these results suggest that receptor uptake depends on both recruitment of individual receptors and recruitment of heterogeneous protein complexes. Moving forward, this work suggests novel approaches for amplifying signaling in therapeutically relevant pathways, such as GPCR and apoptotic signaling, by promoting interactions between target receptors and receptors undergoing active internalization.

1408-Pos Board B317

Modeling the Flat to Curved Transition during Clathrin Mediated Endocytosis

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Clathrin-mediated endocytosis is essential for the cellular uptake of receptors and nutrients. Although under investigation since decades, the exact sequence of structural and molecular events remains elusive. There are two basic models that have been suggested for the way it proceeds. In the constant curvature model, it is assumed that clathrin-coated pits grow with constant curvature, determined by the geometry of clathrin triskelia. In the constant area model, it is assumed that clathrin triskelia first assemble into flat hexagonal arrays that later invaginate at constant surface area. This second model implicitly assumes that during bending, some hexagons are converted into pentagons. Here, we integrate data sets from correlative electron and light microscopy and quantify the sequence of ultrastructural rearrangements of the clathrin coat during endocytosis in mammalian cells with the help of some simple mathematical growth laws. Our main assumption is that the clathrin domain can grow only over the boundary. In the case of flat arrays, this requires some balancing process to prevent a run-away process, which we assume to grow in proportion to the domain area. In the case of curved arrays, pit closure is sufficient to limit growth and thus an additional process is not required. Our analysis shows that clathrin-coated structures initially grow flat but start to acquire curvature when 70% of the final clathrin content is reached. We find that this transition correlates with a change in the ratio of clathrin to adaptor protein AP2, and that membrane tension suppresses this transition. Hence, our analysis suggests that elements of both suggested models are present and that mechanical and cellular factors will decide about the relative weights of growth versus curvature formation.

1409-Pos Board B318

Clathrin Coat Controls Vesicle Acidification by Blocking Vacuolar ATPase Activity

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At neuronal synapses, exocytosed membrane and proteins are internalized predominantly by clathrin-mediated endocytosis. Newly formed synaptic vesicles (SVs) are rapidly acidified by vacuolar ATPases, generating a proton electrochemical gradient across the membrane that is subsequently used by vesicular transporters to fill the vesicles with neurotransmitters. To date, it is unclear when endocytosed vesicles acidify and refill at the synapse. While some have suggested that endocytic coated vesicles do not have acidic internal pH, other studies have shown that membrane-permeable pH-sensitive weak bases accumulate in the lumen of clathrin-coated vesicles (CCVs), indicating that acidification occurs in the presence of the coat. To weigh in on this debate, and to inspect whether vATPase is active at the CCV, we isolated CCVs from the mouse brain and used our recently developed single-vesicle imaging assay (Farsi, et al. Science, 2016) to perform full characterization of the electrochemical gradient in single CCVs. We have observed that the ATP-induced acidification of CCVs was strikingly reduced in comparison to SVs. Remarkably, when the clathrin coat was removed from CCVs, uncoated vesicles regained ATP-dependent acidification, demonstrating unequivocally that CCVs contain the functional vATPase, yet its function is inhibited by the clathrin coat. Considering the known structures of the vATPase and clathrin coat, we propose a model in which the formation of the clathrin coat surrounds the vATPase and blocks its activity. Such inhibition of vATPase by clathrin coat is likely fundamental for the proper timing of SV refilling that is driven by the electrochemical gradient, as well as for the regulation of vATPase activity during the endocytic process.

1410-Pos Board B319

Role of Actin and Membrane Tension in Regulating Modes of Exocytosis

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Exocytosis is a critical biological process in which secretory vesicles fuse with the plasma membrane, expelling their contents into the extracellular space. Recent experimental evidence implicates a role for actin as a modulator of local membrane tension and setting the balance between ''kiss-and-run'' vs. ''full-fusion'' modes of exocytosis. How exactly physical parameters like vesicle size, spontaneous curvature, membrane tension, turgor pressure and external forces come together to control the balance of these two exocytic modes is unclear. To address this question, we use a general continuum-mechanical model of lipid bilayers to simulate membrane shapes following the fusion of an exocytic vesicle with the plasma membrane. We find that a local increase in membrane tension will either cause the membrane to flatten or shrink the fusion pore as determined by a balance between vesicle size, bending rigidity and magnitude of spontaneous curvature. Furthermore, an increase in turgor pressure can provide a complementary driving force for flattening the vesicle membrane post-fusion which could compensate for a lack of actin-induced membrane tension. We also show that dissipation of spontaneous curvature as well as membrane tension contribute to shrinking of an omega-shaped membrane profile even while the fusion pore is pinched by an external force. A localized pressure on an omega-profile is also sufficient to induce shrinking, indicating other hypotheses for the role of actin in compressing the exocytic vesicle to expel its contents are also plausible. Importantly, the length scales over which these mechanisms are relevant provides insight into why actin might differentially contribute to the mode of exocytosis in different cell types.

1411-Pos Board B320

Force Generation by Curvature-Generating Molecules in Cells with Turgor

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Curvature-generating molecules (CGMs) are central to a variety of biological processes. Proteins such as clathrin and BAR-domain proteins help provide forces and moments to drive membrane bending, as in the case of endocytosis. We develop a discrete equilibrium mechanical model of the shape of a small CGM-membrane complex that incorporates the effects of cell wall elasticity and turgor pressure, as well as a simple continuous model that also incorporates the effects of the wall force. We calculate the dependence of the force generated by the CGPs on various parameters, including the bending modulus, the patch size, and the turgor pressure, and find evidence of transitions as a function of external turgor pressure and intrinsic curvature that are discontinuous for the force and continuous for the displacement. We develop simple formulas for the maximum force generated by the CGM patch in terms of the patch size, the bending rigidity, and the preferred curvature. Comparison of the discrete model to previous continuous models of CGP forces reveals important corrections to the continuous models. We find that the spatial distribution of the forces depends on the strength of the turgor pressure relative to bending energy, with forces being localized at the edges for high turgor pressure, and more widely distributed for low turgor pressure. In addition, the energy exhibits a minimum at small numbers of molecules. Further, for realistic values of the bending rigidity and curvature, CGPs alone are insufficient to initiate endocytosis against turgor pressure, consistent with previous findings.

1412-Pos Board B321

Membrane Tension Dictates the Spatiotemporal Heterogeneity of Endocytic Clathrin Coat Dynamics in Cells

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The dynamics of endocytic clathrin coat assembly can be widely divergent, not only among cells within the same culture but even within distinct regions of the