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Neuropilins Lock Secreted Semaphorins onto Plexins in a Ternary Signalling Complex
Tomas Malinauskas[1], Bert J.C. Janssen[2], Greg A. Weir[1], M. Zameel Cader[1], Christian Siebold[1], E. Yvonne Jones[1].
[1]University of Oxford, Oxford, United Kingdom, [2]Utrecht University, Utrecht, Netherlands.
Semaphorin-Plexin cell-cell signalling plays key roles in axon guidance, immunity and cancer. Co-receptors provide an additional level of complexity essential for many of these biological functions. The large Semaphorin 3 class of secreted Semaphorins requires a co-receptor, Neuropilin, to signal through the Plexin A receptor class. It is not known whether Semaphorin 3s interact directly with Plexin As, and if so what the characteristics of these interactions are, what the role of Neuropilin is, or how the extracellular regions of these three proteins act together to trigger signalling. Here we present the 7 Å crystal structure of a mouse Semaphorin 3A-Neuropilin 1-Plexin A2 complex. The ternary complex consists of six subunits: the dimeric Semaphorin, two copies of the Plexin and two copies of the Neuropilin arranged as a dimer-of-heterotrimers. In each heterotrimer, one subunit of the Semaphorin 3A directly contacts a Plexin A2, as in co-receptor independent Semaphorin-Plexin signalling complexes. The Neuropilin 1s ''lock'' the Semaphorin 3A and Plexin A2 together into the complex by cross-bracing the two halves of the assembly, bridging Semaphorin 3A and Plexin A2 subunits from the two component heterotrimers. Surface plasmon resonance-based and cellular analyses of wild-type and mutant proteins suggest that this Neuropilin binding mode stabilises a canonical, but weak Semaphorin 3A-Plexin A2 interaction, thereby adding a co-receptor based level of control onto the mechanism by which receptor dimerization and/or oligomerization triggers signalling.
Correspondence: E.Y.J. Equal contribution: T.M. and B.J.C.J.

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Visualizing the Dynamics of SRC Homology 2 (SH2) Domain containing Proteins on the Live Cell Membrane
Dongmyung Oh, Ogiue-Ikeda Mari, Jadwin A. Joshua, Machida Kazuya, Mayer J. Bruce, Yu Ji.
University of Connecticut Health Center, Farmington, CT, USA.
Epidermal growth factor (EGF) stimulation triggers downstream signaling cascades through binding of Src homology domain 2 (SH2)
containing proteins to the phosphorylated tyrosine (p-Tyr) sites of the EGF receptor (EGFR). This tyrosine signaling plays an essential role in cell proliferation, metastasis, survival, tumorigenesis, etc. However in vivo kinetics and dynamics of this process are not well quantified. Here we use total internal reflection (TIR) microscopy and single molecule imaging to visualize interactions between individual SH2 modules and p-Tyr sites near the cell membrane which provides us with the statistical kinetics and dynamics parameters, dissociation and diffusion rate. We found that surprisingly a constant apparent dissociation rate (Δ) of SH2 modules within the TIR illumination field and it is significantly longer than predictions based on chemical dissociation rate constants, suggesting that SH2 modules quickly rebind to p-Tyr site in the vicinity of the previous binding site after dissociation. We also found that the effective diffusion coefficient, (D_{eff}), is positively correlated with the Δ value for different SH2 modules and Δ is negatively correlated with the local density of receptor tyrosine kinase (RTK)
phosphorylation. These results are theoretically well explained with diffusion-controlled rebinding model, describing the SH2 molecule competes in the diffusion and re-association event after dissociation. The studies suggest a mechanism whereby signal output can be regulated through the spatial organization of multiple binding sites, which will prompt reevaluation of many aspects of RTK signaling, such as signaling specificity, mechanisms of spatial control, and noise suppression.

