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Welcome

Dear Participants, Dear Guests!

We are pleased to welcome you at Pécs for the EMBO|FEBS Lecture Course on Novel Biophysical Approaches in the Investigation of the Cytoskeleton.

This meeting is the 27th European Cytoskeletal Forum conference and this year's event continues the tradition of gathering researchers to discuss recent results of cytoskeletal research, emerging new technologies and future directions of scientific development. We believe that the meeting will provide an excellent occasion for investigators of the cytoskeleton and related fields to exchange ideas and scientific information and hopefully to initiate new collaborations. Indeed advancing life sciences has increasingly become multidisciplinary and requires team work. We hope that bringing you all together at this meeting, where science and technology merge, will foster new collaborative efforts. Special attention goes to young scientists. Becoming a good scientist is more than being a successful experimentalist. It is also about sharing creativity and learning from each other. Try to envisage the different research possibilities and technical approaches to tackle your particular scientific question. Grab this unique opportunity and discuss this with your peers or with the more senior scientists present at this meeting. To facilitate this we have scheduled 46 oral and 45 poster presentations. These are from participants from 26 countries and highlight different aspects of cytoskeletal research paying attention to fundamental scientific questions and/or deregulations during diseases.

We hope that you will benefit from the scientific programme, that you savour the beauty of Pécs, and take the opportunity to spend constructive moments with colleagues from the cytoskeleton research community. Enjoy your meeting.

Sincerely,

The Organizers
Miklós Nyitrai, Department of Biophysics, University of Pécs, Hungary
Christophe Ampe, Department of Biochemistry Ghent University, Belgium
Laura Machesky, The Beatson Institute for Cancer Research, United Kingdom

Organisers

Main organisers

Miklós Nyitrai
Department of Biophysics, University of Pécs, Hungary

Christophe Ampe
Department of Biochemistry Ghent University, Belgium

Laura Machesky
The Beatson Institute for Cancer Research, United Kingdom

Local organisers

Beáta Bugyi
Department of Biophysics, University of Pécs, Hungary

Gábor Hild
Department of Biophysics, University of Pécs, Hungary

András Lukács
Department of Biophysics, University of Pécs, Hungary

László Grama
Department of Biophysics, University of Pécs, Hungary

Official travel agency of the Conference

Tensi Aviation Kft. - Congress Department
H-7621 Pécs,Teréz u. 17.
Tel.: +36 72 513 983, 513 990
Fax.: +36 72 510 497
www.tensipecs.hu
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General information

Invited speakers

Victoria Allan
University of Manchester, UK

Alexander D. Bershadsky
Weizmann Institute of Science, Israel and Mechanobiology Institute, Singapore

Laurent Blanchoin
Universite Joseph Fourier, Grenoble, France

Francesca Cella Zanacchi
Istituto Italiano di Tecnologia, Genova; Department of Physics, University of Genoa, Italy

Edward Egelman
University of Virginia, Charlottesville, USA

Michael A. Geeves
University of Kent at Canterbury, UK

Kenneth Holmes
Max Planck Institute for Medical Research, Heidelberg, Germany

Anne Houdusse
Institut Curie - Section de Recherche, France

Miklós Kellermayer
Semmelweis University, Budapest, Hungary

Mihály Kovács
Eötvös Loránd University, Budapest, Hungary

Frank Kozielski
The Beatson Institute for Cancer Research and Glasgow University, Scotland, UK

Laura Machesky
The Beatson Institute for Cancer Research and Glasgow University, Scotland, UK

Yuichiro Maeda
Nagoya University, Japan

Alf Mansson
School of Natural Sciences, Linnaeus University, Sweden

Dietmar Manstein
Medizinische Hochschule Hannover, Germany

Justin Molloy
MRC National Institute for Medical Research, London, UK

Judit Ovádi
Biological Research Center, Hungary

Ewa Paluch
Max Planck Institute of Molecular Cell Biology and Genetics, Germany

Robert Robinson
Institute of Molecular and Cellular Biology, Singapore

James Spudich
Stanford University, Stanford, California, USA

Claudio Sunkel
Universidade do Porto, Portugal

Péter Tompa
Biological Research Center, Hungary

Maria Vartiainen
University of Helsinki, Finland

Sven Vogel
Biotechnologisches Zentrum der TU Dresden, Germany

Venue

Hotel Palatinus City Centersuperior***
Király utca 5.
H-7621 Pécs, Hungary
GPS Coordinates: N 46°4'46" E 18°13'18"

General information

Registration

Saturday, 3 November	12:00 - 19:00
Sunday, 4 November	08:00 - 17:00
Monday, 5 November	08:00 - 16:00
Tuesday, 6 November	08:00 - 15:00
Wednesday, 7 November	08:00 - 16:00

Registration fee

The registration fee includes

- admission to lecture and poster sessions,
- course materials, abstract book, meeting bag,
- use of meeting room dedicated for poster sessions and poster-mounting kits,
- dinners and lunches during the conference,
- accomodation (5 nights, 3-7 Nov, breakfast included),
- Welcome dinner (3 Nov), Gala dinner in Villány (7 Nov), coffee breaks, wine and cheese during poster sessions,
- shuttle bus transfer between Budapest airport and Pécs for arrival (3 Nov) and departure (8 Nov).

Registration fee does not include travel to Hungary.

Registration fee for accompanying person includes

- accommodation for 5 nights in double room shared with the participant,
- dinners (4-6 Nov),
- Welcome dinner (3 Nov) and Gala dinner (7 Nov).

Registration fee for accompanying person does not include the shuttle bus transfer between Budapest Airport and Pécs (30 EUR/person/one-way).

Shuttle bus service

Complimentary shuttle service between Hotel Palatinus and Budapest Airport will be provided for those participants, who entered the flight details in their on-line registration. The pick-up time and place is shown on your voucher, which is placed in your conference bag.

Social events

Participation on these events is included in the registration fee.

Saturday, 3rd November, 2012
Welcome Dinner
Hotel Palatinus

Monday, 5th November, 2012
Organ concert and winetasting
30-minutes long organ concert at the Cathedral, followed by snack dinner with winetasting at the Episcopal Winecellar.

Departure time and meeting point: 18:45, Hotel Palatinus reception

Tuesday, 6th November, 2012
Sightseeing tour in Pécs
3-hour sightseeing tour in Pecs with English speaking guide. This tour gets you acquainted with the city's World Heritage Sites, the Cathedral and the Zsolnay Quarter (including a visit to the exhibition of the Golden Age of Zsolnay).

Bus transfer between city center and Zsolnay Quarter will be provided.
Departure time and meeting point: 15:00, Hotel Palatinus reception

Wednesday, 7th November, 2012
Gala Dinner in Villány
Hungary is famous for its great wines. One of the best-known historical wine-districts in Hungary is the Villány Wine-route which is 25 kms from Pécs. During the programme our guests will get to know the production of red wine, visit a winecellar, and able to taste the local culinary specialities and prize-winning wines and listening to traditional local live-music. Bus transfer will be provided from the hotel.

Duration: approximately 4-5 hrs.
Departure time and meeting point: 18:00, Hotel Palatinus reception

General information

Participant badges

For security purposes, participants are requested to wear their personalised badge at all times during the conference.

Information for poster authors

Poster authors are requested to mount their posters on the evening of 3 Nov or the morning of 4 Nov, and leave their posters on the boards during the Conference.

Posters are grouped into two sessions (Sunday and Monday afternoon), indicated by the poster numbers (P1-… or P2-…) in the abstract section of the booklet as well as on the poster boards. Authors are asked to be present at their posters during the Poster session they are assigned to.

Posters will be viewed and rated by a Committee, and the best posters will receive a prize. Results of the poster competition will be announced on Wed, 7 Nov, 17:00.

Information for speakers

The time allocated for talks shown in the programme includes the time for questions. Please note that all speakers are required to be present at least 15 minutes prior to the starting time of the session so that their presentations can be uploaded. Presentation files may also be uploaded in advance, at the Registration Desk.

Internet access

Free WiFi is available in the lobby and in the rooms of the Palatinus Hotel.
Username: ecf2012
Password: ecf2012

Contact, emergency phone numbers

Conference organizers' mobile phone: +36 70 45 45 600
Ambulance: 104 / Police: 107 / Fire service: 105

ECF 2012 will be featured in a special issue of Cytoskeleton!

We are pleased to inform everyone presenting their work at the "Novel Biophysical Approaches in the Investigation of the Cytoskeleton" meeting that selected works (stemming from invited talks, results presented at the meeting, and/or related topics from invitees' labs) will be published in a special issue of the prestigious journal '*Cytoskeleton*' in 2013. Contributions will be invited from the confirmed list of invited speakers, as well as a select group of young investigators (invitees will be receiving formal invitations to contribute via e-mail shortly). The special issue is tentatively scheduled to publish in September/October 2013. This publication timeline is applicable to the "print" version of the special issue only; individual articles comprising the issue will of course publish online (as "Accepted Articles" and "EarlyView Articles") shortly following their acceptance. To ensure that the target September/October 2013 publication schedule is met,

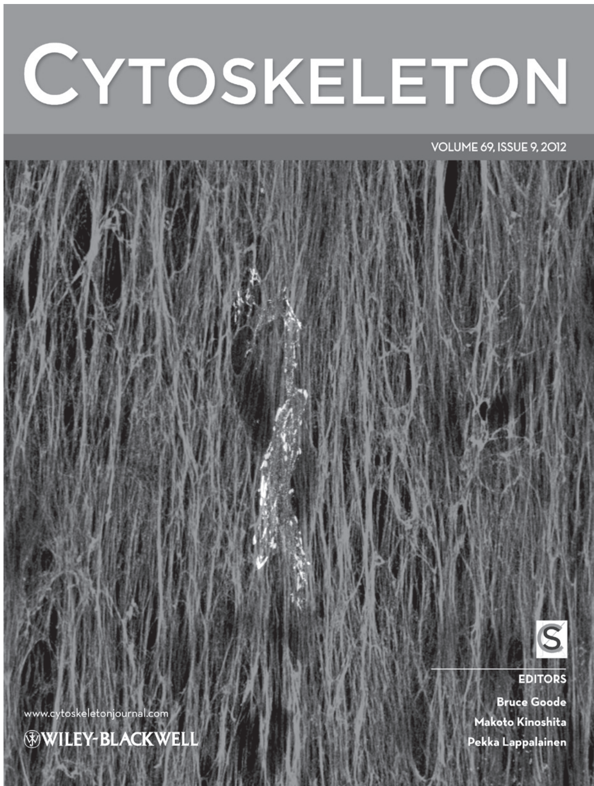
the submission deadline for all articles is February/March 2013.

Submitted manuscripts will undergo the usual *peer review* process, which will be arranged and monitored by the three Guest Editors, ***Laura Machesky*** (The Beatson Institute for Cancer Research, United Kingdom), ***Christophe Ampe*** (Department of Biochemistry Ghent University, Belgium) and ***Miklós Nyitrai*** (Department of Biophysics, University of Pécs, Hungary). The three Executive Editors of Cytoskeleton, ***Bruce Goode***, ***Pekka Lappalainen***, and ***Makoto Kinoshita*** will provide additional oversight. The Managing Editor of Cytoskeleton, ***Michael Wise***, will coordinate everything and serve as the contact person for all authors and editors.

