BP 13: Cell Migration

Time: Wednesday 10:45–13:15 Location: ZEU 260

BP 13.1 Wed 10:45 ZEU 260

Quantitative studies of Dictyostelium discoideum chemotaxis — •Matthias Theves[1], Carsten Beta[1,2], and Eberhard Bodenschatz[1] — [1]Max-Planck Institut für Dynamik und Selbstorganisation, Göttingen — [2]Universität Potsdam

We use microfluidic tools to expose Dictyostelium discoideum amebae, a model organism for eukaryotic chemotaxis, to directional stimuli of cyclic adenosine 3',5' monophosphate (cAMP). We classify the migrational patterns of single cells in stationary linear gradients and quantify the accuracy of directional migration as a function of gradient steepness and varying midpoint concentrations. The results on wild-type chemotaxis serve as a reference to study the altered motility of various cytoskeletal mutants. In particular, we focus on constructs lacking regulators of the Arp2/3 complex, a key player in the formation of a dense cortical actin network at the leading edge of motile cells.

BP 13.2 Wed 11:00 ZEU 260

Force generation in moving fish keratocytes — •Claudia Brunner, Michael Gögler, Daniel Koch, Thomas Fuhs, Allen Ehrlicher, and Josef Käs — Universität Leipzig

A fundamental step in cell migration is the advancement of the cell's leading edge which is hypothesized to be mediated by actin polymerization against the plasma membrane. Our newly established SFM-technique revealed that the force generating mechanism driving this process is indeed actin polymerization. Cells treated with the actin polymerization inhibitor cytochalasin D generated significantly lower forces. Additionally, we directly measured a force associated with the retrograde flow within the lamellipodium, which demonstrates that the protrusion forces are decoupled from the cell body and are generated exclusively at the leading edge. We show that actomyosin interaction is primarily responsible for cell body and traction force generation while myosin II contraction cannot be the dominant force generating mechanism driving retrograde flow in the central lamellipodium.

BP 13.3 Wed 11:15 ZEU 260

Mimicking Cellular Environments: Cells on elastic nanopatterned substrates — •Ilia Louban[1,2], Roberto Fiammengo[1,2], and Joachim Spatz[1,2] — [1]MPI for Metals Research, Dept. of New Materials & Biosystems; Heisenbergstr. 3, D-70569 Stuttgart — [2]Univ. of Heidelberg, Dept. of Biophys. Chemistry; INF 253, D-69120 Heidelberg

The last years, hydrogels based on poly(ethylene glycol) diacrylate (PEG-DA) have been developed to serve as synthetic extracellular matrix analogues with adjustable mechanical and biochemical properties. Their Young's moduli (E) span more than four orders of magnitude (0,6kPa<E<6MPa). Since PEG-DA features protein and consequentially cell repellent properties, the hydrogel surface has to be modified to provide bioactivity. Extended gold nanoparticle arrays, manufactured by block copolymer micellar nanolithography, could be transferred to the hydrogel surface providing single anchor points for biofunctionalization. The interparticle distance (30nm<ΔL<200nm) on the substrate can be varied independently from its rigidity. To promote integrin mediated cell adhesion of rat embryonic fibroblasts, gold nanoparticles were functionalized with a cRGDfK peptide specific for $\alpha V\beta 3$ integrin. The effect of variation of substrate compliance and interparticle distance, tuned at the same time, was investigated. Our experiments reveals a significant decrease in cell spreading area on soft substrates (E<10kPa) and substrate with high interparticle distance (ΔL>70nm) after 6, 12 and 24 hours of adhesion respectively. Additionally we performed atomic force spectroscopy to quantify cellular adhesion to these surfaces.

