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Symposium: Response of Single Molecules to Force: Bridging Length Scales

2067-Symp

How the Mechanics of Single Molecules Determine Cell Adhesive Behavior

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Many blood cells and pathogens bind to other cells or tissues in the presence of flowing fluid. These diverse cells have evolved mechanisms to withstand and even utilize the associated drag forces to strengthen adhesion, so that many of them display a shear enhanced adhesion in which they detach at low shear but roll along the tissue surface or even stick firmly at higher shear. In this talk we use *Escherichia coli* as a model system to determine the role of various molecules in this counterintuitive behavior. We use force spectroscopy to characterize the mechanical properties of simple molecular complexes, including the adhesive molecules of *E. coli*, which form catch bonds that are longer lived under increased tensile force, and the tethers anchoring these bonds, which elongate long distances at a constant force. We then incorporate these behaviors into simulations to understand the how each property contributes to the adhesive behavior of cells or artificial adhesives. Other pathogens and blood cells display a remarkable convergent evolution, because their adhesive bonds and tethers catch and elongate with similar mechanical properties as those of *E. coli*, although they have no genetic or structural similarities. This suggests that the principles established from studying this model system can be extended to a large number of cells adhering in flow.

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Force Transmission in the Actin Cytoskeleton

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The actin cytoskeleton plays a crucial role in transmitting forces generated by molecular processes to cellular length scales to control the morphological and physical behaviors of cells. Force transmission from the molecular to cellular scale is well understood in the context of myosin II-mediated forces occurring in highly organized myofibrils found in striated muscle. However, diverse actomyosin organization found in non-muscle cells and utilized to effect shape change and force generation in cell adhesion, migration and division. The mechanical behaviors of such disordered actomyosin networks and bundles are not well understood. Our lab has developed approaches to study these questions by comparing quantitative biophysical measurements on live cells and in vitro reconstitutions with simulation and theory. I will discuss our recent data on force transmission in disordered actomyosin assemblies in the context of cell adhesion and migration.

2069-Symp

Mechanoregulation in Nanoscale Biology: From Hemostasis to Single-Molecule Centrifugation

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Many aspects of biological function and structure are governed by weak non-covalent bonds between and within single molecules. Mechanical force plays a key role in modulating the kinetics of these interactions, which in turn can regulate more complex biological processes. In this talk, I will focus on how forces in the circulatory system regulate hemostasis by acting on the blood protein von Willebrand factor (VWF). Using optical tweezers, we have demonstrated that force acts as a cofactor the enzymatic cleavage of VWF, which in turn down regulates hemostatic potential [1]. We are also developing and applying new approaches for single-molecule manipulation, including single-molecule centrifugation [2] and hydrodynamic trapping, to further investigate how the structure and function of VWF are dynamically regulated by force.

[1] X. Zhang, K. Halvorsen, C.-Z. Zhang, W.P. Wong, and T.A. Springer, ''Mechanoenzymatic cleavage of the ultralarge vascular protein, von Willebrand Factor,'' Science 324 (5932), 1330-1334 (2009).
[2] K. Halvorsen, W.P. Wong, ''Massively parallel single-molecule manipulation using centrifugal force,'' Biophysical Journal 98 (11), L53-L55 (2010).

Symposium: Transporter-Channel Interface

2070-Symp

Gating Ion Translocation through the Na,K-ATPase Pump

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Na,K pumps behave like channels controlled by extracellular- and cytoplasmic-side gates driven to open and close alternately by coupling to a cycle of Na-mediated phosphorylation and K-mediated dephosphorylation. The marine agent palytoxin disrupts that coupling, allowing both gates to sometimes be open, thereby transforming pumps into channels. So, palytoxin enables ion-pathway characterization and assays of gating mechanisms with single-molecule resolution. Palytoxin-bound Na,K pump-channels exposed to Na solutions and millimolar cytoplasmic ATP spend ~90% of the time open, each channel conducting millions of Na ions per second. Cysteine scanning of the transmembrane domain with small hydrophilic reagents reveals an unbranched cation-selective pathway crossing the entire membrane. Replacing external Na with K promptly shuts the extracellular-side gate, like during K transport by unmodified pumps. The high open probability of palytoxin-bound pump-channels in Na solutions falls ≥ 5 fold if ATP is withdrawn. Cytoplasmic AMPPNP or ADP can replace ATP - all acting with low affinity - in supporting this cytoplasmic-side gate opening, echoing acceleration of cytoplasmic K release by these nucleotides in unmodified Na,K pumps. This low-affinity, modulatory, nucleotide binding occurs electrostatically distant from the normally-phosphorylated Asp, as its mutation to Asn, Ala, or Glu leaves nucleotide affinity unaltered. The phosphate analogs BeFx or AlFx close palytoxin-bound pump-channels, apparently in all-or-none manner, quickly when ATP is absent, but slowly in millimolar ATP. This antagonism reflects modulatory nucleotide binding because ADP or AMPPNP mimic it, and it persists after mutating the phosphate-acceptor Asp to Asn. Thus BeFx or AlFx binds most tightly to pump-channel conformations with the cytoplasmic-side gate shut, despite bound palytoxin. Modulatory nucleotide binding, therefore, apparently favors cytoplasmic-side gate opening by weakening the cytoplasmic N-, P-, and A-domain interactions that stabilize gate closure, but modulation fails when BeFx strengthens those interactions. [HL36783]

2071-Symp

CLC Transporters: The Search for Conformational Change

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The conventional alternating-access mechanism for transporter function requires outward-facing, inward-facing, and occluded conformational states. The CLC mechanism has been suggested to deviate from the conventional transporter mechanism and involve almost no global protein movement. However, because this idea is based chiefly on our inability thus far to observe additional conformations crystallographically, alternative strategies for investigating conformational change are essential.

2072-Symp

Glutamate Transporter-Associated Anion Channels

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Excitatory amino acid transporters (EAATs) do not only mediate secondary-active glutamate transport, but also anion-selective currents. EAAT anion currents are small in the absence and increase upon application of L-glutamate due to substrate-dependent gating of EAAT anion channels. Anion channel gating can be described by a kinetic scheme that is based on the glutamate transport cycle and in which anion channel opening is associated with certain states. All voltage- and substrate-dependent conformational changes of EAAT4 anion channels are linked to transitions within the transport cycle, and there are no indications for additional substrate- or voltage-dependent anion channel opening and closing transitions. To account for the substrate dependence of macroscopic currents different transporter states might either exhibit distinct unitary conductances or distinct anion channel open probabilities. Macroscopic current recordings and noise analysis revealed a single channel conductance of 1 pS at symmetrical NO_3^- for EAAT4 anion channels in the absence as well as in the presence of glutamate (Kovermann et al. (2010) *J Biol Chem* 285:23676-23686). Stochastic simulations and noise analysis of simulated currents demonstrated that noise analysis is indeed able to distinguish between variable unitary current amplitudes and open probabilities (Machtens et al. *Channels (in press)).* Our findings indicate that open states branch from the transport cycle, and that - to ensure detailed balance - the uptake cycle does not progress as long the channel is open, resulting in a switch between transport and anion conduction mode. This model correctly describes alterations of anion currents upon variation of voltage and substrate concentrations. It furthermore predicts that conditions that modify the EAAT anion channel open probability modify apparent rate constants in the uptake cycle. To test this prediction we currently employ voltage-clamp fluorometry on EAATs carrying mutations that modify opening/closing transitions of EAAT anion channels.