studies have revealed several components of this process. There is now little doubt that these functional mechanisms involve a combination of changes both in the structure of the receptor and in its intermolecular interactions. However, it has been difficult to put together a coherent and unified mechanism of ErbB receptor activation. The principal reason for this difficulty is that our current understanding is derived from the study of isolated fragments of receptor. Here, we propose to overcome this difficulty by taking advantage of a new residue-level coarse-grained (CG) molecular representation and simulation approach, termed ELNEDYN, which is being developed in our laboratory to investigate these mechanisms in the structural context of ''full'' length receptor constructs. One of our long-term objectives is to identify structural and dynamical mechanisms (e.g. structural rearrangements and modes of receptor-receptor association) that underlie or regulate the signaling function of ErbB receptors. Here we summarize results from these CG 3D-modeling studies that show how the conformational equilibrium properties and structural changes of the extracellular domain modulate the conformational equilibrium of the transmembrane and tyrosine kinase domains of the receptor, thereby providing insight into intramolecular factors that govern and regulate the activation and outside-in signaling mechanism of ErbB receptors.

1890-Plat

A Biophysical Mechanosensor Model for T-Cell Receptor Signaling

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T-cells (cytolytic, helper and others) are vital components in adaptive mammalian immune defense systems. T-cell receptor (TCR) signaling which follows recognition of a peptide antigen bound to an MHC molecule (pMHC) on an antigen presenting cell (APC) is critical for T-cell activation, differentiation, and proliferation. For decades, the mechanism for TCR signaling across the T-cell membrane by this multi-subunit receptor has remained a mystery. Based on our recently obtained structural data on stimulatory and non-stimulatory anti-TCR antibodies and their binding footprint to TCR components obtained by NMR, we present here a simple but all-inclusive ''dynamic torque transducer'' model for TCR signaling. Extracellular mechanical torque can result in quaternary structure changes between the pMHC-binding TCR alpha/beta chains and the non-convalently linked CD3 chains within the TCR complex, triggering downstream signaling via ITAMs in the CD3 cytoplasmic tails. In this process, the pMHC behaves as a detachable effort arm of this complex-lever system; its binding is determined by the specificity of an individual TCR but is not sufficient to mediate signal transduction per se. An external torque is additionally required to provide the energy for this signaling. Such a torque can be generated either via a shear force or vertical pressure between the opposing APC and T cell membranes. The former "bind-and-tug" mode may be responsible for initial signaling during T-cell scanning of APCs, while the latter ''bind-and-bend'' mode is responsible for sustained signaling inside immune-synapses during T-cell activation. Our model can also explain T-cell signaling mediated by antibody or multimeric pMHC cross-linking. This mechanism of converting mechanical energy to a biochemical signal, mediated and controlled by a detachable interface, may be generally applicable in other cell-cell signaling systems both within and outside of the immune system.

1891-Plat

Mechanical Forces in T Cell Triggering

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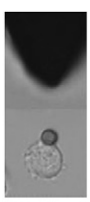
T cell is one of the main player of mammalian immune response. It ensures antigen recognition at the surface of antigen presenting cells (APCs) in a complex highly sensitive and specific process where encounter of T cell receptor with agonist peptide associated with major histocompatibility complex triggers T cell activation. Despite its central role, the mechanism of TCR triggering is still unclear. Several models involving receptor oligomerisation, kinetic proofreading, serial triggering are currently under debate.

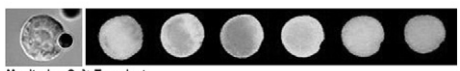
We present here a work aimed to explore experimentally the role of mechanical cues in T cell activation.

We will show the first results of mechanical engagement of TCR in Jurkat cell line using magnetic particles and related cell response as reported by intracellular Ca^{2+} transient.

We will discuss then how these results could provide a mechanistic solution to the TCR triggering puzzle.



Pulling on TCR



Monitoring Ca^{2+} Transient

1892-Plat

Adenosine A_1 Receptor Signaling Unraveled By Particle Image Correlation Spectroscopy (PICS)

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The adenosine A_1 receptor is a typical example of a G protein coupled receptor (GPCR). Despite a wealth of biochemical data the general mechanism of GPCR signaling has not been fully clarified. Whether GPCR signaling takes place in membrane microdomains, and whether the respective G proteins are precoupled, is still heavily debated. Both mechanisms would explain the fast receptor G protein interaction that is observed in experiments. Using single-molecule microscopy in live CHO cells and our recently developed analysis technique (PICS, Semrau, Schmidt., Biophys. J., 2007) we unraveled the first steps of the A_1 receptor signaling. We found that at least 7% of the receptors are precoupled to the G protein already before stimulation with an agonist. Furthermore, 9% of the receptors translocate to membrane microdomains upon agonist stimulation. These domains, which are about 150 nm in size, are related to the cytoskeleton. We believe that this knowledge about the molecular mechanisms of GPCR signaling will open up new ways to manipulate GPCRs and develop new, potent drugs.

