Symposium 7: Protein and Cellular Mechanics

1097-Symp
How Mechanical Forces Can Switch On and Off Protein and Cell Binding Sites
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How do cells sense whether proteins are stretched or relaxed? While mounting evidence exists that cells and tissues sense mechanical stimuli and convert them into biochemical signals, knowledge about the underpinning mechanisms is sparse. A multitude of structural mechanisms have evolved among extracellular and cytoplasmic proteins that are part of force-bearing protein networks, each enabling distinct modes of mechano-chemical signal conversion. The structural motives include designs by which force can destroy recognitions sites, or alternatively open up cryptic sites that can then recruit other proteins in a force-up-regulated manner. Here we will discuss how the stretching of fibronectin fibers, which form the most extensible protein fibers known so far, can activate or destroy protein and cell binding sites over a wide range of mechanical strains. Stretching of fibronectin fibers thus not only increases their Young's moduli, over orders of magnitude until they rupture when stretched to a few MPa, but their biochemical display is altered in intricate ways as well. Deciphering the underlying engineering design principles by which extracellular matrix proteins can serve as mechano-chemical signalling switches is not only essential to learn how cells sense and respond to mechanical forces, and probe the physical properties of their environments. It has far reaching implications in tissue engineering, systems biology and medicine.

1098-Symp
Nanoscale Protein Architecture of Focal Adhesions
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Focal adhesions (FAs) mediate cell interactions with their extracellular matrices (ECMs) and consist of integrin ECM receptors linked to the actin cytoskeleton via plasma-membrane-associated protein plaques. Despite their fundamental importance in multicellular organisms, the three-dimensional organization of proteins within FAs is unknown. Here we determine FA molecular architecture by using 3D superresolution microscopy (interferometric Photo-Activated Localization Microscopy) to map nanoscale protein organization. We find that the FAs consist of partially overlapping proteinspecific vertical layers of 15-50 nm thickness, with integrins and actin separated by a 30-50 nm FA core which is spanned by talin tethers. This reveals a structural basis for FA function whereby a multilaminar core architecture mediates the interdependent cell processes of adhesion, signaling, force transduction, and actin cytoskeletal regulation.

1099-Symp
Force Probing the Molecular Mechanics of Cell Rounding
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During mitosis tissue culture cells undergo a dramatic shape change, from essentially flat to nearly spherical. The forces and mechanisms that drive this shape change remain unexplained. Here we use assays based on atomic force microscopy to measure the height and rounding force of single mitotic cells. We show that under our conditions, human cells exert forces approaching 100 nN when they round up. The force depends not only on the actomyosin cortex but also on trans-membrane ion gradients. In further experiments we demonstrate which membrane proteins are coupled to and regulated by the actomyosin cortex to establish a hydrostatic pressure that rounds up the cell. By using single-molecule force spectroscopy we look inside these individual membrane proteins to quantify by which interactions and mechanisms they are functionally regulated. Based on these results we introduce an advanced model of cell rounding in which a hydrostatic outward pressure, and contractile actomyosin cortex forces govern shape.

1100-Symp
Regulation of Mechanical Equilibrium in Multicellular Arrangements
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We investigated the physical laws governing the mechanical equilibrium of multi-cellular arrangements. Breaking and maintaining this equilibrium are the fundamental basis for embryonic morphogenesis and tissue homeostasis. It notably plays a key role in epithelium-mesenchymal transition (EMT) during normal development and tumor transformation. Since multi-cellular equilibrium relies on a spatial regulation of the force balance between cell-cell and cell-extra cellular matrix (ECM) adhesions, we studied human epithelial cell pairs confined on defined ECM micro-patterns geometries. We developed an automated tracking method to quantify the cell movements in high throughput time-lapse acquisitions. We found that cell pairs could adopt different behaviors depending on pattern geometries. A complete survey over many different geometries showed that cells adopted all graded phenotypes from continuous cell migration to static mechanical equilibrium. After induced EMT the stability of cell pair configuration was affected.
We used cytoskeleton observations and physical modeling to identify the physical parameters implicated in the establishment of mechanical equilibrium. Current physical models of multi-cellular equilibrium, which consider a constant line tension along the perimeter, surface tension of the membrane and adhesion energies could not account for spatial arrangements we observed. Immuno-fluorescent labellings and in vivo expression of actin marker revealed three types of actin cables: cables above adhesive regions connecting two ECM adhesion sites and cables above non adhesive regions connecting either two ECM adhesion sites or one ECM and one cell-cell adhesion site. Preliminary nano-ablation experiments to severe actin cables suggested that tension could vary in each type of cable. It seems that cells develop an anisotropic distribution of line tension in response to local adhesiveness. We currently investigate with experimental and numerical approaches whether anisotropic distribution of tension could be the regulator of the spatial arrangement we observed in various microenvironment geometries.

