deformations around invaded cells, knowledge of the matrix rheology is necessary.

Confocal images of collagen gels (2.4mg/ml of a 1:1 mixture of bovine and rat collagen type I) showed a narrowly distributed pore size of Ø1μm. Macrorheological measurements using a parallel-plate rheometer revealed predominantly elastic behavior that was approximately linear for strains <5%, with a shear modulus G' of 80Pa, a loss modulus G" of 11Pa, and a weak frequency dependency of both moduli according to a power-law with exponent 0.09. Microrheology was measured by applying a 21nN "point" force to a ferrimagnetic Ø4.5μm bead, and tracking the resulting displacements of Ø1μm fluorescent beads dispersed in the gel (average distance 10μm) in three dimensions. Alternatively, we applied a distributed force using a steel sphere (Ø100μm) placed onto the gel surface, or we sheared the gel with a parallel glass plate. Under all three conditions, the microscopic gel deformations closely followed that of a linear elastic, isotropic and homogeneous continuum with a Poisson ratio of 0.34. In summary, for small strains and length scales down to typical bead separation distances, marker positions in reconstituted collagen type I networks deform as expected for an affine, predominantly elastic, isotropic, homogeneous continuum.

3234-Pos Determination of Cell Elasticity through Hybrid Ray Optics and Continuum Mechanics Modeling of Cell Deformation in the Optical Stretcher

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The optical stretcher is a dual-beam trap capable of stretching individual cells. At this time there is no direct method for measuring the optical stress distribution that is responsible for the action of the optical stretcher. Previous studies have used either ray- or wave-optical models to compute the optical pressure on the surface of a spherical shell (1–2). We have extended the ray-optics (RO) model to account for focusing by the spherical interface and the effects of multiple internal reflections. Using the exact ray-optics solution for the stress distribution, cellular deformation in the stretcher is determined by a numerical solution of the Euler-Lagrange equations appropriate for thin spherical shells. Our simulation results for red-blood cells (RBCs) show that internal reflections can lead to significant perturbation of the deformation. Even in the absence of internal reflections, the RO model produces stress distributions that can deviate from the cosine-squared approximation, potentially leading to a systematic error in the determination of cellular elasticity. Calibration studies with polystyrene spheres show excellent agreement between model predictions and experimental escape force measurements, and RBC elasticity measurements are consistent with literature values. We have also used the stretcher to measure the elasticity of murine 2T3 osteoblast-like cells, and find these cells are approximately 20 times stiffer than red blood cells. Results from our current efforts to use fluorescence monitoring to assess physiological changes in stretched cells will also be discussed.

This project was supported by NIH P20 RR016469 from the INBRE Program of the National Center for Research Resources.

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Signal Transduction in Contractile & Motile Cells

3235-Pos The Giant Sarcomeric Protein Obscurin as a Potential Regulator of RhoA Signaling in Skeletal Muscle

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Board B538

Obscurin is an 800 kDa protein of the titin superfamily that organizes myofibrils and the sarcoplasmic reticulum in striated muscle. Unlike titin, obscurin surrounds myofibrils at the level of the M-bands and, to a lesser extent, the Z-disks. The multidomain protein contains 49 immunoglobulin domains and 2 fibronectin-III-like domains at the amino-terminus, followed by an IQ domain, more immunoglobulin domains as well as some nonmodular sequence, an SH3 domain, a guanine nucleotide exchange factor (RhoGEF) domain and tandem plextrin homology (PH) domain, and several consensus phosphorylation motifs for ERK kinases at the carboxy-terminus. As aberrant signal transduction has been linked to numerous pathophysiologies, including cardiac hypertrophy, we have begun to investigate the role of the RhoGEF domain of obscurin in muscle development and physiology. Through co-localization studies and co-immunoprecipitation of exogenously over-expressed proteins in COS-7 cells, as well as immunofluorescence of endogenous proteins in developing myotubes and adult rat striated muscle, we identified the small GTPase, RhoA, as a ligand of the RhoGEF domain. The RhoGEF domain does not interact with several other related Rho family members, including Cdc42. RhoA appears to organize with obscurin at the same time developmentally, when the two proteins both concentrate primarily at the M-band. Over-expression studies indicate that obscurin is critical for RhoA organization in developing myotubes. Both proteins also concentrate at M-bands in adult rat tibialis anterior muscle, but RhoA redistributes to other sites when muscle is injured in high strain lengthening contractions. Our results suggest that RhoA activity is regulated by its interaction with the RhoGEF domain of obscurin.