Special issue of Cytoskeleton

The goal is to publish a nice mix of '***research articles***', '***review articles***', and '***technique articles'*** stemming from work presented at the meeting (or related topics from invitees' labs). Instructions for the preparation of all three article types can be found by following the "Author Guidelines" link on the Cytoskeleton homepage (http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1949-3592). The special issue will open with a brief meeting review written by one or more of the Guest Editors, possibly in conjunction with other meeting participants. There are no publication costs associated with these papers. With regard to color images, authors will receive up to three "pages" of color images "free of charge" (in the "print" version of articles). It is important to bear in mind that in many cases more than one color image may appear on a single page. Also, please be aware that "all" submitted color images will publish in color (free of charge) in the "online" version of articles). To recap, "all" color will publish free of charge in the online version of articles, while essential color images will be limited to three "pages" (free of charge) in the print version of articles.

Please, do consider and keep in mind this excellent opportunity when planning your publications over the next few months!



SATURDAY, 3 NOVEMBER

18:00 - Opening ceremony

First evening plenary lecture

James A. Spudich
One path to understanding energy transduction in biological systems, and where do we go from here?

19:00 - Welcome dinner at Hotel Palatinus

SUNDAY, 4 NOVEMBER

Session I - Cytoskeletal interactions at the molecular level
(chairs: Yuichiro Maeda, Japan, *Beatriz García Fernández,* Portugal, *Antoine Jégou*, France)

8:55 - 9:00	Chairman's introduction
9:00 - 9:30	Ewa Paluch Actin cortex mechanics and cell shape instabilities in cytokinesis
9:30 - 10:00	Mike Geeves Rapid kinetic methods to study motor proteins
10:00 - 10:20	Isabel Van Audenhove Fascin loss-of-function by intracellular delocalisation with nanobodies
10:20 - 10:40	Beatriz García Fernández The role of β-catenin in postnatal heart development under stress and aging
	Coffee break
11:10 - 11:40	Alexander D. Bershadsky The actin cytoskeleton plays a key role in tissue growth events by regulating mechanodetection and mechanotransduction events
11:40 - 12:10	Alf Månsson Materials properties of cytoskeletal filaments and bundles - relations to motor driven transportation

12:10 - 12:30 Ksenia Astanina
Cytoskeletal dynamics in TNT formation and functioning in endothelial cells

12:30 - 12:50 Tina Zupancic
Keratin inclusion bodies as potential vehicles for recombinant protein delivery into skin cells

Lunch

14:50 - 15:10 Laura Machesky
Invasion and migration of cancer cells: Similar and opposing processes

15:10 - 15:30 Antoine Jégou
Studying formin dynamics on individual actin filaments using microfluidics

15:30 - 15:50 Mónika Ágnes Tóth
SALS, a WH2-protein in sarcomeric actin assembly

15:50 - 16:30 Yuichiro Maeda
Conformational and energetic cycle of actin in polymerization and depolymerization

16:30-17:00 Judit Ovadi
Moonlighting TPPP/p25 mediates ultrastructure-controlled function of the microtubule cytoskeleton

17:30 - 19:00 Poster session 1 (wine and cheese)

Senior-junior researcher discussion forum (beer and sandwich)

19:00 - Dinner

MONDAY, 5 NOVEMBER

Session II - Structural methods in the investigation of the cytoskeleton (chairs: Bob Robinson, Singapore/UK, *Louis Renault*, France, *Thomas Bornschlögl*, France)

8:45 - 8:50 Chairman's introduction

8:50 - 9:30 Kenneth Holmes (EMBO Plenary Speaker)
The structural basis of muscle contraction

9:30 - 10:00	Péter Tompa Concept of disordered proteins and their cytoskeletal functions
10:00 - 10:20	Teresa Bonello Characterising the impact of tropomyosin-targeting compounds on the actin cytoskeleton
10:20 - 10:40	Louis Renault Structural basis for the functional versatility of ß-Thymosin/WH2 domains in actin self-assembly
	Coffee break
11:10 - 11:40	Anne Houdusse How myosin motors produce force - new insights from a reverse-direction motor
11:40 - 12:10	Bob Robinson The structural basis and dynamics of actin polymerization: From bacteria to eukaryotes
12:10 - 12:30	Cora-Ann Schoenenberger Unraveling the role of actin lower dimer in supramolecular actin assembly by TIRF microscopy
12:30 - 12:50	Francesco Difato A study of cytoskeleton dynamics in developing mouse neurons by optical tweezers force-spectroscopy
	Lunch
14:50 - 15:20	Edward Egelman New Insights Into the Unusual Properties of Actin
15:20 - 15:40	Thomas Bornschlögl How Filopodia Exert Force
15:40 - 16:00	Veronika Kollár The effect of mouse Twinfilin-1 on the structure and dynamics of actin
16:30 - 18:00	Poster session 2 (wine and cheese)
	Senior-junior researcher discussion forum (beer and sandwich)
18:45 -	Departure to organ concert at the Cathedral, followed by snack dinner with winetasting

Programme

TUESDAY, 6 NOVEMBER

Session III - Novel approaches in imaging the cytoskeleton
(chairs: Dietmar Manstein, Germany, *Sharissa Latham*, Australia)

8:35 - 8:40	Chairman's introduction
8:40 - 9:10	Christophe Ampe Beta-actin a player in cell migration and in cell homeostasis; what can we learn when biased and unbiased approaches meet?
9:10 - 9:30	Marleen Van Troys Quantitative analysis of cell migration dynamics in 3D-matrices at higher throughput
9:30 - 10:00	Dietmar Manstein Hybrid Analysis of the Actomyosin System
10:30 - 10:50	Sharissa Latham Unique roles for cytoplasmic actin isoforms in mechanically regulating endothelial microparticle formation
10:50 - 11:10	Max Nobis Intravital imaging of Src response to Dasatinib treatment using FLIM-FRET in vivo
	Coffee break
12:00 - 12:30	Sven Vogel Actin pattern formation and lipid/protein dynamics in reconstituted actomyosin cortices
12:30 - 13:00	Miklos Kellermayer Manipulation of filamentous biomolecular systems with force-clamp optical tweezers
13:00 - 13:30	Francesca Cella Zanacchi Optical nanoscopy and individual molecule localization methods towards multimodal imaging of the cell
13:30 - 14:00	Justin Molloy (EBSA speaker) Single Fluorophore Imaging Reveals the Abundance, Distribution, Mobility and Oligomeric State of M_2 Muscarinic Acetylcholine Receptors in Live Cardiac Muscle
14:00 - 14:50	Lunch

15:00 -	Guided sightseeing tour in Pécs
19:00 -	Dinner

WEDNESDAY, 7 NOVEMBER

Session IV - Molecular motors, cellular motility and related methods (chairs: Michael A. Geeves, UK, *Ruth F. Sommese*, United States, *Vaishnavi Ananthanarayanan*, Germany)

8:55 - 9:00	Chairman's introduction
9:00 - 9:30	Victoria Allan Role of dynein in endosome movement in living cells
9:30 - 10:00	Mihály Kovács Elucidation of the mechanisms of action of DNA helicases and other motors traveling along nucleic acid strands
10:00 - 10:20	Hans-Peter Wollsheid Molecular basis of ubiquitin-mediated regulation of Myosin VI in cell migration
10:20 - 10:40	Ruth F. Sommese Molecular Level Understanding of Cardiomyopathy Mutations on the Regulated Actomyosin Interaction
	Coffee break
11:10 - 11:40	Maria Vartiainen Dynamics of nuclear actin: from form to function
11:40 - 12:10	Laurent Blanchoin Directed actin self assembly and contractility
12:10 - 12:30	Feng-Ching Tsai Shape transition of liposomes induced by actin bundles
12:30 - 12:50	Geraldine O'Neill Tropomyosin and actin-dependent protein trafficking to focal adhesions
	Lunch
14:50 - 15:20	Claudio Sunkel The function of the kinetochores in microtubule attachment and checkpoint function

15:20 - 15:40	Marina Kriajevska S100A4 binds to the extended and compact forms of non-muscle myosin IIA in A431 cells undergoing epithelial-mesenchymal transition
15:40 - 16:00	Vaishnavi Ananthanarayanan Characterization of dynein by single-molecule investigations in vivo
16:00 - 16:30	Frank Kozielski How does kinesin-1 enter into ‘energy saving mode’?
17:00 - 17:30	Young Investigators’ Forum/Prize Ceremony/Suggestions and remarks
18:00	Departure to Gala Dinner in Villány

Programme overview

Saturday, 3 Nov	Sunday, 4 Nov	Monday, 5 Nov	Tuesday, 6 Nov	Wednesday, 7 Nov
	Session I *Cytoskeletal interactions at the molecular level*	***Session II*** *Structural methods in the investigation of the cytoskeleton*	***Session III*** *Novel approaches in imaging the cytoskeleton*	***Session IV*** *Molecular motors, cellular motility and related methods*
	8:55–10:40 Talks	8:45–10:40 Talks	8:35–11:10 Talks	8:55–10:40 Talks
	10:40–11:10 Coffee break	10:40–11:10 Coffee break	11:10–12:00 Coffee break	10:40–11:10 Coffee break
	11:10–12:50 Talks	11:10–12:50 Talks	12:00–14:00 Talks	11:10–12:50 Talks
	12:50–14:50 Lunch	12:50–14:50 Lunch	14:00–14:50 Lunch	12:50–14:50 Lunch
	14:50–17:00 Talks	14:50–16:00 Talks	15:00 Guided sightseeing tour in Pécs	14:50–16:30 Talks
18:00 Plenary lecture	17:30–19:00 Poster session 1	16:30–18:00 Poster session 2		17:00 - 17:30 Prize ceremony, closing remarks
19:00 Welcome dinner at Hotel Palatinus	19:00 Dinner	18:45 Departure to organ concert at the Cathedral, followed by snack dinner with winetasting	19:00 Dinner	18:00 Departure to Gala Dinner in Villány

One path to understanding energy transduction in biological systems, and where do we go from here?

James A. Spudich
Stanford University, Stanford, California, USA

Who could not be fascinated by the myriad biological movements that define life? From cell migration, cell division, and a network of translocation activities within cells to highly specialized muscle contraction, molecular motors operate by burning ATP as a fuel. Three types of molecular motors, myosin, kinesin and dynein, and nearly 100 different subtypes transduce that chemical energy into mechanical movements to carry out a wide variety of cellular tasks. Understanding the molecular basis of energy transduction by these motors has taken decades. What more needs to be done? Where are the major opportunities for future research? Here I present my perspectives on these and related questions.

Abstracts (talks)

Session I - Cytoskeletal interactions at the molecular level

Sunday, 4 November

Actin cortex mechanics and cell shape instabilities in cytokinesis

Ewa Paluch
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
and International Institute of Molecular and Cell Biology, Warsaw, Poland

Cytokinesis relies on tight regulation of the mechanical properties of the cell cortex, a thin acto-myosin network lying under the plasma membrane. At anaphase onset, the cortex accumulates into an equatorial ring that drives furrow ingression. Although most studies of cytokinetic mechanics focus on force generation at the constriction ring, a contractile acto-myosin cortex remains at the poles of dividing cells throughout cytokinesis. Whether polar forces influence cytokinetic cell shape and furrow positioning is poorly understood. Using a combination of experiments and theory, we demonstrate that the polar cortex makes cytokinesis an inherently unstable process, where any imbalance in contractile forces between the two poles compromises the accurate positioning of the constriction ring. We show that limited asymmetric polar contractions occur during normal cytokinesis, and that perturbing the polar cortex leads to cell shape oscillations, resulting in furrow displacement and division failure. A theoretical model based on a competition between cortex turnover and contraction dynamics accurately accounts for the oscillations. Taken together, our findings reveal an inherent instability in the shape of the dividing cell, indicating that polar cortex contractility must be tightly controlled to ensure successful cytokinesis.

