and RhoA/PKC-mediated adducin phosphorylation at Ser726 (Zhang et al, *J Biol Chem* 278:35644-50, 2003). Adducin, together with actin and spectrin, is a major component of the OHC's cortical cytoskeleton. Thus, activation of RhoA/PKC pathway could induce changes in OHC length and motility associated with structural modifications in this cytoskeletal structure. In this study, we aimed at determining if PKC inhibitors were able to regulate RhoA/PKC-adducin mediated effects on OHC motility. Actin-dependent changes in cell length (slow motility) and the amplitude of electrically induced changes in cell length (fast motility) were measured in isolated guinea pig OHC, patch clamped in whole-cell mode and internally perfused through the patch pipette with inhibitor of PKC while being externally stimulated with LPA. OHC motility was monitored by high-speed video microscopy, whereas confocal microscopy was used to investigate effects of PKC inhibitor on RhoA/PKC-Adducin signaling pathway. We found that LPA induced expression of cPKCα and nPKCζ, with cPKCα but not nPKCζ phosphorylating adducin both of Ser725 and Thr445. However, treatment with specific pharmacological inhibitors of PKCα reduced adducin phosphorylation only at Ser726. Also, we determined that activation of the RhoA/PKCα-mediated signaling pathway by LPA induced OHC shortening and a simultaneous increase in the amplitude of fast motility. Our results support a positive role for cPKCα in OHC motility and strongly suggest a link between cPKCα, adducin phosphorylation and the regulation of OHC motility.

This work was supported by NIDCD/NIH grant R01 DC010146. Its content is solely the responsibility of the authors and does not necessarily represent the official view of the National Institutes of Health.

1811-Pos Board B541

FMG1-B as a Eukaryotic S-Layer

Puey Ounjai[1,2], Kenneth H. Downing[1].
[1]Life Sciences Division, Lawrence Berkeley National Lab, Berkeley, CA, USA, [2]Faculty of Science, Mahidol University, Bangkok, Thailand.

Interactions of planktonic cells with solid surfaces govern various microbiological phenomena, for example in the early stages of biofilm formation. However, the mechanism governing the interaction of motile eukaryotic cells with surfaces remains poorly understood. In unicellular algae, the flagellar surface plays an important role in cell-surface adhesion and gliding through several flagellar membrane proteins. Using electron microscopic imaging, we have obtained direct evidence that the glycocalyx layer of Chlamydomonas reinhardtii flagella is a eukaryotic version of a bacterial S-layer. This component of the flagellum, previously characterized by conventional electron microscopy as a ''fuzzy layer'', can be seen by cryo-electron microscopy and tomography as consisting of associated proteins that sometimes appear in a regular hexagonal array. Immunolabelling indicates that the layer is formed by the flagellar membrane glycoprotein FMG1-B, which has been implicated in surface contact and gliding. There are clear differences between bacterial S-layers and the Chlamydomonas flagellar layer. For example, the latter appears to be quite fluid, while bacterial S-layers have been visualized as regular arrays of rigidly interlocked proteins. However, the questions of how these proteins adapt to a dynamic and irregularly shaped surface suggest the notion of convergent evolution, and it will be interesting to develop an understanding of the functional relation between these surface layers. These observations also provide insights on eukaryotic cell-surface adhesion that may lead to a better understanding of biofilm formation by algae and also offer an alternative platform for nanotechnology applications of S-layers.

1812-Pos Board B542

Regulation of Actin Filament Turnover in Brain Tumor Cell Motility

Brannon R. McCullough, David J. Odde.
Biomedical Engineering, University of Minnesota, Minneapolis, MN, USA.

Cell migration and invasion is driven by turnover of the actin cytoskeleton, a process initiated by cofilin activation. Active cofilin concentrations correlate with the grade of primary brain tumors, tumor cell migration and brain invasion (Nagai S, Moreno O, Smith CA, Ivanchuk S, Romagnuolo R, Golbourn B, Weeks A, Seol HJ, and Rutka JT. 2011, Genes Cancer. Sep;2(9):859-69). To investigate how cell migration and invasion is enhanced in high grade brain tumors by cofilin we have developed a physical model based on the molecular mechanisms for actin turnover already known to be enhanced by cofilin. The model is then used to fit actin distributions in eGFP-actin transfected U251 brain tumor cells using model convolution microscopy. The best fits of the model yield estimates for the kinetic parameters for actin turnover in high grade brain tumor cells. These parameters are similar to isolated in vitro measurements made in the presence of cofilin and suggest that actin turnover is stimulated throughout the cell by cofilin. Further analysis of the model is used to elucidate potential mechanisms to regulate brain tumor cell motility.

