demonstrated that this mechanosensory response is tunable by varying the lever arm length of myosin-II heavy chain, showing that myosin-II is an active force sensor in this mechanosensory system. We now focus on how mechanical inputs mediated through myosin-II lead to changes in biochemical signaling pathways, specifically the cortexillin-I regulatory and spindle signaling pathways. Rac1A (a small GTPase), IQGAP1, and IQGAPA (GTPase effectors) can form complexes with cortexillin-I. In the absence of both IQGAP1 and IQGAPA, cortexillin-I does not localize normally to the cleavage furrow during cell division. However, IQGAPA, but not IQGAP1, is required for myosin-II mechanosensing. Kif12, a mitotic-kinesin-like protein in Dictyostelium cells, is part of the chromosomal passenger complex, including INCENP and Aurora kinase. Kif12 is also recruited to the cell cortex inside the micropipette in a myosin-II-dependent and/or IQGAPA-dependent manner during cell division. Thus, mitotic spindle signaling proteins are responsive to mechanical stress sensed by myosin-II. Overall, myosin-II is a key mechanical stress sensor and these mechanical inputs are fed back to the mitotic spindle signaling system.

836-Pos

Mechano-Chemical Feedbacks Play a Major Role in Regulating Actin Mesh Growth in Lamellipodial Protrusions

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During cell motion on a substratum, eukaryotic cells project sheet-like lamellipodia which contain a dynamically remodeling three-dimensional actin mesh. A number of regulatory proteins and subtle mechano-chemical couplings determine the lamellipodial protrusion dynamics. To study these processes, we constructed a microscopic physico-chemical computational model, which incorporates a number of fundamental reaction and diffusion processes, treated in a fully stochastic manner. Our work sheds light on the way lamellipodial protrusion dynamics is affected by the concentrations of actin and actin-binding proteins. In particular, we found that protrusion speed saturates at very high actin concentrations, where filament nucleation does not keep up with protrusion, resulting in sparse filamentous networks, and, consequently, high resistance forces on individual filaments. We also observed maxima in lamellipodial growth rates as a function of Arp2/3, a nucleating protein, and capping proteins. We provide detailed physical explanations behind these effects. In particular, our work supports the actin funneling hypothesis explanation of protrusion speed enhancement at low capping protein concentrations. Our computational results are in agreement with a number of related experiments. Overall, our work emphasizes that elongation and nucleation processes work highly cooperatively in determining the optimal protrusion speed for the actin mesh in lamellipodia.

837-Pos

In Silico Study of Formation and Collapse of T-Killer Cell Synapse Mediated by Receptor Recycling and Actin Network

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T-killer cells of the immune system eliminate virus-infected and tumorous cells through direct cell to cell interactions. Reorientation of the killing apparatus inside the T-killer cell to the interface with the target cell ensures specificity of the immune response. Several research works were reported to explain the mechanism of reorientation but the most adversary situation, when the cell's initial orientation is complete opposite to the desirable direction, always left skepticism toward the suggested mechanism. We have constructed a computational model that incorporate T-killer cell receptor dynamics and all the possible mechanical properties which involve not only intrinsic physiology of T-killer cell but also the synapse formation with target cell. The model studies show that the actin network nucleation and degradation is a crucial part in the T-killer cell synapse formation. Furthermore, the role of actin network provides a safety feature for the T-cell reorientation mechanisms by allowing T-cells to detach from the target cell when they are stranded in situations in which reorientation is not available. Our computational model also provides insights into the actin network near the T-cell synapse including retrograde flow development.

838-Pos

Intimacy Between Actin Network Flow and Turnover in the Lamella of Crawling Fragments

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Elucidating the dynamics of the actin cytoskeleton that generates the driving force for cell migration is fundamental to understanding the mechanisms of actin-based cell migration, which is important to various physiologically relevant processes including metastasis and angiogenesis.
In this study, based on the hypothesis that actin cytoskeleton in migrating cells is a spatiotemporally self-regulating structure, we aimed at elucidating the dynamic coupling between actin network flow and turnover by focusing on flow dynamics in the lamella of one of the simplest but elegant motility systems; crawling fragments derived from fish keratocytes. Using a combination of fluorescent speckle microscopy and particle imaging velocimetry, we have succeeded in quantitatively mapping the flow in the lamella of these simple motility systems where it was previously reported to be stationary. Moreover, by correlating network flow with turnover, we have demonstrated that whereas polymerization mediates network assembly at the front, surprisingly, network flow convergence modulates network disassembly toward the rear of the lamella, suggesting that flow and turnover are coupled during migration. Furthermore, we found that polymerization is not just limited to the usually reported narrow rim along the leading edge, but occurs over an extended ~8 ∈ 1/4m wide region at the posterior of the lamella. We suggest that this is necessary to maintain the structural integrity of the lamella for rapid cell migration, as in fish keratocytes. These results obtained using simple but remarkable motility systems present new interesting concepts about actin network dynamics during cell migration that will definitely have profound impact on cell migration research. We believe that this study will make a major contribution toward biophysical understanding of cell migration, and aid in the development of quantitative models for exploring the yet unknown mechanisms of the process.