Rapid kinetic methods to study motor proteins

Michael A. Geeves
School of Biosciences, University of Kent, Canterbury, UK

Motor proteins are involved in both the movement of material around the cell and in generating mechanical forces inside and between cells. Both motor activities are mediated through the cytoskeleton. While many of the motor proteins operating in a cell have been identified, understanding the details of the activity and the regulation of the activity of any one of the multitude of motors in a cell remains a challenge. As indeed is the challenge of understanding why there is such a variety of motors on the cell. Rapid kinetic approaches are one of the tools which can be used to define the range of activities a specific motor protein can undertake, how that activity is modulated by binding partners and identifying the potential physiological role of the motor. The approach is essential to understanding how different motors are adapted for specific cellular function.
All protein undertake a series of conformational changes in response to the binding of small ligands, interacting with other proteins and in response to changes in the physical environment. Understanding these conformational changes, the speed (or rate of the process) and energetics (thermodynamics) of the process are central to unlocking the nature of protein function. This can be done for may isolated pure motor proteins. More challenging is to define methods that can be used equally well to study the proteins in solution and in the environment of the cell. Rapid perturbation of the cell environment is one approach that has great promise.

Fascin loss-of-function by intracellular delocalisation with nanobodies

Isabel Van Audenhove[1,2]*, Joël Vandekerckhove*[1,2]*, Ariane De Ganck*[1,2] *and Jan Gettemans*[1,2]
[1]Department of Medical Protein Research, VIB, Ghent, Belgium;
[2]Department of Biochemistry, Ghent University, Faculty of Medicine and Health Sciences, Albert Baertsoenkaai 3, Ghent, Belgium

Fascin is an actin bundling protein which is considered as a metastatic marker and therefore an important therapeutic target. We have generated a unique set of tools, nanobodies, enabling us to study endogenous fascin in cancer cells. Nanobodies, present in species of the *Camelidae*, correspond with the smallest antigen binding fragments which fully retain their binding affinity. We identified a fascin nanobody, FASNb5, which potently inhibits the actin bundling activity of fascin *in vitro*. When expressed in PC-3 prostate cancer cells, this nanobody prevents formation of filopodia, fingerlike protrusions important for adhesion and migration. FASNb5 also inhibits matrix degradation, an invasive process during which an underlying extracellular matrix is degraded. Another fascin nanobody, FASNb2, has no effect on actin bundling because it binds another epitope in fascin. This nanobody has no influence on filopodia formation and matrix degradation. However, as an alternative strategy we tagged this nanobody, causing delocalisation of the nano-body and fascin towards the mitochondrial outer membrane. Under these conditions, FASNb2 significantly reduces both filopodia formation and matrix degradation, thereby establishing a clear correlation between the subcellular localisation of fascin and its role in filopodia formation and matrix degradation. This reveals a new way to promote a protein functional knock-out using high affinity nanobodies, at the level of the endogenous protein.

The actin cytoskeleton plays a key role in tissue growth events by regulating mechanodetection and mechanotransduction events

***García Fernández, Beatriz**, Jezowska, Barbara Zofia and Janody, Florence*
Instituto Gulbenkian de Ciência. Rua da Quinta Grande 6. 2780-156 Oeiras. Portugal.

Cellular mechanodetection and mechanotransduction are key events in providing cells with information about their environment. It is still unclear what most of these mechanodetectors are and how mechanotransduction is coupled to developmental programs. The actin filament (Factin) system and its regulators are arising as mediators of these events. We showed that proper regulation of Factin is required to suppress inappropriate tissue growth through the regulation of the tumor suppressor pathway Hippo. Our results suggest that a regulatory loop exists between Factin and cell proliferation that is mediated by the transcription factor Yorkie. Thus, it seems that tension exerted by neighboring cells can be sensed at the cell membrane by Factin and is translated into cell proliferation by the Hippo pathway.

Interestingly, we have also discovered an interplay between Factin and the Src proto-oncogene to avoid tumor formation. The Factin network is known to control Src activity and in turn, Src modulates the Factin cytoskeleton. Moreover, Src activation can be driven in response to mechanical forces and cytoskeleton stretch. Upregulation of Factin processes are linked to aberrant cell migration in Src tumor-dependent events. We have data arguing that Factin dynamics is not only involved in the control of Src-dependent invasion but also in earlier events. In *Drosophila* epithelia, knocking-down Capping Protein (CP), which regulates Factin polymerization, recapitulates the behavior of cells overexpressing Src64B and synergizes with increase Src64B levels to promote apoptosis. Moreover, both loss of *CP* or increase Src activity promote Factin accumulation. Interestingly, overexpression of CP rescues growth defects induced by Src-overexpression suggesting that Src-dependent growth events are Factin dependent. I will discuss our ideas on how Factin dynamics modulates the activity of oncogene and tumor suppressor pathways to regulate growth, through biophysical properties.

Forces and signaling in the self-organization of actin cytoskeleton and focal adhesions

Alexander D. Bershadsky[1,2], *Yee Han Tee*[2], *Tom Shemesh*[1], *Alexandra Lichtenstein*[1], *Masha Prager-Khoutorsky*[1], *Visalatchi Thiagarajan*[2], *Ramaswamy Krishnan*[3], *Benjamin Geiger*[1], *Michael M. Kozlov*[4]

[1]Weizmann Institute of Science, Rehovot, Israel; [2]Mechanobiology Institute, National University of Singapore, Singapore; [3]Harvard School of Public Health, Harvard University, Boston, MA, USA; [4]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Polarization in fibroblasts is associated with a development of an array of parallel actin filament bundles (stress fibers) with focal adhesions (FAs) at their ends. The cytoskeletal and signaling mechanisms underlying this process are poorly understood. Here, we demonstrate that fibroblast polarization and formation of stress fiber arrays depends on FA mechanosensitivity, and does not occur on compliant substrates. To gain insight into control mechanisms regulating these processes we used a siRNA screening approach. siRNA-mediated knockdowns of 85 known human protein tyrosine kinases (PTKs) revealed distinct alterations in the cell polarization response associated with the changes in the substrate rigidity-dependence of cell traction force generation and FA formation. Thus, stages of cell polarization are regulated via multiple, PTK-regulated force-dependent checkpoints. To dissect cytoskeletal events preceding cell shape changes, we further studied the actin cytoskeleton self-organization in fibroblasts confined within cell-sized circular adhesive islands. The process begins with formation of a radially symmetrical actin cytoskeleton, comprising radial (R) and transverse (T) actin bundles (fibers). The radial organization was transient and was followed by a chiral swirling of the entire system of R- and T-fibers. We propose that contraction of myosin IIA-containing T-fibers generates stresses, which result in a centripetal motion of these fibers, as well as force dependent growth of the R-fibers from the focal adhesions. The noise in the system then causes a spontaneous symmetry breaking, resulting in swirling motion. The chirality of swirling is explained as a consequence of helical symmetry of actin filaments in the radial fibers.

Materials properties of cytoskeletal filaments and bundles - relations to motor driven transportation

Alf Månsson[1]*, Elina Bengtsson*[1]*, Hideyo Takatsuki*[1]*, Saroj Kumar*[1]*, Malin Persson*[1]*, Lasse ten Siethoff*[1]*, Mercy Lard*[2]*, Heiner Linke*[2]
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The persistence length (Lp) of a polymer or a sliding filament path is the length scale/sliding distance over which the "memory" of the tangent angle along the polymer/path is maintained. Theory suggests that Lp for a heavy meromyosin (HMM) propelled actin filament path in the in vitro motility assay is quantitatively equal to Lp of the free leading end of the filament, i.e. directly determined by actin filament materials properties (Young´s modulus, mass distribution over filament cross-section). However, path Lp may be a complex function of the materials properties if these vary along the filament due to short distance propagation of structural changes induced by myosin head binding. Additionally, off-axis motor forces may cause torques around the filament long axis adding to effects of thermal fluctuations. Here, we first describe the theoretical basis for expected equivalence of filament and path persistence lengths and then report experiments (in vitro motility assays at different [ATP] and HMM surface densities and on nanostructured surfaces etc.) evaluating this idea in relation to complicating factors. We also describe Monte-Carlo simulations of filament paths assuming different actin filament material properties and different degrees of HMM induced sideways filament movements. The results are consistent with limited effects of the latter movements at high HMM density and millimolar [ATP]. Under all conditions tested the path persistence lengths were similar to those expected from the filament materials properties but more studies are needed for full understanding of details particularly at low [ATP].

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Cytoskeletal dynamics in TNT formation and functioning in endothelial cells

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Tunneling nanotubes (TNT) are long cellular protrusions containing cytoskeleton filaments. The proposed function of TNTs is the cell-to-cell trafficking of organelles and signals, whereby a highly cell-type specific structure and function has been suggested.
We here describe TNT-like structures in human microvascular endothelial cells (HMEC-1). The identified TNTs were studied with CLSM, live cell imaging, and environmental scanning electron microscopy (ESEM). At least three types of nanotubes could be distinguished: (i) actin-containing TNTs, (ii) tubulin-containing TNTs, and (iii) nanotubes with both actin and tubulin filaments. Whether these nanotubes are of different types or whether they correspond to different stages of TNT maturation is still unclear. However, the cargo of different TNT types seems to differ.
To clarify the role of cytoskeleton in TNT formation the cells were treated either with depolymerizing agents (cytochalasin D, nocodazole), or stabilizing substances (jasplakinolide, taxol). Unexpectedly, the effects of the actin-stabilizing drug were similar to the actin-depolymerizing one: both treatments resulted in a significant increase in the number and average length of TNTs. Moreover, the role of the Rho GTPase cytoskeletal regulators RhoA, Rac1, Cdc42 has been studied in TNT formation. Here, dominant-negative mutants of Rac1 and Cdc42 showed increased formation of long and thin TNTs, whereas in constitutively active mutants the nanotube formation was abolished.
The findings described in this study give first important information about the structure, formation and functioning of TNTs in endothelial cells and will be extended to fully elucidate the mechanisms of TNT formation and functioning in endothelial cells.

Keratin inclusion bodies as potential vehicles for recombinant protein delivery into skin cells

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We present the results of a study developing a method for a possible new protein therapy approach. It is based on recombinant protein production of model protein keratin 14 (K14), an intermediate filament cytoskeleton component, in the form of so-called inclusion bodies. K14 inclusion bodies were electroporated into SW13 cells grown in culture together with a “reporter” plasmid containing EYFP labeled keratin 5 (K5) cDNA. As SW13 cells do not normally express keratins and keratin filaments are built only of keratin heterodimers, short filamentous structures may be obtained only if: a) both inclusion bodies and plasmid DNA are transfected simultaneously into the same cell(s); b) once inside cells, K14 protein is released from inclusion bodies; c) released K14 is functional, able to form heterodimers with EYFP-K5. This is the first time *in vitro* evidence is presented for inclusion bodies being used as nanoparticles, vehicles for protein delivery into epithelial cells.

