

prostaglandin receptors (EPs) selectively couple to AC2 in non-raft domains. Using genetically encoded FRET-based biosensors targeted to the bulk cytosolic compartment as well as lipid raft and non-raft membrane domains, we monitored changes in cAMP activity in response to β AR and EPR stimulation in HASM cells overexpressing (OE) AC2 or AC6. We found that OE AC2 selectively enhanced cAMP responses to EPR stimulation in non-raft locations, while having no effect on cAMP produced by β ARs anywhere. These results suggest that AC2 is exclusively coupled to EPRs in non-raft domains of the plasma membrane. In sharp contrast, OE AC6 selectively inhibited cAMP responses to β AR stimulation in lipid raft-associated locations, while having no effect on cAMP produced by EPRs anywhere. Furthermore, the effect of OE AC6 on β AR responses could be blocked by inhibition of phosphodiesterase type 4 (PDE4) activity, or by disrupting A kinase anchoring protein interactions with protein kinase A (PKA). These data suggest that AC6 is selectively coupled to β ARs in lipid raft domains and that OE AC6 upregulates PDE4 activity due to PKA-mediated phosphorylation or recruitment in lipid raft microdomains.

2298-Pos Board B314

Effects of Cell Cortex-Based Transient Confinement on Transmembrane Protein Interactions in Intact Cells

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Modifying the diffusion of membrane receptors transiently changes their local concentration which affects their rate of interaction, and hence the cell signal. Underneath the plasma membrane bilayer is the cell cortex, a meshwork of actin and related filaments, which actively and passively interact with proteins in the plasma membrane. Diffusing transmembrane proteins are temporarily corralled between these filaments, and traverse across these filaments via hop diffusion. Here we demonstrate how binned-imaging fluorescence correlation spectroscopy (bimFCS) can quantify the corraling, and show via experiments and simulation how this corraling affects the cell signaling. A novel technique developed by our lab, we use bimFCS to measure diffusive behavior at multiple length scales simultaneously. Coupling this technique with the principles of total internal reflection fluorescence (TIRF), excitation of fluorescently-tagged transmembrane proteins are restricted near the basal membrane. Autocorrelation data is analyzed with two-component fit to separate fast short range diffusers from slower long range diffusers interacting with actin filaments. Data is interpreted through FCS Law in order to derive the average confinement strength and size of corrals. Through simulation, we also explore the effect that corrals have on protein interactions. Because the cortical actin structure is constantly undergoing changes due to factors such as thermal fluctuations, ATP-driven remodeling, and intracellular and extracellular interactions, the influence this variability has on protein interactions is of great interest. Parallel to computational results obtained by Kalay, et al. of Kyoto University, our simulation suggests that, while the overall quantity of protein dimerization events is independent on how strongly the proteins are confined, the frequency of dimerization events is dependent on the confinement strength, which has implications towards amplification of a cell signal.

2299-Pos Board B315

The Role of Cortical Actin in the Regulation of Eph Receptor Signaling

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Actin cytoskeleton filaments form a mesh lying in close proximity to membrane (cortical actin) that is able to compartmentalize receptors and other membrane proteins. It has been previously reported that the spatial organization of Eph receptors and their ligands (ephrin) at the cell-cell contact interface modulate signaling cascades in breast cancer cell lines, impacting tumor aggressiveness. In particular, it has been suggested that the formation of clusters of Eph receptors on the membrane correlates with an invasive phenotype. Following this evidence, we hypothesize that cortical actin regulates Eph receptor signaling by tuning the organization of receptor at the membrane. By employing ephrin decorated DNA nanostructures rendered from polyhedral flat sheets, we are studying the role of the cortical actin in the stimulation of Eph-A2 receptor with their ligands. The shape and size of the nanostructure will impact on the lateral association of the ligands and hence the receptors. DNA flat sheets are functionalized with monomeric ephrin-A5 conjugates following two designs that allow or not the dimerization of ligand bound receptor, depending on the proximity between ligands on the nanostructures. Proximity Ligation Assay is used to monitor the phosphorylation levels of the receptor upon treatment with drugs (Latrunculin A, Jaspilakinolide) that modify the size of the cortical actin mesh. High-resolution microscopies (AFM and STORM) are used to monitor the mesh size and the clustering of the receptors. All together,

this approach will point out molecular mechanisms of spatial organization of ligands and receptors during clustering and the effects of cytoskeleton on membrane receptor-mediated signaling.