Supported by T32 HL 072751 (D.L.F.) and by grants to R.J.B. from the Muscular Dystrophy Association (#3932) and the NIH (RO1 HL64304).

3236-Pos Re-formulation Of Langevin Dynamics For Semi-flexible Rods And Application To Actin Filament Dynamics

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Board B539

We present a technique for the coarse-grained modeling of the fluctuations of semi-flexible filaments, based on the Langevin dynamics method but reformulated for short-filament subunits. Brownian force distribution on the filament sub-units is determined from experimental data on the diffusion of short filament segments, short enough to be considered rigid and analyzed by conventional diffusion dynamics. We show that for long rigid filaments modeled as a series of short-filament subunits, the translational and rotational diffusion coefficients can be accurately simulated. Also, with the distribution of Brownian forces on a semi-flexible filament now known, for given stiffness properties, the fluctuation dynamics of a filament can be simulated by simple balancing of forces. We show that fluctuation-dissipation theorem is obeyed in the filament response to external force. The strength of the method is in avoiding the conventional approximation of filaments to a series of infinitely-small spheres, which increases accuracy and decreases computational time. We use this technique to simulate the conformation space of a fluctuating actin filament and compare against that documented in literature.

3237-Pos Homologous Receptor Desensitization Is Required for Chemotactic Navigation in Competing Gradients

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Board B540

All known G-protein coupled chemoattractant receptors signal transiently upon ligand binding to effect cell orientation and motility, but then are removed from the signaling receptor pool in a ligand-dependent process termed homologous receptor desensitization. The physiologic importance of ligand-induced desensitization has been unclear, since molecularly modified nondesensitizable chemokine receptors mediate efficient cellular chemotaxis, comparable to that directed by wild type receptors. We hypothesized that homologous receptor desensitization might be required for cellular navigation in fields of competing attractants. Modeling of cells expressing wild type or mutant, nondesensitizable receptors predicts that receptor desensitization is required for integration of competing attractant signals: Cells expressing normal receptors orient preferentially to distant gradients and are predicted to seek an intermediate position between balanced agonist sources, and can be repositioned between chemoattractant-defined microenvironmental domains by modest changes in receptor number. In contrast, in the absence of desensitization, orientation is dominated by local agonist sources, precluding continued navigation. In experimental studies using human lymphocytes transfected with receptors for the chemokine IL-8, we find that chemotaxis to a second chemokine is unaffected by a competing IL-8 gradient when cells express wild type receptors, but is inhibited when cells express a nondesensitizable receptor. We conclude that homologous receptor desensitization is critical for integration of competing chemoattractant signals, and thus for multi-step cellular navigation in complex chemoattractant fields.

3238-Pos Ionic Strength Influence on Troponin Dissociation from Skeletal Myofibrils

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Board B541

Activation of striated muscle is a cooperative process initiated by Ca2+ binding to troponin (Tn). It is a solid-phase signal transduction process involving 3 states of the thin filament: a blocked (B) state preventing myosin interaction, a closed (C) state allowing weak myosin interaction and favored by Ca2+ binding to Tn, and an open or M state allowing strong cross-bridge formation and activation. Previous studies proposed that low ionic strength (IS) induces thin filament activation by promoting a transition from the B to C state without Ca2+ (Head et al., Eur. J. Biochem. 227:694). To further test this proposal, we investigated the influence of IS on Tn dissociation rate from myofibrils using fluorescently labeled Tn exchange. Fluorescence intensity ratio (non-overlap region/overlap region, NO/OL) was measured to determine the location and rate of Tn exchange. Globally, low IS increases Tn dissociation rate in both B and C state regions. At pCa 9.0, the NO/OL fluorescence intensity in standard buffer (IS = 200 mM) was only 0.4 at 16 min, consistent with our previous studies showing that most Tn in the NO region is in the B state (J. Mol. Biol. 361:420). However, the ratio increased to 0.9 when IS was decreased to 50 mM. At pCa 4.0, the NO/OL fluorescence intensity also increased from 1.4 to 2.3 when IS was decreased from 200 to 50 mM. These data suggest that lowering IS increases Tn dissociation rate and favors actin-tropomyosin (Tm) activation in the non-overlap region. Between 200 and 300 mM IS, the intensity ratio was mostly independent of IS at both pCa 9.0 and 4.0. Our study further confirms that low IS ($<$ 200 mM) favors thin filament activation, likely resulting from a transition of actin-Tm from B to C state.