839-Pos

Quantitative Analysis of Cell Edge Dynamics and Cell Shape in Non-Polarized Fish Epidermal Keratocytes

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Cell migration requires the coordination of several processes such as leading edge protrusion, adhesion formation and disassembly, and trailing edge retraction. The quantitative analysis concerning the correlation between these processes will be useful to understand the coordination mechanism. A major difficulty in the quantitative analysis stems from the complex morphology of migrating cells. To cope with this difficulty, we selected the non-polarized fish epidermal keratocytes as a simplified experimental system that includes basal migratory mechanisms. We acquired a mixture of non-polarized stationary keratocytes and polarized highly motile keratocytes by disaggregating the large epidermal sheets. The time-lapse micrographs of non-polarized keratocytes were used to analyze cell edge dynamics and cell peripheral shape. We adopted the protrusion and retraction rate and cell peripheral curvature as quantitative parameters. Non-polarized keratocytes did not exhibit net translocation, however, active protrusion and retraction were observed around the cell periphery. Protrusion rate was negatively correlated with the cell peripheral curvature. In contrast, retraction rate was positively correlated with the cell peripheral curvature. The plot of protrusion and retraction rates over the entire cell periphery showed that protrusion and retraction waves were traveling laterally in both directions along the cell periphery. The lateral traveling velocity of each wave was constant. The wave persistence varied from 10 s to 100 s. These results indicate that the cell has the positive feedback mechanism maintaining stable protrusion and retraction and that the rate of protrusion and retraction is related to the cell peripheral shape. Quantitative analysis together with theoretical and molecular biological studies will shed light on the mechanism of cell migration.

840-Pos

Mechanisms Underlying Protrusion-Retraction Waves at the Leading Edge of Migrating and Spreading Cells

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Motility is fundamental to many cell types and plays key roles in immune response, tissue development, and cancer metastasis. Recent studies of migrating mouse embryonic fibroblast cells revealed protrusion-retraction cycles and lateral waves at the leading edge [Giannone G, et al, Cell, (2004); Giannone G, et al, Cell (2007)]. Each cycle entails membrane protrusion powered by actin polymerization, interrupted every ~24 s by ~5 s partial retraction episodes attributed to myosin II proteins which pull back the growing lamellipodial actin network until the latter separates from the leading edge membrane. We developed a mathematical model and extended our experimental observations in order to quantitatively describe the mechanisms underlying this motility behavior. We find the retraction waves are caused by propagation of myosin powered tears between the lamellipodium and cell membrane. Once a tear is nucleated its

propagation initiates self-sustaining periodic behavior at the leading edge. The model and experiment are in quantitative agreement.

841-Pos

Force Transmission in Migrating Cells: Gripping at the Front, Slipping at the Back

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During cell migration, forces generated by actin cytoskeleton are transmitted through adhesion complexes to the substrate. To investigate the mechanism of force generation and transmission, we analyzed the relationship between the velocity of actin network movement and the stress applied to the substrate over the entire cell using a simple model of persistently migrating fish epidermal keratocytes. Lateral stresses at the cell sides and forward stress at the back of the cell were largely proportional to actin velocity, with higher coefficient of proportionality for lateral stresses than for the forward stress. In contrast, backward propulsive stress at the cell front exhibited significant velocity independent component. These results suggested that the mechanism of conversion of actin dynamics into the substrate stress depended on the region of the cell and on the direction of the stress: frictional slippage was characteristic of the back and sides of the cell, and elastic gripping, of the front. Analysis of substrate stress and cell motion in the presence of inhibitors of actin/myosin system cytochalasin D and blebbistatin indicated that cell translocation could be driven by two different processes: actomyosin contraction, and actin assembly, the former associated with significantly larger substrate forces than the latter.

842-Pos

WITHDRAWN

843-Pos

Continuum Elastic Model of Epithelial Sheet Migration

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A key component of a wound healing process is rapid migration of epithelial cells that covers the wound area and helps to protect the underlying tissue. In our recent paper* we have developed a one-dimensional continuum mechanical model of intestinal epithelial cell layer, that incorporates lamellipod forces at both the wound edge and in the interior of the layer, adhesion forces between the cell layer and the substrate, and elastic stress within the cell layer. Here, the model is extended to two-dimensions and solved numerically using a level set method, which tracks the moving wound edge on a fixed grid. The model is calibrated by comparing the position of wound edge with experimentally observed positions in scratch-wound assay experiments. These comparisons show good qualitative agreement between model results and experimental observations. The models supports experimental observations that the time to wound closure varies with initial wound shape and area, and that the closure is possible, albeit slower, if boundary lamellipod formation is inhibited.

* Qi. et al., One-dimensional elastic continuum model of enterocyte layer migration, *Biohys. J.*, **93**, 3745-3752 (2007).