Invasion and migration of cancer cells: Similar and opposing processes

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The WASP family control Arp2/3 mediated actin polymerization. Each family member regulates different actin-based processes, but despite many years of study, their full physiological roles remain unclear. N-WASP is implicated in generation of filopodia, lamellipodia, and clathrin coated pits, but loss of N-WASP causes only slight effects in each. Here we describe a central role for N-WASP in 3D cancer cell invasion through polarized trafficking of the major collagenolytic matrix metalloprotease MT1-MMP. N-WASP is crucial for formation of invadopodia, both in 2D and in 3D matrix and invadopodia are important for establishment of polarized MT1-MMP trafficking. Another WASP-family actin nucleation promoting protein, WASH, is also involved in cell invasion, at least in part via the trafficking of integrin alpha5 beta 1 to the leading pseudopodia of invading cells. Our studies of N-WASP and WASH point to polarised trafficking into leading invasive pseudopodia as key for invasive migration in 3D. Scar/WAVE complex, on the other hand, seems to have no positive role in cancer cell invasion and acts rather as an invasion suppressor. Overall, our studies suggest that cancer cell invasion and migration share in common that they require actin assembly to generate force, but that they are in many ways completely different processes with different limiting factors and machineries. This has interesting implications for development of in vitro assays for studying cancer cells leading to drug target identification and future therapies for metastasis.

Studying formin dynamics on individual actin filaments using microfluidics

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The study of actin assembly dynamics at the scale of individual filaments provides key information on the molecular mechanisms at play. Using microfluidics, we have developed experimental configurations which provide a straightforward and accurate monitoring of individual filaments in vitro, with an extensive control of their biochemical environment. We have recently used this setup to elucidate ATP hydrolysis in actin filaments (Jégou et al. PLoS Biology 2011) and the occurrence of photo-induced pauses during depolymerization (Niedermayer et al. PNAS 2012, accepted).
Here, we use our microfluidics setup to investigate the processive elongation of actin filaments by formin mDia1. By measuring elongation rates and processivity of formins in different conditions, we unveil molecular details of actin subunit addition at the barbed end. We take advantage of the friction force exerted by the flowing fluid on the actin filament to investigate how formins respond to mechanical tension. These experiments provide insight into mechano-chemical coupling in formins, and help decipher the processive motor activity of formins.

SALS, a WH2-protein in sarcomeric actin assembly

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The morphology and dynamics of sarcomeric actin filaments are essential for proper muscle development and function. However, it is not completely understood how sarcomeric actin filaments are stereotyped in length and dynamics. Recently, SALS (*sa*rcomere *l*ength *s*hort) a WH2-domain-containing protein was identified in *Drosophila* as an important regulator of the assembly of sarcomeric actin structures. Disruption of *sals* by RNAi leads to lethality at the embryonic stages. This phenotype results from muscle defects caused by the improper organisation of sarcomeric actin filaments in SALS mutant embryos. Further loss-, and gain-of-function studies indicate that SALS influences sarcomere length by promoting actin filament assembly at the pointed ends and by antagonising with the pointed end-binding protein, tropomodulin. However, the exact role of SALS in muscle development has not been revealed.
To dissect the mechanism by which SALS contribute to the establishment of sarcomeric actin structures first we investigated the interaction of the WH2 domain containing fragment of SALS (SALS-WH2) with actin using biochemical and biophysical approaches.
We found that SALS-WH2 inhibits the assembly of actin monomers to filaments and enhances the disassembly of actin filaments even in the presence of tropomyosin. SALS-WH2 influences actin dynamics by shifting the monomer : filament equilibrium towards monomeric actin.
To reveal the biological role of SALS further studies are needed. The results can contribute to the understanding of the mechanisms underlying muscle function and the multifunctionality of WH2 domain proteins.

Conformational and energetic cycle of actin in polymerization and depolymerization

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We obtained a high resolution structure of polymerized F-actin, which revealed for the first time substantial conformational changes of actin molecule (1). In monomeric G-actin, either side of the central Me-Nd binding cleft is twisted by 20 degrees relative to each other, whereas in F-actin the molecule is untwisted and flat.
Taking the G-to-F conformational transition into consideration, we propose a basic cycle of actin molecule. In the turn-over (tread-milling) of pure actin, individual actin molecules repeat this cycle, whereas in cells each actin binding protein regulates a particular step of the cycle.
Strain energy: It is highly probable that the flattened F-form stores mechanical strain energy within the molecule, whereas the entire F-actin structure is stabilized by interactions between surface loops which become possible by the conformational transition.
Energy sources: Since ADP-bound G-actin as well as Nd-free G-actin polymerizes, the conformational transition is not driven by the actin ATPase but is driven by the collision of a G-actin to an end of F-actin, and thereby the transition is propagated at the end.
Role of ATPase: The actin ATPase does not drive polymerization. The opposite should be true: The G-F conformational transition induces the actin ATPase (2). Upon release of Pi, ADP-F-actin becomes less stable with a higher critical concentration, which may be due to storage of additional strain energy within F-actin molecule. The ATPase (Pi-release) drives depolymerization and thereby drives the actin turn-over.
Polymerization initiation: If the G-to-F transition is propagated, an important question arises about where and how “the first” actin molecule is transformed. It should be emphasized that mechanisms of this transformation by polymerization initiators, like Arp2/3 complex or formin, remain unknown.
It is worth noting that the basic actin cycle is driven not only by chemical energy (released by the ATPase) but also by mechanical energy. In other words, there is no one-to-one correspondence between each actin species in the cycle and chemical species of bound Nd. It is also remarkable that this basic cycle of actin is very much similar to the basic cycle of tubulin.

Moonlighting TPPP/p25 mediates ultrastructure-controlled function of the microtubule cytoskeleton

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A major constituent of the cytoskeleton is the microtubule system that displays multiple activities due to its decoration by stably and dynamically associated proteins. A newly discovered brain-specific, microtubule associated protein is the Tubulin Polymerization Promoting Protein (TPPP/p25) displaying moonlighting function originated by its distinct macromolecular associations at physiological and pathological conditions.
TPPP/p25 is an *intrinsically unstructured protein* with its extended unstructured N- and C-terminals straddling a flexible region comprising binding domains involved in physiological (tubulin, GTP, zinc) or pathological (α-synuclein, α-amyloid) interactions. The dimerization of TPPP/p25 with favorable physiological functional potency is proposed to play significant role in the fine tuning of TPPP/p25-mediated microtubule assembly.
In normal brain TPPP/p25 is predominantly expressed in oligodendroglial cells (OLGs); it is indispensible for the differentiation of the progenitor cells by its rearrangement role in the microtubular network in the course of elongation of microtubule-based projections necessary to the axon ensheathment. Under pathological conditions the destructive potential of TPPP/p25 likely the monomers are involved in the formation of toxic protein assemblies leading to the etiology of CNS diseases such as Parkinson and Alzheimer diseases or multiple sclerosis. This *neomorphic moonlighting* features of TPPP/p25 makes it a powerful diagnostic marker and potential drug target.

Abstracts (talks)

Session II - Structural methods in the investigation of the cytoskeleton

Monday, 5 November

The structural basis of muscle contraction

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Myosin is a product-inhibited ATPase. Product release is greatly accelerated by binding to filamentous-actin. Lymn and Taylor elucidated the cross-bridge cycle: in the absence of ATP the myosin cross-bridge binds strongly to actin (rigor); ATP binding to the cross-bridge leads to rapid release from actin; a conformational change of the cross-bridge (the recovery stroke) permits hydrolysis; the cross-bridge then rebinds to actin (allowing phosphate release) and subsequently undergoes a conformational change that drives actin passed myosin (the power stroke); at the end of the power stroke ADP is released, ATP can rebind to repeat the cycle.
X-ray crystallography showed that the myosin cross-bridge consists of a large catalytic domain, often called the motor domain, based on a central 7-stranded beta-sheet that carries the ATP binding site and the actin binding site. A long lever arm is connected to the C-terminus of the motor domain. The catalytic mechanism of the cross-bridge is similar to the G-proteins: the active site contains a P-loop and switch 1 and switch 2 elements. The lever arm was later found in two different conformations (so called pre-power-stroke and post-rigor) allowing one to see how switch 2 movement was coupled to a swing of the lever arm. The swinging lever arm is the basis of the rowing-like movement of myosin along the actin filament. This mechanism appears to be common to all myosins. A number of crystallographic studies, notably by Anne Houdusse, have given insight into the function of myosin as a molecular machine.
ATP binding sequesters switch 1 to open the large cleft in the motor domain thereby breaking actin binding site. Conversely, the strong binding to actin, together with the subsequent power-stroke, causes a movement that opens the nucleotide binding site, bringing about the release of nucleotide.
Decorated actin (the rigor complex) allows one to study the strong F-actin-myosin interaction. Most recently high resolution cryo-EM reconstructions of decorated actin from Raunser's group and from Namba's group have produced atomic models of the actin myosin interaction in the strong-binding state, allowing one to postulate a power stroke mechanism. Unfortunately, these projections are incomplete since the structure of the ephemeral "beginning of the power stroke" remains enigmatic.

Concept of disordered proteins and their cytoskeletal functions

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Intrinsically disordered proteins (IDPs/IUPs) exist and function without a well-defined 3D structure, defying the classical structure-function paradigm [1]. Structural disorder is widespread in eukaryotic proteomes and correlates with important functions such as signal transduction and transcription regulation. Whereas currently increasing effort is focused on these proteins, most of our concepts regarding their structure and function stem from *in vitro* data. In my talk I will briefly overview the field of structural disorder, with a major focus on the special functional modes it enables, and also touch upon evidence for their existence *in vivo*. I will show how bioinformatics tools can be used to address important issues with respect to their physiological existence, function and regulation. Special attention will be paid to cytoskeletal IDPs, which, in different ways, are involved in the assembly and regulation of the actin cytoskeleton, intermediate filaments and microtubules alike. Overall, the message of my talk is that despite much controversy about the physiological importance of these proteins, the evidence is overwhelming that their unusual structural state is a highly evolved feature enabling exciting biology in the cell.

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Characterising the impact of tropomyosin-targeting compounds on the actin cytoskeleton

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The actin cytoskeleton is organised into functionally distinct compartments by the tropomyosin family of actin-binding proteins. Transformed cells rely on a restricted repertoire of tropomyosins, including the isoform Tm5NM1. There is a need to elucidate how individual tropomyosin isoforms expressed in cancer cells contribute to actin filament function. Targeting tropomyosin to disrupt specific actin-filament populations would serve as both a valuable biological tool and potential therapeutic strategy. Using *in silico*-homology modelling, we have developed small molecules against the C- and N-termini of Tm5NM1. Ascertaining the specificity of these small molecules presents some challenges given that Tm5NM1 is a structural protein with no simple functional readout by which to assess activity. To address this, we have taken both a biochemical and cell-based approach to investigate the nature and specificity of the Tm5NM1-compound interaction. *In vitro*, lead anti-tropomyosin compounds reduced the binding affinity of recombinantly expressed Tm5NM1 for filamentous actin. Further to this, the presence of compound was found to affect Tm5NM1-regulated actin filament elongation kinetics. To investigate how Tm5NM1-targeting compounds alter actin filament dynamics in the cell we have utilised a high content imaging platform. Here images of compound-treated cells were analysed using an algorithm to quantitate actin filament organisation. Cells treated with Tm5NM1-targeting compounds showed a reduction in both actin filament number and filament length, consistent with cellular fractionation studies showing a decrease in total levels of filamentous actin. Taken together, this data supports targeting distinct tropomyosin isoforms to perturb actin filament function.

