BP 33: Biological Machines & Motor Proteins

Time: Friday 10:15–13:00 Location: ZEU 250

Invited Talk BP 33.1 Fri 10:15 ZEU 250

Clamping DNA Strands Together: The Mechanics of Single-strand Annealing — •Erik Schäffer and Marcel Ander — Biotechnology Center (BIOTEC), TU Dresden, Dresden, Germany

Homologous recombination is the fundamental biological process for exchanging DNA segments. It serves to repair DNA breaks, re-launch stalled replication forks, and maintains genetic diversity by mediating gene transfer mechanisms. Two mechanisms are known: strand invasion and single-strand annealing. While the former ATP-dependent mechanism promoted by RecA/Rad51 has been characterized to some extent, the latter ATP-independent mechanism is not understood on the molecular level. Using optical tweezers, we investigated the single-strand annealing mechanism using Redß as a model system. We discovered that despite Redß's efficiency in promoting single-strand annealing, it defaults to kinetic inhibition of DNA annealing. Instead, it is active towards the 3'-end of a single-stranded DNA in the following way: If sufficient complementarity towards another single-stranded DNA is given, presumably a monomer of Redß nucleates clamping of DNA strands. The clamping leads to a large energetic gain and resistance against DNA unzipping. Sequence conservation patterns suggest the existence of three distinct superfamilies: Redß, ERF, and Rad52. For the human version of the latter, we have also indications for the clamping mechanism suggesting that it is perhaps the underlying general mechanism of DNA single-strand annealing, irrespective of the protein family.

BP 33.2 Fri 10:45 ZEU 250

Exceptional *in vitro* and *in vivo* motility of the S. cerevisiae Kinesin-5 Cin8 — •Christina Thiede[1], Adina Gerson-Gurwitz[2], Natalia Movshovich[2], Vladimir Fridman[3], Maria Podolskaya[3], Tsafi Danieli[4], Stefan Lakämper[1], Christoph F. Schmidt[1], and Larisa Gheber[2,3] — [1]Drittes Physikalisches Institut, Georg-August-Universität Göttingen, Göttingen, Germany — [2]Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva, Israel — [3]Department of Clinical Biochemistry, Ben-Gurion University of the Negev, Beer-Sheva, Israel — [4]Protein Expression Facility, Wolfson Centre for Applied Structural Biology, Hebrew University of Jerusalem, Jerusalem, Israel

Members of the conserved Kinesin-5 family fulfill essential roles in mitotic spindle morphogenesis and dynamics. The mechanisms that regulate Kinesin-5 function are not well understood. In this study, we have examined *in vitro* and *in vivo* functions and regulation of the *Saccharomyces cerevisiae* Kinesin-5 Cin8. Using *in vitro* single-molecule fluorescence motility assay in whole-cell extracts, we found that Cin8 motility is exceptional in the Kinesin-5 family. In high salt, Cin8 moved fast along microtubules ($\sim$ 22 μm/min) for a Kinesin-5. In low salt, Cin8 was slower and moved more diffusively. We further found that a unique 99 amino acid insert, located in the Cin8 motor domain, increased Cin8 binding to microtubules, affected its motile properties and *in vivo* controlled its localization and function during anaphase spindle elongation.

BP 33.3 Fri 11:00 ZEU 250

Cooperative Transport by Two Molecular Motors — •Florian Berger[1], Corina Keller[1], Melanie J. I. Müller[1,2], Stefan Klumpp[1], and Reinhard Lipowsky[1] — [1]Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany — [2]Department of Physics, Harvard University, Cambridge, MA 02138, USA

Intracellular transport of cargos is achieved by molecular motors, which pull the cargo along cytoskeletal filaments. Due to the thermal environment, these motors unbind from the filament resulting in a finite run length of the cargo. Assuming non-interacting motors the run length increases exponentially with the number of motors attached to the cargo [1]. However, in a recent in vitro experiment two kinesins coupled via a DNA interfere in such a way that they pull each other from the filament [2]. Using a discrete stochastic model based on well established single motor properties together with a spring like coupling, we study the origin of this interference in a motor pair of two identical motor proteins. Additionally, we consider a cargo transported by one active microtubule based motor, kinesin-1, and one diffusive actin based motor, myosin V resulting in an enhanced processivity of the cargo in agreement with a recent in vitro experiment [3].