2300-Pos Board B316

Control Neurotrophin Signaling using Light during PC12 Cell Differentiation and *Xenopus* Embryonic Development

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Growth factor-mediated signaling pathways regulate neuronal survival, proliferation, differentiation, and apoptosis. The mitogen-activated protein kinase (MAPK) signaling pathway is a primary signaling cascade downstream of the binding of neurotrophins to their receptors. Evidence suggests that signaling output of the MAPK pathway varies with its temporal kinetics. A quantitative delineation of signaling kinetics is limited due to a lack of tools that allows precise temporal control of the MAPK pathway. The emerging non-neuronal optogenetics, which utilizes light to control intracellular signaling pathways, provides a new modality for spatiotemporal signaling control. We have developed an optogenetic system that allows reversible activation of the MAPK signaling pathway in intact cells and in developing *Xenopus laevis* embryos. In PC12 neuronal cell lines, light-controlled, intermittent MAPK activity reveals a memory effect in light-induced neurite outgrowth. In *Xenopus* embryos, developmental stage-specific MAPK activation implies that this pathway can reprogram cell fate after germ layer specification, a crucial time window during which destination of cell fate is set. Our strategy can be generalized to control other kinase pathways with a similar activation mechanism. Results from our research will help resolve intracellular mechanisms of neurotrophin-regulated signal transduction during cell differentiation and embryonic development.

2301-Pos Board B317

Structural Basis for Growth Differentiation Factor 5 (GDF5) Signaling Inhibition by Repulsive Guidance Molecules (RGMs)

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Repulsive guidance molecules (RGMs) are cell surface proteins that play key roles in cell migration, neuron regeneration and iron homeostasis. RGMs modulate two signaling pathways: the Neogenin and the Bone morphogenetic protein (BMP)/Growth differentiation factor (GDF). Here, we present crystal structures of the N-terminal domains of three human RGM family members in complex with GDF5. All three RGMs occupy the binding site for GDF5 type 1 transmembrane receptors. We show that mutations of RGM residues that cause the iron-overload disease are located in the GDF5-binding interface and weaken RGM-GDF5 interactions. Our RGM-GDF5 crystal structures, structure-guided biophysical binding studies and cellular assays suggest that RGMs inhibit GDF5 signaling by competing with GDF5 type 1 receptors.

2302-Pos Board B318

Cell Surface Calreticulin-LRP1 Binding and its Role in Apoptotic Cell Engulfment

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Phosphatidyl serine (PS) lipids in inner leaflet of cell membrane are externalized and become exposed in cholesterol-rich domains during apoptosis and co-localized with cell surface calreticulin (CRT) (J Immunol. 148:2207-16, 1992; Cell. 123:321-34, 2005). Association of cell surface CRT in apoptotic cells with LDL receptor-related protein (LRP1) in phagocytes plays an important role in apoptotic cell engulfment (Cell. 123:321-34, 2005). Whether cell surface CRT directly binds to LRP1 in apoptotic cells and what role of CRT-LRP1 binding in apoptotic cell engulfment is needs to be addressed. Such studies could identify a potential site for regulation of apoptotic cell removal in tissue remodeling and tumor immunity. In this study, we characterized CRT-LRP1 binding in apoptotic or viable wild type mouse embryonic fibroblasts (K41 MEFs), apoptotic CRT-null MEFs (K42 MEFs) and apoptotic recombinant CRT-rescued K42 MEFs with co-immunoprecipitation experiments and determined engulfment of apoptotic wild type MEFs or