Structural basis for the functional versatility of ß-Thymosin/WH2 Domains in Actin Self-Assembly

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Widespread ß-thymosin (ßT) and WH2 (Wiskott-Aldrich syndrome protein Homology domain 2) domains are found as single or repeated units in a large number of multi-domain actin-binding proteins involved in developmental processes, synaptic plasticity, polarized cell migration or intracellular pathogen infections. They are archetypes of small, intrinsically disordered actin-binding modules with highly variable sequences of 25-55 residues. Intrinsically disordered proteins exist in their unbound native, functional state as dynamic ensembles of interconverting structures and their specific regulatory mechanisms defy the structure-function paradigm. In the last years multi-domain protein organizations with ßT/WH2 domains have emerged as multifunctional regulators of actin self-assembly dynamics *[1]* but understanding the structure-function relationship governing their versatile or multiple functions remain very challenging *[2]*.
We combined mutational, functional, and structural analysis by X-ray crystallography, SAXS and NMR on Thymosin-ß4, Ciboulot, TetraThymosinß, the long WH2 domain of WASP-interacting protein (WIP), N-WASP and Spire WH2 tandem repeats.
We show how the structurally plasticity of WH2 domains is partially conserved and functional in their G-actin bound state. We describe how the distribution of static and dynamic interactions in different 1:1 WH2:actin complexes controls their ability to control either unidirectional assembly like profilin or to sequester G-actin in an ionic strength dependent fashion. At physiological ionic strength the local interaction dynamics are primarily controlled by strong electrostatic interactions of a single residue along their sequence *[3]*. In larger complexes, low resolution SAXS structural analyses suggest how WH2 tandem repeats stabilize actin-actin spatial arrangements which approach those of filaments.

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How myosin motors produce force – new insights from a reverse-direction motor

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Force production in myosin motors is tightly coupled to transitions promoted by actin binding that allow release of phosphate followed by release of ADP. While the release of phosphate is the rate limiting step for conventional muscle myosin II, it is much faster for several unconventional myosins that walk as single molecules. Coupling structural and kinetic studies for different myosins has shown that they populate similar structural states. These studies have elucidated how certain transitions of the actin-myosin catalytic cycle, including the ATP-induced dissociation of myosin from actin and for the ATP hydrolysis step that allows priming of the lever arm. Not yet visualized however are the structural rearrangements triggered by actin binding that are coupled to force generation and product release. While phosphate is produced in the pre-powerstroke (ADP.Pi) state, it is also trapped in this state until an actin-activated transition opens a back-door to promote its release from the active site. It is unclear, however, which of the three elements of the active site (Switch I, Switch II and the P-loop) play a role in this transition and whether the major cleft within the motor partially closes to allow this release while keeping the lever arm primed. We have crystallized a new structural state for myosin VI in the presence of MgADP and phosphate that has an open back-door due to a Switch II rearrangement and a lever arm primed. Thus it has the expected features of the previously unseen Pi release state of myosin. Kinetic and structural studies on a number of mutants from different myosins are currently being performed to evaluate this possibility. The structure is consistent with cleft closure occurring subsequent to Pi release, which is also being tested. An interesting finding in this new structure is that the SH1 helix kinks to allow the converter to stay in the primed position, the signature of a state at the beginning of force production. These structures of myosin VI prior to the lever arm swing also reveal regions of compliance within this lead head. The location of the compliance restricts the possible actin binding sites and predicts the observed stepping behavior of myosin VI. The model reveals that an uncoupling of the lever arm of the lead head occurs when it attaches strongly to actin. Thus, myosin VI, unlike plus-end directed myosins, does not use a pure lever arm mechanism, but instead steps with a mechanism analogous to the kinesin neck-linker uncoupling model.

The structural basis and dynamics of actin polymerization: From bacteria to eukaryotes

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Eukaryotic actin is highly conserved. From humans to frogs, a period of approximately 320 million years, alpha-actin has remained unchanged. In contrast bacterial actin-like filament systems have diverged wildly. One evolutionary pressure that maintains the immutable character of eukaryotic actin is that it is the universal force provider. In order to precisely regulate the spatial and temporal control of actin polymerization, and to harness the force generated within a range of biological processes, a host of actin-regulators and binding partners are required. In the background of this multitude of constraining interactions the central molecule, actin, has had little latitude to evolve. In contrast, bacterial actin-like proteins often have a single biological function, such as, providing force for plasmid segregation. In prokaryotic systems, the evolutionary pressure may be exerted towards divergence. For instance, two different plasmids within a single bacterium will require two distinct actin-like polymerizing motors to ensure unerring inheritance. In this talk, I will compare the structure and dynamics of actins from prokaryotes and eukaryotes and discuss how the differences may affect biological function.

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Unraveling the role of actin lower dimer in supramolecular actin assembly by TIRF microscopy

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Plasma membrane protrusion and the movement of intracellular vesicles require dynamic remodelling of the actin network that involves distinct supramolecular actin assemblies. Subunit configurations other than those present in F-actin filaments may be essential contributors to this structural plasticity. Unconventional actin configurations have been detected in vitro and their significance in actin-based cell functions is receiving increased attention (Schoenenberger et al., 2011). For example, we have recently demonstrated the presence of the so-called 'lower dimer' (LD) or antiparallel dimer in cells by means of an LD-specific antibody. Immunofluorescence studies revealed a relationship of the LD arrangement with the endolysosomal pathway and with the leading edge of migrating cells (Silván et al., 2012). Here we use TIRF live microscopy to elucidate the mechanism how the LD configuration participates in suparmolecular actin assembly. In particular, we monitor the incorporation of chemically crosslinked LD into polymerizing actin filaments *in vitro*. Our studies reveal the ability of LD to establish contacts between filaments that might increase the stiffness of the resulting actin network. Based on our in vitro polymerization experiments we propose a molecular model for the LD-mediated organization of supramolecular structures in vivo. Moreover, preliminary data suggest that a redox mechanism might play a role in regulating LD-mediated actin patterning.

A study of cytoskeleton dynamics in developing mouse neurons by optical tweezers force-spectroscopy

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During development, cells rearrange the cytoskeleton and modulate its dynamic to redistribute intracellular forces, and to adapt to the mechanical properties of the extracellular matrix.
Several studies exploited optical tweezers to resolve forces and motion in the molecular range, and to address the function and role of molecular and polymer motors. Detecting the activity of such molecules in their intracellular milieu represents an important issue to understand how their function is affected in aging and disease.
The integration of an optical tweezers and a laser dissector in an optical surgery workstation, in conjunction with the development of an interferometric system, working in position and/or force clamp condition, allows touching and cutting the living cell while sensing and/or imposing the force perturbation that the cell has to face during optical manipulations.
We measured the force produced by a navigating growth cone of hippocampal neurons, and how its motility is affected by the application of an external force.
We quantified the adhesion of the axon to the substrate, by measuring the release of tension during laser axotomy. Subsequently, we detected the cytoskeleton dynamics against a microsphere mimicking the extra-cellular matrix, with calibrated stiffness, during axonal regeneration.
In conclusion, we developed an experimental model to study cytoskeleton dynamics in developing neuron during two finely regulated tasks: the growth cone exploratory motion, and cytoskeleton reorganization during regeneration of an injured axon. The presented system will open the possibility to analyze how these processes are affected in disease bearing cytoskeleton dysfunction, and in traumatic injury.

New Insights Into the Unusual Properties of Actin

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An unsolved puzzle has been why the sequences of all eukaryotic actins have been so exquisitely conserved over large evolutionary distances (e.g., no amino acid changes from chickens to humans in the skeletal muscle isoform, a distance of ~ 350M years), but the bacterial actin-like proteins show no such sequence conservation, and have diverged so much that many are as different from each other as they are from eukaryotic actin. We have been using cryo-EM of F-actin and complexes of F-actin with other proteins. We can show that actin filaments exhibit large amounts of cooperativity in structural states, as well as allosteric relationships within the subunit. Interestingly, some of the most dramatic allosteric couplings involve elements in actin that are not present in the bacterial actin-like proteins, such as the N-terminus, the C-terminus, the DNase I-binding loop, and the "hydrophobic plug". We suggest that these insertions, which have been stable over all eukaryotic evolution, provide for the extraordinary properties of actin, allowing actin filaments to form highly organized structures such as muscle sarcomeres, stereocilia of the inner ear, microvilli, stress fibers, etc. In contrast, the bacterial ParM protein forms a very different filament than F-actin, which accounts for why that filament behaves very differently: it shows dynamic instability, and the growth at the two ends is very similar. We can show how actin's structure and dynamics are modulated by proteins such as cofilin, and that the ability of actin-binding proteins to change actin's structure depends upon the intrinsic plasticity and cooperativity of actin. These observations give insights into many questions in cell biology, such as how different proteins can be targeted to different actin filaments, how information can be communicated through actin filaments, and how actin can specifically interact with more than 100 binding partners.

How Filopodia Exert Force

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Filopodia are very dynamic, cylindrical cell protrusions that play important mechanical and sensory roles for different cell processes. They are involved in cell migration, wound healing and in the dorsal closure during the embryonic development. Filopodia have been recently observed while actively pulling the invasive bacteria Shigella towards the host cell before infection occurred. In all these examples mechanical forces are involved but the exact mechanics behind the filopodial retraction are far from being understood. We use an optical trap together with confocal microscopy to place beads with different coatings close to the tip of a preexisting filopodia. We observe directly after binding filopodial retraction which shows that adhesion to the filopodial tip is sufficient to trigger its retraction. Filopodia can pull up to a stall force of around 20 pN where they can be abruptly stalled. The stall force decreases with a decreasing number of adhesive links between filopodial tip and bead. Below the stall force single filopodia retract with constant velocity in a force independent manner over their whole length. We present a model where the filopodial retraction force is produced by membrane tension as well as by active retrograde flow of its internal actin core. For forces that are higher than the membrane tension the force transduction via the actin abruptly fails at the filopodia tip.

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The effect of mouse twinfilin-1 on the structure and dynamics of actin

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The actin cytoskeleton of eukaryotic cells plays a key role in many processes. The structure and dynamics of the cytoskeleton are regulated by a large number of proteins that interact with monomeric and/or filamentous actin. Twinfilin is a 37-40 kDa actin-binding protein composed of two ADF-homologous domains connected by a short linker.
The effects of the mouse twinfilin-1 (TWF1) on the monomeric actin were studied by biophysical techniques. The affinity of TWF1 to the ATP-actin monomer was determined by fluorescence anisotropy measurements (K_D = 0.015μM). The fluorescence of the actin bound ε-ATP was quenched by acrylamide in the presence and absence of TWF1. Twinfilin reduced the accessibility of the bound ε-ATP which indicates that the nucleotide binding cleft shifted toward a closed conformational state. Stopped-flow experiments confirmed that the kinetics of nucleotide exchange of actin decreased in the presence of TWF1. The thermodynamic properties of actin monomer were also investigated with differential scanning calorimetry in the presence of TWF1. The results indicate that TWF1 stabilized the structure of the monomeric actin.
These results can help to understand in more details the regulation of G- and F-actin by actin binding proteins.

Abstracts (talks)

Session III - Novel approaches in imaging the cytoskeleton

Tuesday, 6 November

Beta-actin a player in cell migration and in cell homeostasis; what can we learn when biased and unbiased approaches meet?