[1] S. Klumpp, and R. Lipowsky, PNAS 102:17284 (2005)
[2] A. R. Rogers et al., PCCP 11:4882 (2009)
[3] F. Berger, M. J. I. Müller, and R. Lipowsky, EPL 87:28002 (2009)

BP 33.4 Fri 11:15 ZEU 250

Using single-molecule FRET to determine the stepsize of the rotating c-ring of F_oF_1-ATP synthase with DCO-ALEX — •Eva Hammann, Stefan Ernst, Andrea Zappe, Jörg Wrachtrup, and Michael Börsch — 3.Physikalisches Institut, Universität Stuttgart, Pfaffenwaldring 57, 70569 Stuttgart, Germany

ATP production is essential for life. The enzyme F_oF_1-ATP synthase performs this task by proton-driven internal subunit rotation. F_oF_1-ATP synthases comprise a membrane-embedded F_o part and a protruding F_1 part in the inner membranes. The F_1 part consists of 5 different subunits with the stoichiometry $\alpha_3\beta_3\gamma\delta\epsilon$. The α- and β-subunits form three catalytic binding sides for the reaction of ADP and phosphate to ATP and for the reversed ATP hydrolysis direction. The F_o part consists of three different subunits. The a- and b_2- , δ-, α- and β-subunits form a stator part. The 10 c-subunits (in E. coli bacteria) are arranged in a ring and form the rotary motor with the γ- and ϵ-subunits. The driving force is a proton current through the a-subunit and the c-ring. The rotation of the c-ring can be visualized by labeling the a-subunit and one c-subunit with two different fluorophores and measuring steps by single-molecule Förster resonance energy transfer (FRET). For ATP synthesis activity the protein must be reconstituted in an artificial membrane, or for longer observation times, has to be immobilized on a Ni-NTA-surface. Here we show rotary motion of the c-ring by FRET using an duty-cycle optimized alternating laser excitation scheme (DCO-ALEX).

15 min. break

BP 33.5 Fri 11:45 ZEU 250

Towards in vitro reconstitution of motor-driven nuclear oscillations — •Manuel Neetz[1], Stefan Diez[1,2], and Iva Tolic-Norrelykke[1] — [1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden — [2]B CUBE, Dresden

Molecular motors are necessary for fundamental cell functions such as cell division and intracellular transport. These vital processes rely on the interplay of a multitude of motors exerting force on microtubules, which leads to concerted movements in the cell. The one dimensional nuclear oscillations in the fission yeast Schizosaccharomyces pombe represent an easily accessible model process to study intracellular movements driven by molecular motors and microtubules. Similar oscillations have been observed in other organisms during mitosis. For the oscillations in S. pombe the minus-end directed motor protein dynein is necessary, which generates pulling forces by binding to microtubules and the cell cortex [Yam 06]. In S. pombe microtubules grow in opposite directions from the spindle pole body, and the movement of dynein all along the microtubules gives rise to antagonistic pulling forces [Vog 09]. We investigated the resulting dynamics by studying the gliding of cross-linked anti-parallel microtubules in vitro. Currently we are working with stabilized microtubules and the plus-end directed motor protein kinesin [Led 10]. We will extend the approach to anti-parallel dynamic microtubules gliding on yeast dynein.

[Yam 06] Yamamoto et al., J. Cell Biol., 145 (1999); [Vog 09] Vogel et al., PLoS Biology, 7 (2009); [Led 10] Leduc et al., PRL, 105 (2010);

BP 33.6 Fri 12:00 ZEU 250

Actin filaments undergo local structural transitions at random sites — •Thomas Niedermayer[1], Antoine Jegou[2], Emmanuele Helfer[2], Guillaume Romet-Lemonne[2], Marie-France Carlier[2], and Reinhard Lipowsky[1] — [1]Abteilung Theorie und Bio-Systeme, Max-Planck-Institut für Kolloid- und Grenzflächenforschung, 14424 Potsdam, Germany — [2]Laboratoire d'Enzymologie et Biochimie Structurales, CNRS, 91198 Gif-sur-Yvette, France

After the polymerization of actin monomers into filaments, the actin-bound ATP is hydrolyzed into ADP, a process that is believed to decrease the filament stability. Recent experiments suggest the opposite behavior, however, namely that actin filaments become increasingly stable with time. Several mechanisms for this unexpected behavior have been proposed, ranging from structural transitions of the whole

filament helix to pure artifacts arising, e.g., from the capping or surface attachment of the filament ends. We performed novel fluorescence microscopy experiments on single filaments to clarify this controversial issue. We find that filaments do indeed cease to depolymerize in an abrupt manner, and that this transition happens on relatively long time scales that exceed those of both ATP cleavage and phosphate release. We also developed a theory that allows us to distinguish the different possible transition mechanisms. A detailed comparison of theory and experiment implies that the sudden truncation of the shrinkage process does neither arise from artifacts nor from a collective transition of the whole filament. Instead, our results provide strong evidence for a local transition process occurring at random sites within the filament.