Symposium 8: Structure and Dynamics of Membrane Transporters

1101-Symp
A Dynamical View of Membrane Transporter Function at Sub-Angstrom Resolution
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Membrane transporters provide the main mechanism for active exchange of materials between the cytoplasm and a cell's environment in a highly selective manner. These complex molecular machines present a structurally diverse group of pumps evolved to efficiently couple various sources of cellular energy to the selective transport of different molecules. Depending on the source of energy used and the type of the substrate transported, different protein architectures and, thus, different mechanisms are employed by membrane transporters. Active transporters undergo various degrees of protein conformational changes (induced, e.g., by ATP hydrolysis or by binding of the substrate and the co-transported ions) during their transport cycle. In other words, they adopt distinct conformational states during their function. Due to the difficulties associated with structure determination of membrane proteins, however, for the majority of structurally-known transporters only one of the major functional states has been structurally characterized. Substrate binding and translocation along the transport pathway in membrane transporters are closely coupled to numerous stepwise protein conformational changes of various magnitudes and forms that are induced by and/or coordinated with the energy-providing mechanisms. A detailed description of the mechanism of membrane transporters, therefore, relies on high-resolution methodologies that can describe the dynamics of the process at an atomic level. In this talk, latest results of molecular dynamics simulations performed on a number of membrane transporters with diverse mechanisms, and the molecular events involved in their function revealed by these simulations will be presented.

1102-Symp
Ion Transport by the Sodium Pump
Hanne Poulsen.
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The first crystal structure of the Na+,K+-ATPase revealed the potassium-bound form of the pig kidney enzyme at 3.5 Å resolution. This large membrane protein complex consists of an alpha subunit similar to the Ca2+-ATPase, a heavily glycosylated beta subunit and a small regulatory gamma subunit (also known as FXYD2). The electrogenic transport performed by the Na+,K+-ATPase causes extrusion of three sodium ions and uptake of two potassium ions per ATP split. The gradients thus formed are of fundamental importance in physiology as they

control ionic conditions in the cell and energise osmotic potentials, secondary transport schemes and ionotropic signalling.
A surprising finding from the Na+,K+-ATPase structure was the docking of two conserved tyrosine residues at the C-terminus of the alpha subunit into the transmembrane domain, hinting that this was a previously unidentified regulatory element. Several mutations causing human neurological syndromes have subsequently been mapped to the C-terminal structure element, also clearly indicating that conservation of the structure is important for pump function.
Mutational analysis confirmed this and prompted our further analysis by electrophysiology and molecular dynamics simulations, which have shown a profound effect of the C-terminus on the electrogenic transport properties. We further propose that the C-terminal region forms a binding pocket that can be exploited for pharmacological intervention in cardiovascular and neurological disease.

1103-Symp
Alternating Access Mechanism of Glutamate Transporters
Olga Boudker.
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In the central nervous system, glutamate transporters are responsible for the glutamate clearance following rounds of neurotransmission. They are molecular pumps, which utilizes the energy of pre-existing electrochemical gradients of ions to drive substrate uptake against steep concentration gradients. Sodium coupled aspartate transporter from Pyrococcus horikoshii, GltPh, is a homologue of the mammalian transporters and has served as a model system, within which to understand the molecular details of transport. The previously determined crystal structures of GltPh revealed the substrate and sodium binding sites located near the extracellular solution leaving the question of how they reach the cytoplasm unanswered. Recently, we have determined the crystal structure of a double cysteine mutant of GltPh, captured by cross-linking in a novel conformational state. In this state the substrate-binding sites are near the cytoplasmic surface of the protein. These findings suggest a novel and unexpected mechanism, by which GltPh and, by analogy mammalian glutamate transporters catalyze trans-membrane transport of their substrates.

1104-Symp
Alternating Access of the Maltose Transporter
Jue Chen.
Purdue Univ, West Lafayette, IN, USA.
No Abstract.

Platform R: Channel Regulation & Modulation

1105-Plat
Photopharmacology: Controlling Native Voltage-Gated Ion Channels with Light
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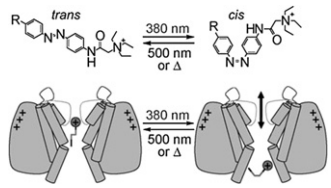
Optical control of proteins provides critical advantages for studying cell signaling and offers great promise in biotechnology and biomedical research. We have developed a series of photochromic ligands (PCLs) that target voltage-gated ion channels. They possess an azobenzene photoswitch connected on one side to a quaternary ammonium ligand (internal blocker for potassium, sodium and calcium channels) and on the other side to a variety of chemical groups. The azobenzene photoisomerizes between cis and trans configurations using different wavelengths of light, thereby repetitively turning on and off ion flow.
Alteration of the R group makes our approach very modular. First, increasing hydrophobicity allows better membrane penetration and therefore greater potency of the PCL. Second, PCLs with a charged R group require hydrophilic pathways to cross cell membranes and can be specifically targeted to cells expressing entry-route proteins. Third, selectivity for certain ion channels can be attained, allowing a more precise control over cellular excitability. Fourth, some PCLs act as cis blockers, offering the advantage of being silent in the dark. Finally, modifying the R group can be used to tune the spectral characteristics of the PCL, with potential interest for vision restoration.



