compartments and participate in a diversity of cellular roles. Natural cellular machines provide tools and inspiration for biomimicry and for resolution of societal grand challenges through redirected applications. Using tools available through the semiconductor industry and materials science we are generating artificial centrosomes (ACENs) from nanoparticles to act as simplified nucleation centers for use in synthetic self-assembly paradigms.

730-Pos Board B499
Mesoscale Coordinated Dynamics of Cytoskeletal Components at Mechanosensory Podosomes Shown by Time Resolved STICS
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Extensive literature is available on how focal adhesions sense and respond to environmental cues during fast migration in polarized cells. In contrast, little is known about how cells feel and translate environmental cues during slow mesenchymal migration through tissues (e.g. dendritic cells, macrophages). These cells form many more podosomes than focal adhesions and use them as mechanosensors to probe and remodel the extracellular matrix. Each podosome consists of a dense, protrusive actin core and an adhesive ring of integrins and cytoskeletal adaptor proteins such as vinculin and talin. Podosomes are highly dynamic, and their actin content continuously fluctuates mediating their protruding activity. Podosomes are organized in large clusters. Electron microscopy showed that podosomes are interconnected by cytoskeletal fibers, suggesting the existence of a mesoscale organization. By combining image correlation spectroscopy (ICS) techniques such as raster ICS (RICS) and time-resolved spatiotemporal ICS (trSTICS), we investigated the collective dynamic behavior of podosome clusters. We compared the dynamic behavior of the mechanosensitive protein vinculin and the mechano-insensitive protein talin. RICS showed similar diffusion (D=2.4 and 2.0μm^2/s) and binding (t=0.024 and 0.026s) properties for vinculin and talin. In contrast trSTICS revealed significant differences in their flow patterns. While waves of correlated flow, with speeds ranging from 0.01–0.16 μm/min, are visible throughout the podosome cluster for vinculin, talin dynamics show no clear directionality. Moreover, podosome formation and dissolution are accompanied by characteristic flow patterns of vinculin, suggesting that localized recruitment of mechanosensitive proteins could coordinate podosome protrusive forces. Finally, using cross correlation STICS we show that vinculin, but not talin, dynamics correlate to flow patterns in the actin network. Taken together, our data demonstrate coordinated behavior of mechanosensitive adhesion proteins in podosome clusters providing evidence for mesoscale coordinated protrusive dynamics.

731-Pos Board B500
Inhibition of Contractility and RhoA Deactivation Trigger Podosome Formation
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Development of integrin-mediated cell-matrix adhesions is important in the regulation of cell growth and differentiation. Activated integrin clusters are sites of actin polymerization, and the physical properties of extracellular matrix ligands play critical roles in the development of adhesion structures. Previously, we have utilized RGD-membranes, continuous supported bilayers with freely diffusing lipid-anchored RGD ligands, to activate integrins and investigate force dependence of initial integrin clustering during early adhesion formation (Yu et al., PNAS 2011). At later times (>45 minutes) on RGD-membranes, we found that fibroblasts natively form podosomes as adhesion structures when cell-matrix traction force cannot be stabilized. Podosome core assembly was initiated by Arp2/3-mediated actin polymerization locally within integrin clustering sites that were characterized by integrin-associated proteins, such as vinculin, paxillin, and talin. In addition, myosin-1E/1F and CARMIL1 were found as new markers at podosome cores. We utilized a FRET-based RhoA biosensor to measured differential RhoA activities in cells forming podosomes on RGD-membranes versus focal adhesions on RGD-glass. RhoA-GTP levels were indeed low in cells forming podosomes on RGD-membranes, whereas RhoA-GTP levels were high in the cells on RGD-glass. Constitutively active RhoA-Q63L mutant blocked podosome formation on RGD-membranes. Furthermore, fibroblasts on RGD-membranes with dense nano-partitions (1μm-pitch line partitions) in RGD-membranes provided mechanical barriers that enabled adhesion maturation that was not possible with wider line-partitions (4μm) in RGD-membrane. When a single cell adhered over both regions of high and low density, podosomes formed over low-density regions only. From our data, we suggest that development of podosomes as adhesion structures requires inhibition of traction forces between integrin receptors and matrix ligands. Activation of myosin contractility at integrin clusters activates focal adhesion formation and inhibits podosome formation at integrin clusters, giving rise to a mutually exclusive behavior of focal adhesions and podosomes.

732-Pos Board B501
The Molecular Basis for Cellular Contractility Driven Mechanosensing
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Internal and external mechanical forces govern many cellular processes in normal and diseased states such as muscle contraction, tissue invasion and metastasis of tumor cells. Thus a cell's ability to sense and respond to mechanical stimuli is critical for cell behavior and survival. In Dictyostelium, the mechanoenzyme myosin II and the actin crosslinker cortexillin I work cooperatively to control cellular contractility in response to deformation. The scaffolding proteins IQGAP1 and IQGAP2, which can bind to cortexillin I, further regulate this mechanosensory system through feedback loops. The IQGAPs are not required for mechanosensing, but affect the stress-dependent myosin accumulation in the cortex. IQGAP1 inhibits mechanosensing, while IQGAP2 is required for suppressing this inhibition. A molecular understanding of how this mechanosensory system works to modulate contractility remains elusive. By measuring protein dynamics using fluorescence recovery after photobleaching (FRAP), we observed stress-dependent reduction in the mobility of many of the proteins in the mechanosensory system. Further, these dynamics are dependent on the integrity of the mechanosensory system, as mutant cells with disrupted mechanosensing typically show slower protein dynamics and increased cortical association of the proteins than in wild type cells. In addition, we are also characterizing the biochemical interactions important for mechanosensory response. These experiments will allow us to develop a molecular model to explain how mechanical forces are transmitted within the cell. The knowledge of the molecular underpinnings of this myosin II-based feedback control system will be applicable to other contractile systems such as those involved in development and morphogenesis of multicellular organisms.

Microtubules & Microtubule-associated Proteins

733-Pos Board B502
Efficient Small Scale Preparation of Fluorescently Labeled Microtubules
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Large-scale functional tubulin purification methods have been developed for use in the study of microtubule dynamics, mitosis, meiosis, and intracellular transport. These methods often start with large quantities (>400 g) of bovine or porcine nervous tissue and generally require more than twelve hours to complete. Here we show a simplified approach to purifying tubulin from small amounts of tissue (<300 mg), which is easily obtained from the central nervous tissues of small rodents. Furthermore, we have cut the preparation time in half while still maintaining an abundant protein yield. In order to visualize the microtubules by epifluorescence, TRITC-labeled tubulin is incorporated in ratios as low as 1:1000 labeled to unlabeled tubulin dimers. We confirmed that TRITC-tubulin was incorporated stoichiometrically and randomly into native microtubules by in-gel fluorescence after electrophoresis, as well as the variance in longitudinal fluorescence observed via video microscopy. Isolated microtubules are stabilized using paclitaxel, which enables multiple-day use of the purified protein. Microtubule-associated proteins are retained during the preparation, but can be removed using a phosphocellulose spin-column. The intrinsic value of this protocol is that it permits the use of single transgenic animal models, as well as biopsy samples, as a source of ''native'' microtubules for in vitro gliding or biochemical assays.

734-Pos Board B503
+Tip-Kinesin Complexes Steer Microtubule Growth In Vitro
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