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Role of FisB-Cardiolipin Interactions in 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 254 amino acid protein which is conserved among spore-forming bacteria. FisB mediates membrane fission during sporulation in 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 possesses a short cytoplasmic N-terminus, one predicted transmembrane domain, and a large extracytoplasmic portion. In FisB knock-out cells, engulfment proceeds normally, but the fission step is severely impaired (Doan et al., Genes & Dev. 2013).
The goal of our research is to understand how FisB mediates membrane fission. Therefore we investigate how FisB is able to form oligomers that translocate to the fission site and sever membranes. The extracytoplasmic domain (ECD) of FisB binds cardiolipin (CL) in a floatation assay and binding is highest at low salt and undetectable at 500 mM NaCl, suggesting electrostatic interactions between FisB and CL. FisB(173-220) was identified as the cardiolipin binding domain.
FisB reconstituted into artificial liposomes efficiently mediates membrane mixing only in the presence of CL. Fluorescently tagged ECD binds to cardiolipin-containing giant unilammellar vesicles and induces aggregation, membrane deformation and collapse. CL is enriched at the poles of B. subtilis, presumably in microdomains that prefer negatively curved regions.
We hypothesize that CL may regulate recruitment and oligomerization of FisB.

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Interaction of Model Lipid Vesicles with Alveolar Macrophages
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Macrophages play key roles in host defense by recognizing and engulfing foreign and apoptotic bodies. To accomplish this task, they rely on complex molecular interactions involving both lipids and proteins. Previous studies have shown that surface exposure of phosphatidylserine by apoptotic cells is required for their successful clearance, suggesting specific lipid-protein interactions at least for the initiation of phagocytosis of apoptotic cells. However, macrophages can engulf foreign and apoptotic bodies that substantially vary in size suggesting that non-specific interactions over a range of length scales may be relevant. Here we investigate the correlation between physical properties of lipid bilayers and their engulfment by macrophages. We use a combination of scattering and spectroscopic methods to quantify lipid interactions and flow cytometry to measure engulfment rates. Our previous engulfment measurements at 1 hour after incubation have shown preference for phosphatidylserine-rich lipid vesicles over phosphatidylcholine. However, the extent of engulfment could depend on incubation time and on the exact state of macrophages. In the current study, we measure the engulfment of lipid vesicles made of either PS or PC lipids as a function of incubation time. This important aspect is relevant to the dynamics of macrophage activity.

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Investigation of the Structure of Dimers of the Voltage-Gated Proton Channel
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Voltage-gated proton channels (Hv1) can operate functionally as monomers; however in vivo they are found as dimers which exhibit cooperative gating. The mechanism by which the cooperativity is enforced however is incompletely understood. However, it is known that removal of the tail region of the dimers, residues 234 to 255 in human Hv1, remove the cooperativity. Our investigation of the structure of the dimers and mechanism by which cooperativity in gating was enforced involved the extension of a homology model for the monomer which has been validated and reported in the literature, Chamberlin *et al* [1], to include a homology model of the dimer tail region based on the crystal structure of Fujiwara *et al* [2]. Subsequent investigation of the tail region of the closed and open state dimers were sampled using coarse-grained methods and further refined using all-atom molecular dynamics simulations. Finally, the gating path was sampled using targeted molecular dynamics simulations.
[1] Chamberlin A, Qiu F, Rebolledo S, Wang YB, Noskov SY, Larsson HP. Hydrophobic plug functions as a gate in voltage-gated proton channels. P Natl Acad Sci. 2014;111:E273-E82.
[2] Fujiwara Y, Kurokawa T, Takeshita K, Kobayashi M, Okochi Y, Nakagawa A, et al. The cytoplasmic coiled-coil mediates cooperative gating temperature sensitivity in the voltage-gated H^+ channel Hv1. Nature Communications. 2012;3.

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Activation of the Ca^{2+}-Activated Chloride Channel TMEM16A
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TMEM16A is a Ca^{2+}-activated chloride channel that is involved in various physiological processes. The functional behavior of the murine ion channel mTMEM16A has been characterized by electrophysiology. mTMEM16A forms anion-selective channels that are activated by Ca^{2+}-binding from the intracellular side. Channel activation by Ca^{2+} was shown to be voltage-dependent with an increase of the EC_{50} at negative potentials. Although studies have identified residues contributing to Ca^{2+} binding, a mechanistic understanding of channel activation and the relationship to the voltage-dependence remained unknown.
To address this question, we have determined the structure of nhTMEM16, a fungal member of TMEM16 family, which functions as lipid scramblase. Due to the close relationships within the family, however, this protein also provides a valuable framework to investigate structure-function relationships in mTMEM16A. The structure of nhTMEM16 reveals a highly conserved Ca^{2+}-binding site within each subunit of the dimeric protein. In this site, up to two Ca^{2+} ions are coordinated by six residues, five of which carry a negative charge. The location of the site within the transmembrane region provides an explanation for the voltage-dependence of Ca^{2+} activation observed in TMEM16A. To probe the importance of the corresponding residues, single mutants of TMEM16A were expressed in HEK293T cells and studied by excised patch electrophysiology. The Ca^{2+} dose-response relationships of all mutants show a shift to higher Ca^{2+} concentrations, indicating that the interaction of these residues with Ca^{2+} is key to channel activation. The structure also hints at a region in the protein, termed the 'subunit cavity', as a potential site of ion permeation. The proximity of the 'cavity' to the Ca^{2+}-binding site and the presence of residues previously shown to affect ion selectivity of the channel, suggests this region as the likely candidate for the ion conduction path in TMEM16 channels.

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Investigating the Effect of PKA Phosphorylation on Intramolecular Interactions in Purified Full Length Wildtype CFTR
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Cystic fibrosis transmembrane conductance regulator (CFTR) is an unique anion channel of the ATP-Binding Cassette (ABC) superfamily. CFTR consists of two nucleotide binding domains (NBDs), two membrane spanning domains (MSDs) and a regulatory (R) domain. There are also alpha-helical extensions and intracellular loops (ICLs) which couple the NBDs and MSDs via ''coupling helices''. The regulation CFTR channel activity involves protein kinase A (PKA) phosphorylation at the R domain. However, not much is known about the effect of phosphorylation on the intramolecular interactions of full length CFTR. We propose that phosphorylation modifies the interactions of full length CFTR, especially at the ICL4:NBD1 interface. Previous studies of a similar ABC transporter, BtuCD, have shown that intrinsic tryptophan fluorescence can detect urea sensitive changes in the coupling between the MSDs and NBDs of full length BtuCD. Thus, we decided monitor intrinsic tryptophan fluorescence to study the effect of phosphorylation on the purified full length Wt CFTR. Interestingly, we found that the urea sensitive changes in the intrinsic tryptophan fluorescence of full length CFTR were modified upon PKA phosphorylation. We were interested to determine whether this result was due to phosphorylation modifying the ICL4:NBD1 interface. In order to specifically study that interface, cysteine crosslinking studies were employed using a short cell permeable cysteine crosslinker on a cys-less CFTR mutant with two cysteines, V510C (NBD1) and A106C7C (ICL4), transfected in HEK293 cells. Phosphorylation induced by cAMP agonists in cells resulted