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We present a cellular model derived from a homozygous beta-actin knock out mouse showing that the conserved isoactins are not redundant at the cellular level. Cells devoid of beta-actin display severely impaired cell migration but curiously have unchanged lamellipodial protrusion rates. Combining microscopy based techniques in conjunction with mass spectroscopy and bioinformatics allow deriving a testable molecular model displaying the altered transcriptional and post translational program. In particular the model suggests increased Rho-ROCK signaling and augmented myosin contractility (in part by expression of alpha smooth muscle proteins). The observed cellular phenotypes are consistent with this molecular model and interfering with ROCK activity or the myosin inhibitor blebbistatin alleviates the migration defect. Collectively the results imply that cells without beta-actin can migrate (if the conditions are appropriate). This, in combination with the changed transcriptome, suggests that the essential role for the beta-actin isoform is not driving cell migration but may rather be its nuclear function.

Quantitative analysis of cell migration dynamics in 3D-matrices at higher throughput

Marleen Van Troys[1], *Lynn Huyck*[1], *Steve De Backer*[2], *Paola Mazusso*[1], *Davy Waterschoot*[1], *Barbara Weyn*[2], *Christophe Ampe*[1]
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Both in healthy and diseased conditions, migration of cells occurs in complex 3D-extracellular matrices of varying density and composition. The search for anti-migratory drugs (e.g. for treating cancer) creates a demand for in vitro assays allowing detailed quantitative analysis of 3D-migration dynamics at higher throughput. Using 3D-assays, a higher physiological relevance of candidate agents is anticipated than by using only 2D- or endpoint-assays.
We present a powerful in vitro migration/invasion platform covering video-microscopy, automated image analysis and quantitative analysis of migration dynamics. It combines high complexity with throughput (48-96 samples). Different migration set-ups are possible (wound-healing-like, random migration, multicellular spheroid-assay), 2D- and 3D-migration can be compared and 3D-matrix properties and treatment regimes can be easily varied.
Quantitative analysis of the image-based data relies on CELLMIA: a newly developed, fully automated software to follow both unlabelled and fluorescently labelled cells in different 3D-matrices. Within one sample, migration parameters of large populations of individually moving cells and of the bulk cell mass are simultaneously extracted. Data quality control and statistical guidelines for in-depth comparison of the multiple tested conditions, have been developed. The platform has been extensively validated a.o. by analyzing the effects of motility-related and/or cytoskeletal drugs on the migration and invasion of cancer cell lines. Output quality in relation to sensitivity, reproducibility and statistical robustness is demonstrated, proving the advantages of the invasion platform for both targeted studies and screening approaches addressing (cancer) cell invasion.

Hybrid Analysis of the Actomyosin System

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Strong links have been established between changes in the activity or abundance of specific myosin isoforms and diseases such as cancer, cardiovascular failure, and disorders of sensory organs and the central nervous system. Detailed characterization followed by targeted intervention at the level of individual myosin isoforms holds therefore a strong therapeutic potential. To study actomyosin function and regulation as it occurs in the context of different cell types, tissues and organs, we employ hybrid approaches combining spectroscopic, hydrodynamic and imaging techniques with overlapping spatial and temporal resolution. The talk will focus in particular on results obtained studying the rigor complexes of specific combinations of muscle and nonmuscle isoforms of actin (A), myosin (M), and tropomyosin (T). Subnanometer-resolution electron density maps of ATM complexes were obtained by cryo electron microscopy. Pseudo-atomic models of the complexes were obtained by fitting crystal structures into the maps. They define the large interface that involves two adjacent actin monomers and one tropomyosin pseudo-repeat per myosin motor domain contact and allow an unambiguous assignment of residues contributing to the ATM interface. Key contact mediating residues are conserved across isoform and species boundaries. Mutations associated with severe cardiac and skeletal myopathies are distributed all over the interface. In addition, we describe the use of hybrid approaches in the elucidation of the molecular mechanism underlying the actions of drug-like effectors of myosin-mediated contractility.

Unique roles for cytoplasmic actin isoforms in mechanically regulating endothelial microparticle formation

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Elevated levels of endothelial microparticles (MP) are observed in numerous diseases, increasingly supporting a role as effectors and valuable marker of vascular dysfunction. Whilst various enzymes, proteins and cellular components, such as the actin-cytoskeleton, have been implicated in vesiculation (MP production), the precise mechanisms of their actions are not understood.
We examined vesiculation in the hCMEC/D3 human cerebral endothelial cell line, investigating the response to inflammatory and immunopathological agonists, including magnetically sorted Plasmodium falciparum-infected erythrocytes. All agonists significantly increased MP release, as seen by flow cytometry and SEM. Data, when combined, provide new insight into in the kinetics, patterns of vesiculating cell recruitment and degrees of response specific to each stimulus.
Stimulation induced reorganisation of cytoplasmic β- and g-actins, F-actin, vinculin and talin, of which the specific subcellular localisations were defined by confocal microscopy. β-actin redistribution into basal stress fibres following stimulation was associated with increased apically situated actin-rich particulate structures. Distribution of these structures directly correlated with electron-lucent membrane protrusions observed by SEM. Inhibition of the Rho-kinase pathway with Y-27632 abolished basal β-actin stress fibre formation, minimising apically associated actin-rich structures, significantly reducing membrane protrusions and MP release to near basal levels. Cytoskeletal protein expression and distribution varied between MP and the mother cell, as determined by western blot and 3D structured illumination microscopy.
Our data strongly suggest that the actin cytoskeleton plays more than a contractile role in vesiculation, with novel actin-rich structures increasing plasma membrane disruption following activation due to their mechanical interaction with basally localised β-actin.

FEBS EMBO

Intravital imaging of Src response to Dasatinib treatment using FLIM-FRET in vivo

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Förster resonance energy transfer (FRET) imaging with a combination of multiphoton and lifetime measurements using a FLIM-FRET Src biosensor was employed in this study to monitor spatial and temporal drug targeting efficacy both *in vitro* and *in vivo* in a live invasive pancreatic ductal adenocarcinoma (PDAC) model driven by mutant *p53* (*p53*^{172H}).
Here, we show that FLIM-FRET analysis allows for accurate, time dependent, live monitoring of drug efficacy and clearance *in vitro* and *in vivo*, which could not be achieved by conventional biochemical techniques. In organotypic assays, we demonstrate that a spatially distinct gradient of Src activity exists within invading tumour cells, which is governed by the depth of penetration into complex matrices. We further show that a gradient of Src activity also exists within the local tumour microenvironment *in vivo,* where Src activity is enhanced at the invasive border relative to the center of the tumour. Upon treatment with the anti-invasive drug dasatinib, we demonstrate a switch in cell activity at the invasive border from a predominantly active to inactive form, correlating with their impaired metastatic capacity *in vivo*. Exploring additional spatial resolution of Src activity existing in live tumours in relation to surrounding vasculature revealed that there is a distinct gradient of Src activity in cells before and after treatment in relation to their distance to the local vasculature. Cells more proximal to the vessels were inhibited to a greater extend by the drug treatment then those distal.
Taken together we demonstrate that FLIM-FRET reporters can be used as pharmacological biomarkers and thus pre-clinical tools to allow for a more accurate evaluation of treatment responses in animal models prior to clinical investigations.

Actin pattern formation and lipid/protein dynamics in reconstituted actomyosin cortices

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In eukaryotic systems the actomyosin cortex is involved in fundamental processes such as cytokinesis and cell polarization and is proposed to control lipid/protein diffusion within the membrane. Cortex remodeling during cell division is a result of myofilament-driven contractility of the cortical membrane-bound actin meshwork. Only little is known about the interaction between individual myofilaments and membrane-bound actin and the role of the membrane during actin cortex reorganization. We therefore developed minimal actin cortices, consisting of membrane bound actin and myosin motors, that allow performing well-defined quantitative experiments, which are difficult to realize in living cells. By directly visualizing the action of individual myofilaments on membrane-bound actin using TIRF microscopy, we found that myosins fragment and compact membrane-bound actin while processively moving along actin filaments. We propose a mechanism by which tension builds up between the ends of myofilaments, resulting in compressive stress exerted to single actin filaments, causing their buckling and breakage. Modeling of this mechanism revealed that indeed sufficient force can be generated by single myofilaments to buckle and break actin filaments. This mechanism of filament fragmentation and compaction may contribute to actin turnover and cortex reorganization during cytokinesis. To further test the impact of the actin meshwork on the lipid/protein diffusion behavior within the membrane we measured the lateral diffusion of lipids/proteins with FCS. We found that actin slows down the diffusion of lipids/proteins in a size dependent manner, pointing to a direct role of actin in controlling the mobility of lipids/proteins within the cell cortex.

FEBS EMBO

Manipulation of filamentous biomolecular systems with force-clamp optical tweezers

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Investigating the nanomechanical properties of cytoskeletal filaments is pivotal in understanding the exact mechanisms behind their structure and function. Usually cantilever-based (e.g., atomic force micrsocopy, AFM) or optical force-field-based (e.g., optical tweezers) devices are used to manipulate filamentous biomolecules and measure their characteristic force response upon stretch in constant pulling velocity experiments. Because in such experiments both molecular extension and force change simultaneously, non-linearly, and as a function of each other, it is often difficult to extract the thermodynamic and kinetic parameters of the force-dependent states and processes. Force-clamp experiments, in which force is kept constant by rapidly adjusting the molecular length in a feedback system, provide an easier access to the rates of force-dependent intramolecular processes and the life-time of intermediate states. We have implemented a force-clamp optical tweezers capable of rapid (ms response time) and large (μm displacements) adjustments of molecular length for exploring the nanomechanics of the giant muscle protein titin and the intermediate filament desmin.

Desmin forms the intermediate filament system of muscle cells where it plays important role in maintaining mechanical integrity and providing elasticity. The molecular details behind desmin's elasticity are far from being fully understood. In force-clamp optical tweezers experiments desmin filaments were gradually extendended while displaying length fluctuations. Stepwise increments in stretch forces up to ~100 pN revealed that desmin extends in a force-dependent manner resulting in an average contour length increment of 14.5 nm. The observed length increment is below the contour length of a coiled-coil desmin dimer (45 nm). Therefore, the incremental extension might be due to the force-driven axial lengthening of a coiled-coil domain or to sliding between staggered desmin dimers. Strikingly, transient shortening phases were also observed during force-clamp, suggesting that desmin is a highly dynamic structure capable of dynamic length adjustments under mechanical load.

Titin is a giant filamentous protein which, due to its sarcomeric arrangement, molecular structure and elasticity, determines the passive mechanical properties of muscle. At low, physiologically relevant passive forces (<30 pN/molecule) titin is thought to extend continuously at the expense

of reducing the configurational entropy of its tandem-immunoglobulin regions and PEVK domain as in a random polymer chain. Structural transitions, such as the unfolding of the component titin domains, are thought to begin only at forces well exceeding the physiological regime (typically >100 pN). By manipulating individual skeletal-muscle titin molecules with high force- and time-resolution optical tweezers operating either in constant-velocity or constant-force mode, we show that discrete, stepwise structural changes may occur at forces well below 30 pN. Strikingly, the transitions appear reversible on the physiologically relevant few-second time scales, indicating that they may play a role in rapid sarcomeric stress adaptation.
Force-clamp optical tweezers measurements thus allow us to gain insight into mechanically driven structural changes of filamentous biomolecular systems with high time and spatial resolution.