BP 13.4 Wed 11:30 ZEU 260

Three-dimensional micro scaffolds for single cell experiments — •Joachim Fischer[1], Franziska Klein[2], Clemens Franz[2], Georg von Freymann[1], Martin Bastmeyer[2], and Martin Wegener[1] — [1]Institut für Angewandte Physik, Universität Karlsruhe (TH), 76128 Karlsruhe, Germany — [2]Zoologie I, Universität Karlsruhe (TH), 76128 Karlsruhe, Germany

The extracellular matrix with its specific mechanical and chemical properties has an important influence on eucaryotic cell adhesion, migration and differentiation behaviour. Investigating these cues *in vitro* requires a suitable method to fabricate three-dimensional micro environments with desired geometry, elasticity and functionality. Direct Laser Writing (DLW) allows the highly-reproducible fabrication of nearly arbitrary, three-dimensional polymer-microstructures with feature sizes between 100 nm and several microns. The structures' mechanical responses to cellular contraction forces can be controlled by using different photoresists or by varying the thickness of individual elements. As a proof-of-principle, we demonstrate that chick cardiomyocytes cultured in these structures can rhythmically deform our elastic 3D-templates. Furthermore, we show a method to characterize the mechanical properties of these structures with an atomic force microscope. In the future, shaping the laser focus via phase- and/or amplitude modulation might allow for bigger and even smaller features and hence make DLW even more versatile for micro scaffold fabrication.

15 min. break

BP 13.5 Wed 12:00 ZEU 260

Single cell motility in tunable environments — •Sravanti Uppaluri[1], Jan Nagler[1], Markus Engstler[2], and Thomas Pfohl[1] — [1]Max Planck Institute for Dynamics and Self Organization — [2]Darmstadt University of Technology

African trypanosomes are parasites that infect a variety of hosts and cause fatal diseases including sleeping sickness in humans. Recent work has shown that trypanosome motility is essential in their evasion of the host immune response [Engstler M et al., Cell 2007]. We investigate the motility of trypanosomes in tunable environments in which we control viscosity (similar to that of blood), physical barriers (ECM-like collagen networks), and nutrient concentration. Despite comparable traveling velocities in all environments, the spread of the parasite, measured by its radius of gyration, is remarkably different among the various environments. In culture medium the trypanosomes move by one of three distinct motility classes: diffusion, directional persistence, and an intermediate class in which they exhibit a combination of both. The distribution of trypanosomes within these classes depends on environmental conditions. We show that the parasites are predominantly directionally persistent in higher viscosities. Analysis of scaling behaviour, corresponding to different motility classes will be presented.

BP 13.6 Wed 12:15 ZEU 260

Stochastic Lamellipodium Dynamics — •Melanie Knorr[1], Daniel Koch[3], Thomas Fuhs[1], Timo Betz[2], Ulrich Behn[1], and Josef Käs[1] — [1]University of Leipzig, Germany — [2]Institute Curie, Paris, France — [3]Georgetown University, Washington

Many processes in the body, such as immune response, wound healing, embryogenesis, and neuronal development rely on both the directed growth and movement of cells. The dynamic behavior of the lamellipodium, a thin veil-like structure at the cell's leading edge, is mainly based on the cytoskeletal processes of actin polymerization and molecular motor-driven retrograde flow. Experimental investigations reveal, that actin polymerization at the leading edge is the driving process of lamellipodial edge fluctuations. Statistical analysis shows that polymerization stochastically switches between "On" and "Off" states, and that both the lifetime of these states and the actin polymerization velocity at the edge determine cell movement. Studying the edge fluctuations of different cell types leads to a classification of cells on the basis of certain parameters that determine the stochastic lamellipodium dynamics. Based on these results we developed a stochastic model that consistently describes the experimentally derived data, including all underlying processes like actin polymerization and retrograde flow.