1893-Plat

Spontaneously Formed EGFR Dimers Are Primed For Activation

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The Epidermal Growth Factor Receptor (EGFR) plays a central role in normal biological processes and disease states such as cancer. Considerable effort has thus been devoted to understanding the mechanisms that control its activation. The conventional model of EGFR activation is that ligand binding induces a conformational change in the receptor, which then leads to dimerization and activation of the intrinsic kinase. Structural studies have identified a key loop protruding from domain II (dimerization arm) of the EGFR ectodomain as a crucial mediator of dimerization. However, a precise mechanistic picture of receptor activation requires time dependent probing of individual molecules on living cells. Here, we report quantum dot (QD)-based optical tracking of single receptor movements on the membranes of living cells. In the absence of ligand, receptors underwent reversible dimerization, indeed dependent on their dimerization arms. However, the dimer concentration is low in cells with normal EGFR expression due to a relatively high dissociation constant (~10-40 μM). Ligand binding stabilized spontaneously formed dimers by reducing the dissociation rate constant, which leads to sustained kinase activation. We found that spontaneously formed dimers can also initiate kinase activation without ligand binding. Moreover, we found that EGFR dimer density was higher in the periphery of the cell versus the center. This difference was reflected in both spatial and temporal heterogeneity of EGFR signaling, where the periphery of the cell serves as an early response site for EGFR activation. Our findings suggest that therapeutic antibodies may be more effective if they increase the dissociation rate constant of EGFR dimers to weaken the activation capability of primed dimers.

1894-Plat

Modeling and Simulation Of A Protein Tertiary Complex: Study of the Interaction Interface and Conformational Dynamics of Dark State Rhodopsin in complex with Transducin

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We report the first all-atom Molecular Dynamics simulation of a transmembrane protein tertiary complex composed of the G-protein coupled receptor (GPCR) rhodopsin and its G-protein intracellular counterpart transducin in a mixed DOPC membrane/water environment. Based on the analysis of our μsec-timescale simulation trajectory starting from a docked conformation of the complex, we characterize the dynamics present in the dark-adapted state and their influence in the properties and stability of the interaction interface

between the protein subunits. Our results demonstrate the large-amplitude collective motions that span across the different protein subunits and indicate the broad range of dynamics present in the dark state that prelude the allosteric changes which take place during the activation of the complex. We suggest that the intracellular domains and cytosolic extensions of the transmembrane α-helices in rhodopsin participate in correlated motions that influence their interaction with key structural elements of transducin, such as the N- and C-terminal α-helices. Despite the very transient nature of the interaction interface, persistent interdomain interactions involving hydrophobic clusters and charged groups are crucial in the stability of the complex. We propose the general structural features of the interaction interface and relate our results with atomic site distance measurements from electronic paramagnetic resonance (EPR) experiments. Our results further suggest novel mutagenesis experiments that can be used to investigate the stability and correlated dynamics of this model receptor system. This work is funded by the NSF, IBM and RPI.

1895-Plat

Differential Voltage-sensitivities Of Agonists At The Dopamine D_{2S} Receptor

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Voltage-sensitivity has recently been demonstrated for agonist potency and affinity at certain G protein-coupled receptors. Several of those studies employed electrophysiology assays coexpressing receptors with G protein-coupled potassium channels (GIRK) in *Xenopus* oocytes. Using this assay, we have previously shown that the potency of dopamine in activating GIRK via the dopamine D_{2S} receptor is reduced by depolarization from -80 to 0 mV. We have now investigated the voltage-sensitivities of a range of structurally related dopaminergic agonists at the D_{2S} receptor. We found β-phenethylamine, *p*- and *m*-tyramine to be voltage-insensitive, while both non-hydroxylated and monohydroxylated *N*,*N*-dipropyl-2-aminotetralin (DPAT) compounds were voltage-sensitive.

There were no differences in the fractional responses to β-phenethylamine, *p*- and *m*-tyramine, relative to the response to a saturating concentration of dopamine, between -80 and 0 mV. Whereas β-phenethylamine, *p*- and *m*-tyramine are all partial agonists at the D_2 receptor, *m*-tyramine behaved as a full agonist, indicating amplifaction in the signal transduction from receptor to channel. The differential voltage-sensitivity did not depend on signalling via distinct G-proteins, since both voltage-sensitive and -insensitive behaviour was observed when signalling occurred exclusively via a PTX-insensitive $G_{\alpha o1}$ subunit. The level of G-protein activation or extent of receptor reserve was not responsible, since voltage-sensitivity of dopamine persisted in the absence of a receptor reserve, and since *m*-tyramine was voltage-insensitive while behaving as a full agonist at GIRK activation. Instead, we speculate that these differences might relate to the structurally constrained binding orientations of DPAT ligands and dopamine, as suggested by mutational binding studies by others, as compared to β-phenethylamine, *p*- and *m*-tyramine, which have been suggested to bind in a more flexible manner. These findings suggest a means of investigating the physiological roles of receptor voltage-sensing and might hint at a mechanism of receptor voltage-sensitivity.