844-Pos

Biophysical Regulation of Astrocytoma Cell Physiology in 2D and 3D Culture

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The rapid progression of high-grade brain tumors is related to diffuse infiltration of single tumor cells into the surrounding brain parenchyma, a process that involves aberrant interactions between tumor cells and the extracellular matrix (ECM). Here, we show that biophysical cues from the ECM regulate key tumor cell properties relevant to invasion in both two-dimensional (2D) and three-dimensional (3D) culture models. We first investigated the role of ECM rigidity in regulating the structure, migration, and proliferation of a panel of astrocytoma cell lines on 2D fibronectin-coated polymeric ECM substrates of defined mechanical rigidity. On highly compliant ECMs, tumor cells appear rounded and fail to productively migrate. As ECM rigidity is increased, tumor cells spread extensively, form prominent stress fibers and mature focal adhesions, and migrate rapidly. Remarkably, cell proliferation is greatly enhanced on rigid versus compliant ECMs. Pharmacological inhibition of nonmuscle myosin II-based contractility blunts this rigidity-sensitivity and rescues motility on highly compliant substrates. We next explore astrocytoma mechanosensitivity in 3D by introducing a novel biomaterial platform in which we progressively modulate the biophysical properties of collagen I matrices by adding agarose. We find that agarose increases the bulk elasticity of 3D collagen ECMs over two orders of magnitude by forming a dense meshwork that intercalates between the entangled collagen fibers. Embedded glioma cells exhibit a pronounced transition to amoeboid motility accompanied by severe limitation of cellular invasion from multicellular spheroids as the agarose content of the hydrogels increases from 0-1% w/v. Our results are consistent with a model in which agarose structurally couples and reinforces individual collagen fibers, simultaneously introducing steric barriers to cell motility while shifting ECM dissipation of cell-induced stresses from the non-affine deformation of individual collagen fibers to the bulk-affine deformation of a continuum network.

845-Pos

Numerical Simulation of Myosin-Triggered Switch in Motile Cells

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Force generation and movement in motile cells are a result of coordinated spatial-temporal organization and segregation of some essential proteins including myosins and integrins. Biochemical signaling coupled with transport underlines the self-organizations of such proteins. It is also expected that the mechanical deformation and restructuring of cell cytoskeleton plays an important role. This is particularly true with proteins that bind and interact directly with cytoskeleton such as myosins and integrins. To see how myosin and adhesion organization is influenced by cytoskeletal dynamics, we use computational modeling to examine simple continuum models of f-actin network mechanics interacting with myosin. We restrict our study to fish keratocyte since it has a highly symmetric and stable shape as it moves. We find that (i) the distribution of myosin is characterized by the left-right symmetry and is biased to the rear; (ii) the f-actin flow induced by myosin contraction is graded (highest at the rear and minimal at the front), (iii) adhesion density is biased to the cell rear and is in antagonistic relation with f-actin. Moreover, there is an effective dynamic switch in cell motile behavior triggered by the overall adhesion/myosin strength. The modeling results are consistent with experimental findings (data courtesy of Julie Theriot group).

Microtubule Motors-Kinesin-related Proteins

846-Pos

Spindle and Pole Mechanisms in Bipolarity and Prophase Control of Spindle Elongation

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Genomic stability through cell division in eukaryotes is enhanced by formation of a conserved mitotic spindle apparatus regulated by multiple Kinesin-like motor protein (Klp) families. While Kinesin-5 and Kinesin-14 opposing forces provide a ubiquitous balance to spindle assembly and stability, Kinesin-6 Klps also contribute. When domain specialization in Kinesin-14 members favors pole localization and mechanisms, Kinesin-6 Klps can provide critical regulation of prophase microtubule cross-linking and spindle elongation. By genetic, cell biological and biochemical approaches including cross-species analysis with human HSET, *Drosophila* Ncd and *Schizosaccharomyces pombe* Pkl1 we are characterizing Klp structure and function in balancing bipolar spindle assembly with regulation of spindle elongation in prophase. HsHSET functionally replaces SpPkl1 in fission yeast, displays similar localization to poles in prophase, and is unable to oppose bipolarity in the presence of a γ-tubulin mutation in a defined Kinesin-14 binding site. DmNcd does not replace SpPkl1 and localizes preferentially to bundled interpolar spindle microtubules, unlike the more uniform spindle and prominent pole localization of HsHSET and SpPkl1. By in vivo analysis of thirty Kinesin-14 derivatives, including Tail, Stalk or Neck-Motor chimeras, for spindle assembly, spindle localization and mitotic progression we defined critical Tail domain regions in SpPkl1 for establishing bipolarity. In fission yeast, Kinesin-6 and Kinesin-14 Pkl1 oppose each other both in regulating bipolarity and in control of prophase spindle length. Flexibility in the design plan of Kinesin-14s, in part through varying Tail elements, broadens their mechanistic possibilities in eukaryotes that include distinct roles in spindle assembly and maintenance. Additional mitotic Klp families, like Kinesin-6, can contribute to critical mechanisms in prophase for spindle assembly and regulation of spindle length to provide the appropriate temporal balance of forces.