Optical nanoscopy and individual molecule localization methods towards multimodal imaging of the cell

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It is well known and established that, for the most popular imaging mode in optical microscopy, i.e. fluorescence, the diffraction barrier does no longer provide an unsurpassable limitation for resolution and localization accuracy. Furthermore, the terms “super resolution” and “optical nanoscopy”, coined earlier, have been implemented in real far field optical microscopes, nowadays available for everyone to use without extreme complexity. Here, we will discuss targeted and stochastic readout methods using both single and multiphoton excitation, in terms of resolution and localization precision accuracy. Individual molecule localization (IML) implemented within selective plane illumination microscopy (SPIM) will be addressed towards 3D super resolution imaging in thick biological samples. STED two-photon excitation microscopy will be discussed reporting about the possibility of using a single wavelength (SW) both for two-photon excitation and STED depletion by implementing a SW-2PE-STED microscope. A further topic will be related to the coupling of STED and Atomic Force Microscopy. So far, a variety of architectures will be outlined in regard to specific applications demanding for multimodal nanoscale investigations.

Single Fluorophore Imaging Reveals the Abundance, Distribution, Mobility and Oligomeric State of M_2 Muscarinic Acetylcholine Receptors in Live Cardiac Muscle

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M_2 muscarinic acetylcholine receptors are canonical GPCRs that modulate cardiac rhythm via regulation of the inward potassium current (through GIRK channels). To increase our understanding of M_2 receptor physiology we used Total Internal Reflection Fluorescence Microscopy to visualize individual receptors at the plasma membrane of transformed CHO^{M2} cells, a cardiac cell line (HL-1), primary cardiomyocytes and tissue slices from pre- and post-natal mice. Receptor expression levels between individual cells in dissociated cardiomyocytes and heart slices was highly variable and only 10% of murine cardiomyocytes expressed muscarinic receptors. M_2 receptors were evenly distributed across individual cells and their density in freshly isolated cardiomyocytes was ~1 receptor per μm^2, increasing at birth (to ~3 mm^{-2}) and decreasing 2-4 weeks after birth (back to ~1 mm^{-2}). M_2 receptors are primarily monomeric but can form reversible dimers. They diffused freely at the plasma membrane, moving approximately 4-times faster in heart slices than in cultured cardiomyocytes.
Knowledge of receptor density and mobility has allowed receptor collision rate to be modeled by Monte Carlo simulations. Our estimated encounter rate of 5-10 collisions per second, may explain the latency between acetylcholine application and GIRK channel opening.

Abstracts (talks)

Session IV - Molecular motors, cellular motility and related methods

Wednesday, 7 November

Role of dynein in endosome movement in living cells

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Microtubules act as tracks for long distance cargo transport within eukaryotic cells. This motility is of fundamental importance, and is carried out by microtubule motor proteins that move either towards the dynamic microtubule plus ends at the cell periphery, or towards their minus ends, which are usually located in the cell centre. Cytoplasmic dynein is the major minus end-directed motor protein and transports many different cargoes. Dynein is crucial for cell division and cell migration, and also plays vital roles in the organisation and function of the secretory and endocytic pathways. These functions are particularly important in neurons, where slight alterations in dynein activity lead to neuronal degeneration and motor neuron disease.

We have analysed dynein's role in moving endocytic organelles, which are responsible for the uptake and degradation of a range of signalling and nutrient molecules. We have focussed on the movement of early endosomes, labelled using green fluorescent protein-tagged Rab5. We use automated particle tracking software (PolyParticleTracker) that generates a spatial resolution of ~10 nm, combined with a suite of MatLab analysis algorithms for global analysis of all particles and detailed analysis of endosomes undergoing translocations of >2 µm. We have characterised dynein-driven endosome movement and find that few (or single) dyneins are able to move endosomes at fast rates. To generate biologically meaningful data, this approach requires: 1) careful control of the level of expression of GFP-Rab5; 2) imaging at sufficiently high frame rates (28 fps) to track fast-moving particles accurately; 3) minimization of photobleaching. Having achieved this, quantitative analysis of particle movement will allow us to determine the contribution of different dynein subunits and accessory factors in dynein motor function.

Elucidation of the mechanisms of action of DNA helicases and other motors traveling along nucleic acid strands

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All living organisms possess a large set of motor proteins that drive processes enabling the maintenance, expression and repair of genetic information. These enzymes are able to specifically modify the structures of DNA and RNA molecules, based on ATP-driven unidirectional translocation along nucleic acid strands. Similarly to cytoskeletal motors, the functional properties of nucleic acid motors are flexibly and specifically shaped by their diverse physiological functions. A variety of ensemble and single-molecule biophysical methods have recently been developed for the quantitative determination of key functional parameters of these enzymes, including mechanochemical coupling, processivity, step size as well as pausing and strand switching behavior. In the presentation, an overview will be provided of the utility and limitations of these methods, issues regarding data interpretation, and recent insights gained into the molecular mechanisms of DNA helicases that utilize ATP hydrolysis to translocate along and separate the strands of multistranded nucleic acid structures.

FEBS EMBO

Molecular basis of ubiquitin-mediated regulation of Myosin VI in cell migration

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Myosins are actin-dependent molecular motors that use the energy of ATP hydrolysis to move along actin filaments. Myosin VI has unique functions in the cell, because it is the only myosin shown to move toward the minus end of actin filaments, in the opposite direction of all other myosins. Given the believed polarity of actin filaments in the cell, with their plus end toward the membrane and the minus end projecting in the cytoplasm, such localizations are consistent with putative roles of Myosin VI in secretion, endocytosis and cell migration. In our lab, we discovered the presence of two ubiquitin binding domains (UBDs) in the C-terminal portion of Myosin VI, making a strong case for involvement of Ub-signaling in the control system of the Myosin VI motor functions. To obtain insight in the pathways regulated by the ubiquitin binding capacity of Myosin VI we performed interaction studies with the isolated UBDs as bait. From this approach we identified a high number of proteins involved in cell migration and adhesion (e.g. KIF2A, Dlg5, etc.). In line with published roles of Myosin VI in migration/invasion, growth factor signaling and endocytosis we are seeking to unravel molecular details in Myosin VI directed processes with the focus on its ubiquitin binding capacity.

Molecular Level Understanding of Cardiomyopathy Mutations on the Regulated Actomyosin Interaction

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In the developed world, cardiovascular disorders are a leading cause of morbidity and mortality. Familial hypertrophic and dilated cardiomyopathies (HCM and DCM) are among the most frequently-occurring inherited cardiac diseases. These diseases and others have been linked to different mutations in the genes expressing the fundamental force generating machinery of the cardiac muscle, namely myosin and the regulated thin filament (RTF). Hence a detailed molecular level study of the effect of these mutations is critical in understanding the biochemical basis of these diseases. Using recombinant human β-cardiac myosin motor domain and thin filament proteins, we are now able to perform detailed functional analysis of the regulated acto-myosin interaction in both bulk assays and on a single molecule level. Our current results suggest that HCM and DCM mutations in cardiac myosin affect the acto-myosin kinetics and the underlying force producing mechanism of the motor whereas those in the RTF alter Ca^{2+} sensitivity. Preliminary enzyme kinetic measurements and single molecule force spectroscopy reveal different mechanisms of action for two different HCM mutations within the cardiac myosin motor domain (R403Q and R453C). Two troponin T thin filament mutations, however, show altered Ca^{2+} sensitivity (E163K and the R205Q). We are currently examining the crosstalk between several myosin mutants with the RTF and vice-versa. Such information will not only help gain insight into the biophysics of cardiomyopathy but will also be invaluable to an improved clinical understanding and therapeutic treatment of these diseases.

FEBS EMBO

Dynamics of nuclear actin: from form to function

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The actin cytoskeleton has an essential role in several important cell biological processes, including cell motility and membrane dynamics. These cytoplasmic functions of actin are well characterized, but the role of actin in the nucleus has been less obvious. Recent studies have, however, identified actin as an essential component of several nuclear complexes, including basal transcription machinery and chromatin remodelers. Moreover, nuclear actin can also function as a signal responsive regulator of specific transcription factors. Nevertheless, the mechanisms by which actin functions in the nucleus are still largely unclear. My lab uses many different methods, from advanced microscopy methods and RNAi screening to molecular biology and fly genetics to study how actin and its binding partners regulate genome organization and expression. I will discuss our recent results on how nuclear actin is regulated, and how we use this information to dissect the functional significance of actin in the nucleus.

Directed actin self assembly and contractility

Laurent Blanchoin
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The organization of actin filaments into higher-ordered structures governs eukaryotic cell shape and movement. Global actin network size and architecture is maintained in a dynamic steady-state through regulated assembly and disassembly. We have developed a micropatterning method that enables the spatial control of actin nucleation sites for in vitro assays (Reymann, Nat Mat, 2010). These actin templates were used to evaluate the response of oriented actin structures to myosin-induced contractility. We determine that myosins selectively contract and disassemble anti-parallel actin structures while parallel actin bundles remain unaffected. In addition, the local distribution of nucleation sites and the resulting orientation of actin filaments regulate the scalability of the contraction process. This "orientation selection" mechanism for selective contraction and disassembly reveals how the dynamics of the cellular actin cytoskeleton is spatially controlled by actomyosin contractility. Further application of the micropatterning method will be presented in particular recent data on the reconstitution of a lamellipodium-type of actin organization.

Shape transition of liposomes induced by actin bundles

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Cytoskeletal networks, composed of microtubules, F-actin and intermediate filaments, support cell shape and are involved in numerous cellular functions, including cell motility and cell division. The cell membrane separates the intracellular from the extracellular environment while intimately interacting with the cytoskeleton to determine cell shape. Bundles of F-actin formed with fascin are for instance involved in the formation of thin filopodial membrane protrusions. To understand how bundles deform the cell membrane, we built a cell-free model system composed of cell-sized liposomes encapsulating actin, fascin, and active myosin II motors. We observed that actin/fascin bundles can dramatically deform liposomes, forming long and thin protrusions. By tuning the osmolarity difference between the internal and external solution, we can tune the liposome shape in good agreement with theoretical models of liposomes deformed by growing rods. When we added myosin II motors, we observed a variety of liposome shapes, which indicated reorganizations of actin bundles by myosin motors. In conclusion, our cell-free model system gives valuable new insights into the physical basis of cytoskeletal-based membrane deformation.

Tropomyosin and actin-dependent protein trafficking to focal adhesions

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Actin filament dynamics are critical for protein trafficking. There is increasing appreciation that the tropomyosin family of actin-associating proteins can impart isoform-specific functions that result in actin filament specialization. However, the role that tropomyosins play in actin-dependent protein trafficking in mammalian cells is currently unknown. Because the tropomyosin isoform Tm5NM1 stabilizes actin filaments and concomitantly reduces phosphorylation of Src kinase substrates at focal adhesions, we hypothesized that Tm5NM1 might cause decreased actin-dependent translocation of Src kinase to focal adhesions. Use of a membrane targeted Src kinase activity reporter confirmed spatially restricted Src kinase activity in cells expressing high level exogenous Tm5NM1. Consistent with a defect in protein trafficking, the kinetics of Src exchange at focal adhesions were reduced. Further confirming that the fault lay in the kinetics of protein trafficking, the GFP-tagged Src Y527F mutant that constitutively traffics to focal adhesions also exhibited reduced molecular exchange at focal adhesions, while paxillin exchange was unaffected. Investigation of the mechanism revealed dys-regulation of recycling endosomes. High level exogenous Tm5NM1 expression led to dispersal of Rab11, VAMP3 and Src positive vesicles throughout the cytoplasm and the formation of enlarged vesicles due to vesicle convergence. Together our data suggest that tropomyosins may regulate protein trafficking to discrete sub-cellular compartments.