BP 33.7 Fri 12:15 ZEU 250

Kinesin-3 (UNC-104) can act as a dimeric motor during axonal transport *C. elegans* neurons *in vivo* — •Volker Christoph Henschel[1], Alessandro Esposito[2], Christoph Friedrich Schmidt[1], Fred Sylvester Wouters[3], and Dieter Robert Klopfenstein[1] — [1]Drittes Physikalisches Institut, Biophysics, Georg-August-University Göttingen, Göttingen, Germany — [2]MRC Cancer Cell Unit, Hutchison/MRC Research Centre, Cambridge, UK — [3]Laboratory of Cellular and Molecular Systems, Department of Neuro- and Sensory Physiology, Georg-August-University Göttingen, Göttingen Germany

Monomeric Kinesin-3 (UNC-104) is responsible for the transport of presynaptic vesicles to synaptic termini in *C. elegans*. To investigate the role of the endogenous coiled-coils, we introduced point mutations in the motors coiled-coil region in the neck promoting either dimer formation of Kinesin-3 or reducing the likelihood of dimerization. We verify dimerization by cross-linking of purified truncated motors *in vitro*. We show by live *in vivo* imaging, that reducing dimerization of Kinesin-3 leads to decreased vesicle transport velocities and affects the control of muscle contraction. *C.elegans* with reduced dimerization properties exhibit a 45% reduction in anterograde velocity. Additionally, severe motility and a significant egg laying defect are observed. To assess dimer formation *in vivo* we combine Foerster Resonance Energy Transfer (FRET) and anisotropy imaging with spinning-disc laser confocal microscopy. Our data suggest a direct link between dimerization status and transport velocities.

BP 33.8 Fri 12:30 ZEU 250

Computational/Genetic approach characterizes the construction of the *Drosophila* ear — •Björn Nadrowski[1,2], Thomas Effertz[2], and Martin Göpfert[2] — [1]Theoretische Physik, Universität des Saarlandes, Campus E2.6, 66123 Saarbrücken — [2]Abt. Zelluläre Neurobiologie, Universität Göttingen, MPI für Experimentelle Medizin, Hermann-Rein-Str. 3, 37075 Götingen

Hearing relies on dedicated mechano-electrical transduction (MET) channels that convert stimulus forces into electrical signals. We present a physical model hat quantitatively links ion channel mechanics and movements of molecular adaptation motors to the dynamics of the entire ear. We fit the model parameters to data obtained from both wild-type and mutant flies. We show that the experimental data obtained in the absence of NompC (a candidate MET gene), can be interpreted as the absence of a transduction channel in a transducer system that consists of two parallely arranged channel populations, one of them constituted by NompC. We further show evidence that NompC might be specifically needed to detect low stimuli amplitudes (i.e. sound stimuli), whereas the other channel population might serve for the detection of wind and/or gravity.

BP 33.9 Fri 12:45 ZEU 250

The molecular basis of filopodial retraction analyzed with photonic force based microscopy — •Felix Kohler[1,2] and Alexander Rohrbach[1,2] — [1]Bio and Nano-photonics, IMTEK, University of Freiburg, Germany — [2]BIOSS Centre for Biological Signalling Studies, Freiburg, Germany

Filopodia are needle-like protrusions of the cell surface. These actin rich protrusions are highly dynamic structures that extend and retract over the timescale of a few seconds. Besides actin polymerization and depolymerization, coordinated transport of molecular motors seems to control filopodial mechanics. Apart from sensing the environment and anchorage of cells to a substratum filopodia are also involved in phagocytosis. We use photonic force microscopy to investigate the mechanical concepts of the filopodial retraction during phagocytosis. The motion of an optically trapped bead is attached to tip of a filopodium and tracked interferometrically in 3D with nanometer precision at a microsecond timescale. The measurement of e.g. the beads mean displacement allows determining the retraction forces of filopodia at various retraction speeds. We have measured F-actin dependent steps inside living cells during filopodial retraction likely belonging to actin-based molecular motors [1]. Steps remain clearly visible even at force regimes clearly beyond the stall force of a single myosin motor. This indicates a kind of inter-motor coupling, a phenomenon which will be presented in this talk and which we try to explain by a stochastic multi-state model.

[1] Kress, H. et al., pnas, 104, 2007, 11633-11638