycol. Additionally, we show through the use of endocytotic inhibitors that the SCB endocytotic route is a clatherin dependent route, which differs from non-specific liposome uptake routes. In these experiments, cells were cultured with liposomes labeled with the membrane fluorescent probe (Texas Red) to measure internalization, and a liposome content marker (calcein) to measure release. Measurements were carried out by flow cytometry and the results confirmed by fluorescence microscopy. Since low density lipoproteins (LDL) have been suggested to participate in SCB transport, we propose that the endocytotic pathway involves the LDL receptor (LDLr). Preliminary results show that SCB endocytosis is inhibited by LDLr specific antibodies.

2656-Pos Board B348

A Molecular View of Lipid Droplet Formation

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Lipid droplets are stores of neutral lipids for membrane regeneration and energy synthesis. They have recently been implicated in diseases such as diabetes, obesity, atherosclerosis, and even virus proliferation. They form as globules within organellar membranes and may even bud off, taking the outer leaflet of the membrane with them. Several models of this process have been proposed, but the formation and budding of these droplets is poorly understood at the molecular level. We have developed molecular simulation techniques that enable us to study such structures. Our simulations provide a molecular view of lipid droplet formation and allow us to propose a mechanism for the budding of lipid droplets and similar organelles. We also discuss the extension of our work to droplets containing synthesized substances such as biofuels and rubber.

2657-Pos Board B349

Interaction of Model Lipid Vesicles with Alveolar Macrophages

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