1813-Pos Board B543

Quantitative Analysis of Cellular Traction Generation and Actomyosin Dynamics in a 3D Fibrin Matrix

Leanna M. Owen, Arjun S. Adhikari, Luv Gupta, Natascha Leijnse, Alexander R. Dunn.
Stanford University, Stanford, CA, USA.

Substantial progress has been made in characterizing the mechanisms of tension sensing, adhesion, and traction in cells migrating on two-dimensional (2D), rigid surfaces. However, there are inherent differences in cellular architecture, signaling, and behavior when cells are embedded in the soft and confined environment of the extracellular matrix. Here we present a quantitative analysis of the dynamics of canonical actomyosin cytoskeletal proteins in primary dermal fibroblasts (HFFs) grown in a three-dimensional (3D) fibrin matrix, and correlate these dynamics to protrusion formation and traction generation during migration through the fibrin network. We observe that fibroblasts adopt an elongated cell morphology with adhesive protrusions that cyclically strain the surrounding fibrin matrix. We use GFP-tagged myosin regulatory light chain (MRLC1) and time-lapse confocal imaging to track myosin dynamics as cells explore the fibrin matrix. We observe highly dynamic alterations in MRLC1 localization coupled to both the extension and retraction of cellular protrusions. During protrusion extension, fibrin strain is often oriented bidirectionally several microns behind the protrusion tip and is transmitted via paxillin-dependent adhesions. ROCK inhibition by Y-27632 causes fibroblasts to develop extended protrusions despite a pronounced reduction in matrix deformation and adhesion maturation as judged by a dramatic reduction in matrix-associated paxillin plaques. Ongoing work characterizes the contribution of cytoskeletal contractility in regulating protrusion and adhesion dynamics.

1814-Pos Board B544

Actomyosin Generated Tension Coordinates Cell Movements during Early Zebrafish Development

Jack Chai, Andrea Hamilton, Michael Krieg, Craig Buckley, Ingmar Riedel-Kruse, Alexander Dunn.
Chemical Engineering, Stanford University, Stanford, CA, USA.

Coordinated cell migration is a fundamental aspect of biological processes ranging from wound healing to embryonic development. How this coordination occurs over length scales much longer than individual cells is poorly understood. We use zebrafish epiboly as a model system to investigate the biophysical underpinnings of coordinated cell migration. During epiboly the blastoderm migrates and spreads from the animal pole of the yolk to converge at the opposite, vegetal pole. At present, very little is known about how cell migration is coordinated along the length of the blastoderm margin (BM), which is ~3 mm at its maximal circumference. We use gentle mechanical deformation to distort the normally symmetric embryo to probe the mechanisms that generate long-range intercellular coordination during epiboly. Geometric distortion causes shape-dependent alterations in the rate of BM migration and realignment of the BM and eventual anterior-posterior (AP) axis away from the initial animal-vegetal axis and toward the new long axis of the embryo. Chemical disruption of the actin band, a contractile ring of actin and myosin immediately vegetal to the BM, restores uniform migration along its length and eliminates AP axis reorientation. A quantitative model that incorporates tension generated by the actin band accounts for these observations, consistent with the idea that physical forces can generate long-range, coordinated cell movements that would be difficult to achieve by biochemical signaling alone. We hypothesize that this mechanism may provide a general means of coordinating long-range cell migration, for example during wound healing and Drosophila dorsal closure. Our data additionally suggest that AP axis specification, a fundamental step in the development of all embryos, is sensitive to mechanical cues. Taken together, these observations support the likelihood that mechanical forces may shape morphogenesis throughout development.

1815-Pos Board B545

Force-Dependent Interactions between the E-Cadherin-Catenin Complex and Actin Filaments

Craig D. Buckley[1], Jiongyi Tan[2], Beth L. Pruitt[3], William I. Weis[4], W. James Nelson[5], Alexander R. Dunn[1].
[1]Chemical Engineering, Stanford University, Stanford, CA, USA, [2]Biophysics Program, Stanford University, Stanford, CA, USA, [3]Mechanical Engineering, Stanford University, Stanford, CA, USA, [4]Structural Biology & Molecular and Cellular Physiology, Stanford University, Stanford, CA, USA, [5]Biology & Molecular and Cellular Physiology, Stanford University, Stanford, CA, USA.

Adherens junctions (AJ) are mechano-sensitive protein complexes essential for many multicellular processes such as tissue morphogenesis and homeostasis.