3239-Pos Local Dynamics Of Chemotactic Receptor In D. discoideum

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Chemotaxis involves three complex and interrelated processes: directional sensing, cell polarization and motility. Directional sensing allows highly migrating eukaryotic cells to chemotax in extremely shallow (<2% across the cell body) gradients of the chemoattractant, cAMP in the case of *Dictyostelium discoideum*. Although directional sensing has been observed as spatially restricted responses along the plasmamembrane, our basic understanding of how cells process the gradient-controlled translocation of proteins during chemotactic movement is still largely lacking. Until now, the dynamics of the chemoattractant-receptor, cAR1, has been neglected in models describing directional sensing mechanisms. We studied in detail the dynamics of cAR1 and found that the receptors show localized differences in mobility across the cell body. In particular an agonist induced increase of mobile receptors was observed at the leading edge of cells performing chemotaxis. We showed this increase to be linked to the uncoupling of the Gα2 protein. Since this response is confined to the anterior of the cell we postulate that locally G-protein activation is enhanced. Enhancement is further facilitated by local clustering of receptors into plasmamembrane domains of ~230nm in size which in turn exhibit a diffusion constant of D=0.01μm^2/s.

Bacterial Sensing, Motors, & Motility

3240-Pos The Mechanochemistry of Mycoplasma Motility

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Board B543

Mycoplasma moves by gliding on solid substrates. Their locomotion has remained mysterious, for it involves no familiar motility related proteins. Located at the neck of the pear-shaped cell, the motility organelle consists of about 400 protein 'legs' spiking from a protein core structure. Each leg binds and unbinds from the substrate at its bulbous distal end. The remainder of the leg presumably acts as an asymmetric spring. As the legs 'row' to and fro, this asymmetry produces a net average driving force that propels the cell forwards.

In the model, the cell is connected to the motility organelle by a molecular motor that cycles through extension and contraction. The interactions between the substrate and the legs can be averaged out to be equivalent to an effective friction coefficient. Because of the asymmetric elasticity in the legs, the effective friction is rendered asymmetric in the direction of motion. The cell thus achieves a net motion through the differential displacements during the cycle of motor extension and contraction.

The model explains most of the experimental data. For example, the velocity of the cell increases nearly ten-fold over a ~30°C temperature range. The net velocity decreases linearly as the external load force increases, and the stall force is nearly independent of temperature. Finally, the result explains the cross-species comparison that longer legs correlate with larger velocity.

3241-Pos Modulation of the Rotational Direction and Speed of the Flagellar Motor by High-pressure

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Flagellated bacteria sense a variety of environmental factors and swim toward their favorable environment by rotating their flagellar filaments. The flagellar motor rotates exclusively in the counter-clockwise direction in the absence of switch-inducing protein CheY. Here, we show that high pressure also induces switching in the rotational direction of flagellar motors. *E. coli* cells that lack CheY proteins, were tethered by their flagellum to the observation window of high-pressure chamber. The rotational motion of the cell body was monitored by a high-pressure microscope, which could be available up to 2000 bar. At less than 800 bar, all cells rotated in the counter-clockwise direction and their speed were not affected. At more than 1000 bar, some cells started to rotate in the clockwise direction, and the rotational speed in both directions decreased steeply with pressure. After decompression, most of the cells recovered the normal activity in the rotational direction and speed. The high pressure is a direct and reversible stimulus for changing the motor function. The pressure-induced reversal in the rotational direction seems to modify the structure of the flagellar motor, as if the rotational direction of the flagellar motor is controlled by association with activated CheY molecules.

3242-Pos

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WITHDRAWN

3243-Pos Elasticity Of Cytoskeleton And Motion Of Magnetotactic Bacteria In AC Magnetic Field

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