BP 24: Actin Dynamics

Time: Thursday 14:00–16:45 Location: C 243

Invited Talk BP 24.1 Thu 14:00 C 243

A biochemical reconstitution approach of the coordinated actin assembly dynamics in motile and morphogenetic processes. — •Marie-France Carlier, Louis Renault, Guillaume Romet-Lemonne, Emmanuele Helfer, Beata Bugyi, Kim Ho Diep Le, Dominique Didry, and Stéphane Romero — CNRS, Gif-sur-Yvette, France

In living cells, the actin cytoskeleton is composed of polar actin filaments that are assembled at a steady state in well organized arrays, and co-exist with a pool of monomeric actin. Filaments turnover via a treadmilling mechanism, in which the pool of polymerizable monomeric ATP-actin, generated by pointed end depolymerization of all filaments in these arrays, drives the growth of filament barbed ends. Filament barbed end growth is also controlled by machineries that link signalling to the actin cytoskeleton. We are interested in understanding the physical chemical principles underlying the coordinated turnover of actin filaments in these different meshworks and their synergetic action in motility and porphogenetic processes. For this we propose a systemic biology approach. I will show a few examples as follows. In vitro reconstitution assays of actin-based propulsion of N-WASP-functionalized beads or liposomes illustrate the role of the interplay between membrane and cytoskeleton dynamics in directional movement ; reconstitution of the rapid processive assembly of filaments profilin-actin by immobilized formins mimicks filopodia extension ; reconstitution of the synergy between Spire and formin suggests a possible functional basis of the genetic interplay between these two proteins in embryogenesis.

BP 24.2 Thu 14:30 C 243

Protrusion force generation of fish keratocytes — •Claudia Brunner, Michael Gögler, Allen Ehrlicher, Bernd Kohlstrunk, and Josef Käs — University of Leipzig

Cell motilitiy is a fundamental process associated with many phenomena in nature, such as immune response, wound healing, and metastasis.On the molecular level, actin polymerization and molecular motors, are involved in cell motility but the mechanism as a whole is not very well understood. Rapidly migrating cells, such as keratocytes, move forward through active protrusion at the leading edge, and retraction/deadhesion of the cells rear, indicating two force generating centers.

Our SFM-based technique uses the vertical and lateral deflection of a modified cantilever and allows direct measurements of the forces exerted by the cell. We present direct measurements of the forward forces generated at the leading edge of the lamellipodium, the cell body and retrograde forces within the lamellipodium. Through selective manipulation of molecular components by addition of different drugs, such as Jasplakinolide, Cytochalasin D, and ML-7 the measured forces and velocity changes can be compared. This leads to new insights concerning the importance of different force generating processes and reveals actin polymerization as the dominant force generating process at the leading edge. On the other hand myosin does not seem to be responsible for the retrograde flow.

BP 24.3 Thu 14:45 C 243

Actin polymerization and ATP hydrolysis kinetics — •Xin Li[1], Jan Kierfeld[1,2], and Reinhard Lipowsky[1] — [1]MPI of Colloids and Interfaces, Science Park Golm, 14424 Potsdam — [2]Technische Universität Dortmund, Lehrstuhl für Theoretische Physik I, 44221 Dortmund

Actin polymerization plays an important role in many aspects of cell dynamics. This active process involves the hydrolysis of ATP molecules, which takes place within an ATP-rich cap and can be spatially separated from the assembly process. In this study, we theoretically compare different mechanism for the coupling between ATP hydrolysis and actin polymerization and describe their effects on experimentally observable quantities, such as cap length, total hydrolysis rate, and actin filament growth rate.

BP 24.4 Thu 15:00 C 243

Actin flow and cellular traction at focal adhesions: measurements and theoretical interpretation — •Benedikt Sabass[1], Margaret Gardel[2], Clare Waterman[3], and Ulrich Schwarz[1] — [1]University of Heidelberg, BQ 0013, Im Neuenheimer Feld 267, D-69120 Heidelberg, Germany — [2]University of Chicago, IL, USA — [3]NIH, Bethesda, MD, USA

The dynamic nature of cell-substrate contacts (focal adhesions) is an essential ingredient of the communication between cells and their environment. In particular the intracellular motion of actin flow and its connection to external cellular traction seems to be crucial for cellular decision making at focal adhesions. We have developed a novel assay to simultaneously measure retrograde actin flow and cellular traction force with high spatial resolution. The resulting data demonstrates a biphasic relationship between actin speed and traction. Moreover, we find that maximum traction is always exerted at a speed level which is independent of biochemical perturbations. This suggests the existence of a robust sensory mechanism relating internal flow dynamics to traction in the environment. We also discuss physical models for bond dynamics which are able to explain the experimentally found data.

15 min. break

BP 24.5 Thu 15:30 C 243

Actin dynamics in SCAR-deficient cells — Hellen Ishikawa-Ankerhold[1], Till Bretschneider[1], Günther Gerisch[1], Annette Müller-Taubenberger[1,2], Robert Insall[3], Eberhard Bodenschatz[4], and •Carsten Beta[5,4] — [1]MPI für Biochemie, Martinsried, Germany — [2]Institut für Zellbiologie, LMU München, Germany — [3]School of Biosciences, University of Birmingham, UK — [4]MPI fuer Dynamik und Selbstorg., Goettingen, Germany — [5]Institut fuer Physik, Universitaet Potsdam, Germany

The dynamical properties of the actin cytoskeleton provide the basis for motility, phagocytosis, and division of eukaryotic cells. Directed polymerization of actin in the cell cortex has been identified as the underlying source of force generation. A key player in the formation of a dense cortical actin network is the seven-subunit Arp2/3 complex that initiates the nucleation of branches on existing filaments. Its activity is controlled by SCAR/WAVE proteins of the WASp (Wiscott-Aldrich Syndrome protein) family that are downstream effectors of receptor-mediated signalling pathways. Here we analyze the temporal patterns of actin polymerization in the cortex of mutant cells lacking members of the pentameric SCAR complex. The results highlight the actin machinery as a self-organizing system that can be described by the concepts of non-equilibrium dynamics. We furthermore report evidence that the cortical dynamics is linked to the chemosensory pathway, so that receptor signals are transmitted to the actin system, even if SCAR is missing.