1896-Plat

"cAMP Sponge": A New Tool For Probing cAMP Microdomains In Living Cells

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Specific buffers for second messengers (e.g. BAPTA for Ca2+ and ''InsP3 sponge'' for InsP3) have proven invaluable for studying complex signaling pathways. A buffer for cAMP would also be highly desirable, but no such tool currently exists. Here we present the generation and validation of a novel genetically encoded cAMP buffer, ''cAMP sponge'', based on the PKA regulatory subunit type 1, subtype beta (RIbeta). In order to avoid possible biological effects of RIbeta, we have engineered a recombinant form consisting of the two C-terminal cAMP binding domains, while the biologically active NH2 terminus was eliminated. In order to quantitatively assess the expression of our ''cAMP sponge'' at the single cell level, we labeled it with the fluorescent protein, mCherry. This allows quantitative and qualitative assessment of our chimera expression (based on fluorescence intensity measured at 610nm emission) on the microscope stage. Evaluation of the cAMP buffering power of our sponge in cell lines stably expressing a FRET-based sensor for cAMP (EPAC H30) showed significant attenuation of the response to PGE2, VIP and FSK. Sponge constructs targeted to cytoplasm, plasma membrane, and nucleus showed significant local attenuation of the response to cAMP-generating agonists. In control experiments, over-expression of mCherry alone or a double mutant of our sponge in which the two cAMP binding sites were inactivated showed no significant attenuation of cAMP signals in PGE2-, VIP- or FSK-challenged cells. Interestingly we found that the extra cAMP buffering power provided by the cAMP sponge can be compensated by second messenger derived from connected neighboring cells that do not express the exogenous buffer, revealing new aspects of gap junction mediated communication. This new tool should also prove valuable for assessing the importance of cAMP microdomains in secretion, migration and transcriptional regulation.

Platform AE: Voltage-gated K Channels - Gating: Structure & Function

1897-Plat

Towards The Chemical Synthesis Of A Voltage Gated K+ Channel

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Understanding the relationship between the atomic structure of a protein and the biological functions requires the ability to perturb the protein structure in a precise manner. This is generally accomplished by means of traditional site-directed mutagenesis. However, the modifications that can be introduced in this manner are limited by the set of naturally occurring amino acids. Chemical synthesis on the other hand facilitates the incorporation of a wide variety of side chain and peptide backbone modifications that enables precise modifications of the structural and electronic properties of the protein. Similar modifications are not possible using conventional mutagenesis, thus making chemical synthesis an important asset in investigations of protein structure and function. The size of the protein has been a major factor limiting the use of chemical synthesis to investigate proteins. In the field of membrane proteins, chemical synthesis has so far been accomplished only for relatively small (< 150 amino acids) proteins. Proteins of interest such as voltage gated ion channels are much bigger and are presently not amenable to chemical synthesis. To overcome this limitation, methods that can be used for the chemical synthesis of large (> 150 amino acid) membrane proteins are required. We are presently developing methodology for the chemical synthesis of the voltage gated K+ channel KvAP. Voltage gated ion channels are responsible for the generation of electrical impulses by excitable cells. They have been the subject of intense research, however, a number of key questions remain about the mechanisms of ionic selectivity and gating in these channels. We believe that using chemical synthesis for protein modification in combination with functional and structural studies will enable us to provide new information on these physiologically important processes.

1898-Plat

Investigating the Electromechanical Coupling in voltage-gated K^+ channels

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While the gating of the voltage sensor as well as the opening of the ion conducting pore in voltage-gated ion channels has been investigated extensively, the electromechanical coupling between the peripheral voltage sensors and the central ion conducting pore needs to be further elucidated. Based on previous work (*Lu et al., 2002*) and the crystal structure of Kv1.2 (*Long et al, 2005*), it has been suggested that the C-terminal S6 is coupled to the S4-S5 linker region, and that this coupling leads to opening of the pore. Here, we set out to investigate the role of this interaction in the coupling mechanism in voltage-gated Shaker K^+ channels. Possible interaction sites were chosen and altered by point mutation. The Shaker channels were expressed in Xenopus oocytes and ionic and gating currents as well as fluorescence changes monitoring the S4 movement were determined using voltage-clamp fluorometry in a cut-open voltage-clamp setup.

We identified two spatially distinct interactions between the S6 and the S4-S5 linker that have a significant influence on the gating properties of Shaker. One interaction acts upon the closed and open state and its disturbance accelerates voltage sensor movement while slowing ionic currents. A second interaction only acts in the open state. Exchange of the responsible residue slows closing of the channel by trapping the voltage sensor in the open state. We are interpreting the interactions in order to form a molecular model for the electromechanical coupling process.

(supported by CIHR MOP-81351 and CRC 202965).