1106-Plat
Receptor and Subunit Specificity in AKAP79/150 Actions On M-Type(KCNQ) K^+ Channels
Jie Zhang, Oleg Zaika, Manjot Bal, Mark Shapiro.
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A-kinase-anchoring protein (AKAP)79/150 mediated PKC phosphorylation of M-type (KCNQ) channels is involved in M current (I_M) suppression by muscarinic M_1, but not bradykinin B_2 receptors (Hoshi *et al.* Nat. Cell Biol. 7:1066-73). In this study, we first explored the involvement of AKAP79/150 in muscarinic suppression of KCNQ currents by co-transfecting AKAP79 with KCNQ1-5 subunits in CHO cells stably expressing M_1 receptors. Expression of AKAP79 sensitized KCNQ2-5 and KCNQ2/3, but not KCNQ1, channels to suppression by the M_1 receptor agonist oxotremorine (oxo-M). Mutation of the PKC phosphorylation site on KCNQ4 (T553A) eliminated the effect of AKAP79, confirming the role of PKC. Co-transfection of wild-type, but not dominant negative, calmodulin abolished the effect of AKAP79 on KCNQ2/3 channels. We asked if purinergic and angiotensin suppression of I_M in superior cervical ganglion (SCG) sympathetic neurons involves AKAP79/150, since purinergic P2Y receptors depress I_M in SCG neurons via a similar mechanism to that of bradykinin, involving IP_3-mediated Ca^{2+} signals, whereas angiotensin AT_1 receptors depress I_M via a similar mechanism as M_1 receptors, by depletion of PIP_2. Transfection of ΔA-AKAP79, which lacks the A-domain necessary for PKC binding, did not affect I_M suppression by the purinergic agonist UTP (2 μM), nor by bradykinin (100 nM), but did reduce I_M suppression by oxo-M (1 μM) and angiotensin II (500 nM). We also tested association of AKAP79 with M_1, B_2, $P2Y_6$ and AT_1 receptors via FRET experiments on CHO cells under TIRF microscopy, which revealed weaker FRET between AKAP79 and $P2Y_6$ or B_2 receptors than for M_1 and AT_1 receptors. Our data suggest AKAP79/150 action generalizes to KCNQ2-5 subtypes, is disrupted by calmodulin, and is involved in angiotensin, but not in purinergic, suppression of neuronal M current. Supported by NIH grants R01 NS043394 and R01 NS065138.

1107-Plat
Potassium Channel Modulation by A Toxin Domain in Matrix Metalloprotease 23
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Peptide toxins found in a wide array of venoms block K+ channels causing profound physiological and pathological effects. Here, we describe the first functional K+ channel-blocking toxin domain in a mammalian protein. Matrix metalloprotease 23 (MMP23) contains a domain (MMP23TxD) that is evolutionarily related to peptide toxins from sea anemones. MMP23TxD shows close structural similarity to the sea anemone toxins BgK and ShK, and the domain blocks K+ channels in the nanomolar to low micromolar range (Kv1.6 > Kv1.3 > Kv1.1 = Kv3.2 > Kv1.4 in decreasing order of potency), while sparing other K+ channels (Kv1.2, Kv1.5, Kv1.7, KCa3.1). Full-length MMP23 suppresses K+ channels with a pattern of inhibition consistent with MMP23TxD activity. Our results provide clues to the structure and function of the vast family of proteins that contain domains related to sea anemone toxins. Evolutionary pressure to maintain a channel-modulatory function may contribute to the conservation of this domain throughout the plant and animal kingdom.

1108-Plat
Differential Redox Regulation of ORAI Channels: A Mechanism to Tune T-Cell Responses
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Phagocytes play an essential role in host defence against pathogens by generating reactive oxygen species (ROS). Effector T helper (Th) cells migrating to sites of infection will be exposed to this highly oxidative environment. Here we show how Th-cells respond and adapt to ROS. Oxidation affects different Ca^{2+}-signalling pathways essential for T-cell function. ORAI1 channels are inhibited with an IC_{50} of ~40 μM H_2O_2, but ORAI3 channels are insensitive. We identify cysteine (C195) of ORAI1, absent in ORAI3, as the major redox