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Effects of Dietary Potassium on Expression of AT1R and ATRAP
Elida Adalgisa Neri, Gabriella Duarte Queiroz-Leite,
Nancy Amaral Rebouças.
University of Sao Paulo, Sao Paulo, Brazil.
Previous studies have shown that potassium depletion enhances AT1 receptor density in the apical membrane, the basal membrane and nuclei of proximal tubular cells. The purpose of this study was to analyze the effect of high K^+ intake and K^+ depletion on Angiotensin II receptor (AT1R) and ATRAP (AT1R receptor-associated protein) expression on kidney cortex. Wistar rats were divided into three groups: 1) normal K^+, 2) high K^+ (9%) and 3) K^+ depletion. The cortical portion was analysed by ''Western/imunobloting'' with anti-AT1R and anti-ATRAP antibodies. ATRAP and AT1R total mRNA were quantified by real-time PCR. using total proteins extract, we observed an increase in AT1R expression of 119% in the overload group and 280% in the K^+-depleted group. ATRAP expression was increased by 82% in K^+-depleted group, whereas in K^+ overload group there were no changes in ATRAP expression. The ATRAP/AT1R expression ratio decreased 38% in overload and 52% in depleted models. In total membrane fractions it was observed an increase of 82% in AT1R and 128% increase in ATRAP expression in depletion model, as ATRAP/AT1R expression ratio was not affected. The results of real-time analysis showed a decrease of 9% in AT1R and 4% in ATRAP in the K^+ overload group. In the K^+-depleted group, AT1R decreased 36% and ATRAP 20%. In conclusion, we showed that both, high K^+ intake or K^+ depletion significantly modifies AT1R and ATRAP protein expression and mRNA abundance, except for ATRAP protein abundance in the overloaded model. Besides, based in our results, we postulate that treatment of animals for 7 days with this dietary may influence the post-translational modifications in AT1R and ATRAP proteins, since we observed an increase in protein expression with reduced mRNA abundance of these proteins.

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Cell Shape can Dynamically Process Information during Signal Flow in Regulatory Pathways
Padmini Rangamani[1], Azi Lipshtat[2], Evren Azeloglu[3], Rhodora Calizo[4], Suzanne Scarlata[4], Susana Neves[3], Ravi Iyengar[3].
[1]UC Berkeley, Berkeley, CA, USA, [2]Gonda Brain Research Center, Tel Aviv, Israel, [3]Mount Sinai School of Medicine, New York, NY, USA, [4]Stony Brook University, Stony Brook, NY, USA.
Shape is often an indicator of cell health; however the role of cell shape in signaling is not well understood. There are many factors that influence the interaction between cell shape and signaling including the cytoskeleton, protein scaffolding and crowding. Here,we determined if cell shape alone could process information during signal transduction at the plasma membrane. Using analytical approaches and numerical simulations we studied elliptical shapes since neoplastic transformation often results in cells that are spindle shaped. Mathematical analyses showed that with increasing eccentricity of the cell, receptors diffusing evenly in the plane of the membrane accumulate transiently at regions of high curvature upon binding ligand. This inhomogeneous distribution of activated receptors is periodic and follows the Mathieu function. This transient inhomogeneity arises from local balance between reaction and diffusion of the soluble ligand and membrane-bound diffusion of the receptor. Numerical simulations for the receptor pathways show that these transient microdomains of activated receptors in the membrane amplify signals to downstream protein kinases. For the growth factor receptor pathway, change in cell shape from circle to ellipse results in a nearly two-fold increase in activated MAP-kinase in the nucleus.
The model predictions were tested experimentally using patterned cells. Experimental measurements of receptor density and diffusivity show that the EGF receptor activation and diffusion is dependent on the local curvature. Additionally, elliptical cells show a transient spatial inhomogeneity in the activation of signaling components when compared to circular cells in vitro.
Thus, cell shape and growth factor signaling can form a multi-scale positive feedback loop that could contribute to the maintenance of the transformed state.

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Role and Control of Cell Membrane Curvature in Receptor Transport
Kathrin Spendier, Joshua B. Baptist, Zbigniew J. Celinski,
Anatoliy V. Glushchenko.
University of Colorado Colorado Springs, Colorado Springs, CO, USA.
The cell membrane is extremely complex, consisting of spatially and temporally dynamic two- and three-dimensional heterogeneities. This changing membrane topography is known to play an important regulatory role in physiological processes, such as cancer. Our primary interest is to understand the implications of membrane curvature in immune signaling by mast cells for which the rat basophilic leukemia (RBL) cell line is used as model system. In tissue, mast cells can cause allergic responses as well as protect our body from parasitic infection. Mast cells have high affinity Fc receptors (Fc-epsilon-RI) specific for immunoglobulin E (IgE). To study the effect of membrane curvature gradients on Fc-epsilon-RI transmembrane signaling, we control membrane curvature with supported lipid bilayer coated three-dimensional glass substrates and observe fluorescently labeled immunoglobulin E loaded Fc-epsilon-RI receptor (IgE-R) motion by single-particle tracking. We show how the dynamics and distribution of IgE-R are affected by curvature-induced changes to the cell membrane thickness.