BP 13.7 Wed 12:30 ZEU 260

Growing Actin Networks Form Lamellipodium and Lamellum by Self-Organization — •Florian Huber, Björn Stuhrmann, and Josef Käs — Universität Leipzig, Linnestr. 5, 04103 Leipzig, Germany

Cell migration is associated with the dynamic protrusion of a thin actin-based cytoskeletal extension at the cell front. This extension has been shown to consist of two different substructures, the lamellipodium and the lamellum, which differ in their kinetic and kinematic properties as well as their molecular composition. While the formation

of the lamellipodium is increasingly well understood, organizational principles underlying the emergence of the lamellum are just beginning to be unraveled. We developed a 2D Monte-Carlo simulation and an analytical description that include chemical reaction kinetics, actin monomer diffusion, and filament transport to investigate the formation of growing actin networks in migrating cells. We demonstrate the system*s ability to form two distinct networks by self-organization. We find a characteristic transition in filament lengths and a distinct maximum of depolymerization, both within the leading 1*2 microns of the cell, in agreement with experimental data. We investigate the complex interplay between ADF/cofilin and tropomyosin and propose a mechanism that leads to spatial separation of, respectively, ADF/cofilin- or tropomyosin-dominated compartments. Tropomyosin is found to play an important role in stabilizing the lamellar actin network.. Furthermore, the influence of filament severing and annealing on the network properties is explored. We contribute to a fundamental understanding of how cells organize their molecular components to achieve movement.

BP 13.8 Wed 12:45 ZEU 260

Microtubule-based neuronal growth cone motility — •Thomas Fuhs[1], Allen Ehrlicher[1,2], and Josef Käs[1] — [1]Universität Leipzig, Soft matter physics, Leipzig, Germany — [2]Harvard University, School of Engineering and Applied Sciences, Cambridge, USA

When creating a functional steering aparatus the individual nerve cells in the brain have to form synapses to pass on informations. Prior to the formation of a synapse the nerve cell has to find some other nerve cell to link to, therefore it sends out an exploratory growth cone. The growth cone is connected to the cell body with the microtubule rich axonal stump while on the front it consists mainly of actin, both as a dense network forming lamellipodia or thick actin bundles (filopodia).

This setup suggests an actin polymerization driven type of motility, as is it observed in fibroblasts. But in contrast to fibroblasts we obeserved inverse durotaxis, contradicting the models used for fibroblasts.

So we developed a theoretical model of how actin bundles steer a growth cone by mechanically stabilizing extending microtubules. Simple physics of anisotropic cytoskeleton elasticity and tube-model based ordering show how microtubules must align with stiff actin bundles, while unaligned one get buckled. Hence the side of the growth cone with fewer actin bundles dissipates more elastic energy in bent microtubules, resulting in a pressure pushing the growth cone in the opposite direction. This model also explains the inverse durotaxis, if more acto-myosin energy is dissipated into substrate deformation, less energy is available to deform the exploratory microtubules, resulting in an preferential extension towards softer materials.

BP 13.9 Wed 13:00 ZEU 260

Vinculin exchange dynamics regulates adhesion site turnover and adhesion strength — •Christoph Möhl, Norbert Kirchgessner, Claudia Schäfer, Kevin Küpper, Rudolf Merkel, and Bernd Hoffmann — Institut für Bio- und Nanosysteme 4: Biomechanik, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

The coordinated formation and release of focal adhesions is a key requirement for effective cell locomotion. New adhesions develop at the cell front and mature over time by changing composition and exchange dynamics of the incorporated proteins. As the cell moves forward, the maturing focal adhesions remain nearly stationary with respect to the substrate. They finally dissolve once the cell's trailing edge comes close. Besides other factors, this adhesion turnover defines the polarization and direction of migration of the cell and is thought to be highly regulated by phosphorylation events.

Here, we analyzed the dynamics of focal adhesions in migrating cells on different time scales. On the long time scale, we measured lifetimes and growth behaviour of focal adhesions, while on the short time scale the exchange dynamics of the focal adhesion protein vinculin was analyzed by FRAP (fluorescence recovery after photobleaching). In parallel, overall focal adhesion phosphorylation was quantified. Additionally, force measurements on moving cells were performed to correlate the maturation state of a focal adhesion with its adhesion strength. Our studies support a direct interplay between phosphorylation, adhesion dynamics and force application.