BP 24.6 Thu 15:45 C 243

A microscopic description of actin-based propulsion of beads — •azam gholami[1], martin falcke[1], and erwin frey[2] — [1]Hahn-Meitner-Institute, Dept. Theoretical Physics, GlienickerStr. 100, 14109 Berlin, Germany — [2]Arnold Sommerfeld Center for Theoretical Physics and Center of NanoScience, Ludwig-Maximilians-Universität, Theresienstr. 37, 80333 München, Germany

Beads propelled by actin polymerization have been widely used as a model system for Arp2/3 dependent actin-based movement. VCA-grafted beads were shown to exhibit the same characteristic motion as the pathogen Listeria monocytogenes. All existing microscopic models, such as the elastic Brownian ratchet model do not explicitly consider geometry, such as the size of object or the curvature. Here, we generalize our simple model of actin-based motility to include the curvature of the obstacle. We find that small and large beads move approximately at the same speed which is different from the tethered ratchet model which predicts faster movement with larger beads.

BP 24.7 Thu 16:00 C 243

Measurement of Force by Actin Gel Polymerization: A Combined AFM and Epifluorescence Study — •Stephan Schmidt[1], Pia Zissman[1], Walter Zimmermann[1], Emmanuèle Helfer[2], Marie-France Carlier[2], and Andreas Fery[1] — [1]Universität Bayreuth, Germany — [2]CNRS-LEBS, Gif-sur-Yvette, France

The ability to generate forces and move actively is one of the key features of micro-organisms and nature has found various pathways to accomplish it. In processes associated with active movement of eukaryotic cells and some bacteria such as Listeria monocytogenes force generation is driven by actin filament growth against the membrane. The

biochemistry of the involved processes are well understood, whereas the molecular scale mechanism of force generation is still matter of debate. We use a simplified in vitro assay composed of purified proteins and artificial colloids. Force measurements on actin networks are performed using colloidal probe AFM techniques, were the growing actin network is clamped between an AFM spring and a solid substrate. Using fluorescence microscopy we observe the gel extension in direct conjunction with the AFM measurement. Results suggest that force stalling is due to buckling and (induced) symmetry breaking at the stressed site of the gel. By changing the composition of the medium we vary the actin density and use different probes to control the size of the gel. In these experiments, the amount of force generated can be well explained by the size and density of the polymerizing network. Further we show experiments and theory concerning confinement effects on symmetry breaking of actin gels.

BP 24.8 Thu 16:15 C 243

Physical principles of self-organized cell locomotion — •Konstantin Doubrovinski — Universität des Saarlandes, Geb E26, Postfach 151150, 66041 Saarbrücken

Experiments on crawling cell fragments of fish keratocytes indicate that cell locomotion can emerge from self-organization of the cytoskeleton. The underlying physical mechaism, however, is still poorly understood. Recent experiments on human neutrophils, which are the most abundant white blood cells, indicate that intracellular spiral waves of the protein HEM, which regulates actin polymerization are essential for driving membrane portrusions at the leading edge [1]. We propose a physical description of spiral waves in human neutrophils, based on experimental findings. A key element of our description is the treatment of interactions between the cytoskeleton and the membrane through a phase-field. In addition to persistent uni-directional locomotion, we find that the system can self-organize into polymerization waves lateral to the membrane. Such waves have been observed in spreading cells [2] and indicate a common mechanism of cell locomotion and spreading.

[1] Weiner et. al. (2007) PLoS Biol 5 e221

[2] Doebereiner et. al. (2006) Phys. Rev. Lett. 97 038102

BP 24.9 Thu 16:30 C 243

Modeling and Mimicking Lamellipodial Actin Network Growth — •Florian Huber, Björn Stuhrmann, and Josef Käs — Institute for Soft Matter Physics, University of Leipzig, Linnéstr. 5, 04103 Leipzig, Germany

Most cells are able to perform directed migration, which is of enormous relevance for many different biological systems and indispensable for multicellular organisms. Typically the cell migrates by formation of lamellipodial structures, i.e. a thin active actin network. Its formation and appearance is regulated by various actin related proteins.

The key molecular players involved in these processes have been identified and have already been used to generate *in vitro* actin network growth. The required next step towards reproducing cellular conditions is to confine the polymerizing actin gel in sub-micron sized structures. These structures are obtained with a combination of several microfabrication techniques that also allow selective functionalization with the polymerization inducing peptide VCA. We use fluorescence microscopy to visualize the emerging actin network. Speckle microscopy will be applied to further analyze its properties.

For the first time we operate with a restricted protein pool that allows to mimic the self-sustaining character of the lamellipodial machinery. Moreover, we directly link experiment to Monte-Carlo simulation and mathematical modeling. All three approaches allow controlled variation of various biochemical and physical parameters in order to better understand the complex interplay between the essential cytoskeletal proteins.