The function of the kinetochores in microtubule attachment and checkpoint function

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Preventing chromosome missegregation during cell division is essential to en sure genomic stability and biological organization. Eukaryotic cells have a surveillance mechanism that monitors chromosome interaction with the spindle and prevents mitotic exit when errors are detected. The Spindle Assembly Checkpoint (SAC) specifically detects absence or abnormal microtubule-kinetochore interaction and produces an inhibitor of the Anaphase Promoting Complex (APC), which cannot promote the degradation of cohesins delaying anaphase onset. There are a number of proteins involved in this signal transduction pathway including Polo, Aurora B, Mps1, Mad2 and BubR1, and how they work in concert to produce a coherent output that inhibits the APC is still poorly understood. Using RNAi, chemical inhibitors and time-lapse confocal microscopy, we have set out to unravel how these different components of the SAC are involved in the sensing and production of the APC inhibitory complex. We find that Polo and Aurora B are involved at the early stage of the pathway giving rise to two separate branches. One involving the protein kinase Mps1 and the eventual phosphorylation of BubR1 and the other the kinetochore localization of Mad2 through the RZZ complex and its binding to Cdc20. The two branches of this pathway meet when the Mad2-Cdc20 complex interacts with Bubr1 producing what appears to be the final and more powerful inhibitor BubR-Cdc20. Our results allow for the first time to integrate all the different components into a coherent sequence revealing the role of Polo in this process.

S100A4 binds to the extended and compact forms of non-muscle myosin IIA in A431 cells undergoing epithelial-mesenchymal transition

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S100A4 protein is a mesenchymal marker that is expressed in several forms of human cancer and implicated in the regulation of cell motility and epithelial-mesenchymal transition. *In vitro*, S100A4 interacts with several molecular targets including non-muscle myosin IIA, a major actin-associated motor protein implicated in cell motility and cytokinesis. Although the non-muscle myosin IIA - S100A4 interaction was proposed to be critical for enhanced motility of mesenchymal cells, it had not been confirmed *in vivo*. Here, we studied the expression and function of S100A4 in A431 cells, in which the epithelial-mesenchymal transition was induced by a transcription factor SIP1. We show that induction of S100A4 expression in this cell model promoted increased cell motility. Using transmission electron microscopy, we demonstrate that S100A4 and non-muscle myosin IIA interact *in vivo*. Importantly, our approach allowed us to discriminate between different conformations of non-muscle myosin within cells. The presence of two major monomeric myosin forms in a solution, compact 10S and extended 6S has been known for many years. However, the *in vivo* relevance of these conformations remains debated. Here we show that cytosol contains 10S and 6S forms of non-muscle myosin IIA, and both these forms interact with S100A4.

Characterization of dynein by single-molecule investigations in vivo

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Cytoplasmic dynein is a motor protein that exerts force on microtubules and in doing so, drives a myriad of intracellular activities from mitotic spindle positioning to chromosome movements in meiotic prophase. To exert force on microtubules, dynein needs anchorage, which is typically found at the cell cortex. The key question is how dynein finds the sites where a microtubule and an anchor protein are close enough for dynein to link them and subsequently pull on the microtubule. Here we directly observe single dyneins in fission yeast and show that they attach in two steps, first from the cytoplasm to a microtubule and then also to cortical anchors. Upon attachment to the microtubule, dynein moves in a diffusive manner along the microtubule. This is a surprising behavior for a minus end directed motor and may help dynein to find cortical anchors. Our work demonstrates that dynein performs three-dimensional diffusion in the cytoplasm and one-dimensional diffusion along the microtubule to find sites where it can exert pulling force on the microtubule.

How does kinesin-1 enter into 'energy saving mode'?

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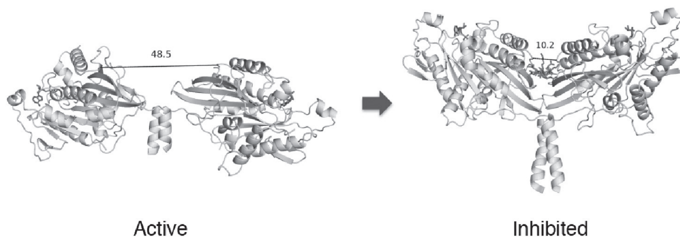
Kinesin-1 (conventional kinesin) is a molecular motor that uses energy from ATP hydrolysis to move cargos processively towards the plus end of microtubules (MTs). When not transporting cargo, kinesin is autoinhibited to prevent squandering of ATP. Although it is widely accepted that the tail domain binds to the motor domain to keep it in a folded autoinhibited state, the molecular mechanism remains unclear and several autoinhibitory mechanisms have been proposed, including steric and allosteric inhibition. An unexpected recent discovery revealed that only one of the two identical tail domains in a heavy chain dimer binds to the motor domain dimer to inhibit ADP release from both motor domains.
We report the crystal structure of a motor domain dimer in complex with its tail domain at 2.2 Å and compare it with a structure of the motor domain alone at 2.7 Å. Based on the crystal structures and supporting biophysical experiments we propose an autoinhibition mechanism for kinesin-1.

Figure: Structure of the kinesin-1 dimer in its active and inactive conformation



Abstracts (posters)

Poster session I

Sunday, 4 November

P1-01

Peripheral neuropathy mutants stabilize microtubules and reveal a novel role for HSPB1 in microtubule nucleation

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The small heat shock protein HSPB1 is a molecular chaperone involved in several cellular processes and human diseases. We have previously demonstrated that a number of mutations in HSPB1, that are causative for inherited peripheral neuropathy, increase chaperone activity and cause increased binding to their client proteins. HSPB1 interacts with the cytoskeleton by binding to tubulin and to the lattice of microtubules (MTs). Mutations in HSPB1 drastically increase binding to MTs and stabilize the MT network: cells expressing mutant HSPB1 are more resistant to cold depolymerization and show a faster recovery after nocodazole depolymerization. Furthermore, in individual MTs, rescue and catastrophe rates are decreased in cells expressing mutant HSPB1.
In addition, we show by a series of experiments in cells and *in vitro* that for wild type HSPB1 the interaction with MTs occurs mostly in small, *de novo* formed MTs during the early phases of polymerization. Moreover, in cells treated with nocodazole, HSPB1 accumulates at sites of non-centrosomal MT nucleation and is absent from the centrosomal region. Knock down or overexpression of HSPB1 has a drastic effect on MT repolymerization, and also *in vitro* HSPB1 increases the number of nucleated MTs, together demonstrating that HSPB1 facilitates non-centrosomal MT formation.
Taken together, we identified HSPB1 as a player involved in non-centrosomal MT nucleation. The misregulation of this function in peripheral neuropathy causing HSPB1 mutants disturbs MT dynamics, which might explain the neuron-specific effects of the mutations.

P1-02

Characterization of the biochemical properties and biological function of formin homology domains of Drosophila DAAM

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We characterised the properties of Drosophila melanogaster DAAM FH2 and DAAM FH1FH2 fragments and their interactions with actin and profilin using various biophysical methods and in vivo experiments. The results show that, while the DAAM FH2 fragment does not have any conspicuous effect on actin assembly in vivo, in cells expressing the DAAM FH1FH2 fragment a profilin-dependent increase in the formation of actin structures is observed. The trachea specific expression of DAAM-FH1FH2 also induces phenotypic effects leading to the collapse of the tracheal tube and lethality in the larval stages.

In vitro both DAAM fragments catalyze actin nucleation, but severely decrease both the elongation and depolymerisation rate of the filaments. Profilin acts as a molecular switch in DAAM function. DAAM FH1FH2, remaining bound to barbed ends drives processive assembly of profilin-actin, while DAAM FH2 forms an abortive complex with barbed ends that does not support profilin-actin assembly. Both DAAM fragments also bind to the sides of the actin filaments and induce actin bundling. These observations show that the Drosophila melanogaster DAAM formin represents an extreme class of barbed end regulators gated by profilin.

P1-03

Interaction of lysophosphatidic acid with PH domains

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The aim of this study is to understand the mechanistic details of some protein - lipid interactions in signaling. Several membrane-associating proteins contain domains which directly interact with lipids, in our case plecktsrin homology (PH) domains. Our attention turned to lysophospholipid mediators, especially lysophosphatidic acid (LPA), as it previously has been reported that LPA binds to gelsolin interfering with its inositol polyphosphate binding.
In the course of our work we aimed at characterizing the interaction between several PH domains and LPA using biochemical and biophysical methods. After multiple sequence alignment we identified a PH domain-like sequence of gelsolin. After expression of this PH domain and the intact protein, fluorescence and CD spectroscopy, QCM, as well as ITC measurements indicated that LPA can bind to the PH domain of gelsolin over its critical micelle concentration. This interaction is specific to LPA. Based on our findings we have looked for other PH domain containing proteins, Vav2, Akt1 and Grp1, seeking for similar interactions. We expressed the PH domains and characterized their interaction with LPA. We also carried out live-cell microscopy with GFP-tagged PH domains of Akt1 and Grp1. We studied the changes in spatiotemporal localization of these GFP-PH domains by modifying the intracellular level of LPA.
Our results show that LPA can act a second messenger-like fashion on membrane surfaces via recruiting protein domains. LPA, in a clustered form mimicked by micelles in vitro, can bind to at least some PH domains, revealing new aspects of lysophospholipid-protein interactions.

P1-04

The effect of Toxofilin on the structure of monomeric actin

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Actin is one of the main components of the intracellular cytoskeleton. It plays an essential role in the cell motility, intracellular transport processes and cytokinesis as well.
Toxoplasma gondii is an intracellular parasite, which can utilise the actin cytoskeleton of the host cells for their own purposes. One of the expressed proteins of *T. gondii* is the 27 kDa-sized toxofilin. The long protein is a monomeric actin-binding protein involved in the host invasion.
In our work we studied the effect of the actin-binding site of toxofilin$_{69-196}$ on the G-actin. We determined the affinity of toxofilin to the actin monomer. The flourescence of the actin bound ε-ATP was quenched with acrylamide in the presence or absence of toxofilin. In the presence of toxofilin the accessibility of the bound ε-ATP decreased, which indicates that the nucleotide binding cleft is shifted to a more closed conformational state.
The results of the completed experiments can help us to understand in more details what kind of cytoskeletal changes can be caused in the host cell during the invasion of the host cells by intracellular parasites.

P1-05

Investigation of the regulation of Tes, an actin cytoskeleton associated focal adhesion protein, by tyrosine phosphorylation

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Tes is a LIM domain protein which has been described as a potential tumor suppressor, as well as an actor in the regulation of the actin cytoskeleton through its interaction with zyxin. Tes activity appears to be highly regulated in cells. The cytoplasmic, "closed" form of Tes sequesters Mena/VASP while the "open" form of Tes is recruited by zyxin into focal adhesions where both proteins participate in the regulation of the actin cytoskeleton. However, the mechanisms which regulate Tes activities remain unknown. Here, we assessed whether the activities of Tes may be regulated through phosphorylation of tyrosine residues located in its first LIM domain. We investigated how amino acid substitutions of putative tyrosine phosphorylation sites may affect targeting of Tes to focal adhesions. Taking into account structural features of Tes LIM domains, we analysed how distinct substitutions (Y/A, Y/F, Y/E) affect Tes activity. The structure-function relationship of the analysed substitutions will be discussed.

P1-06

Actin controls the I-BAR-membrane interaction

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The Inverse BAR (I-BAR) domain is the N-terminal 250 amino acid of the IRSp53 protein that induce negative membrane curvature both *in vitro* and in cells. Generation of membrane curvature by I-BAR proteins often works together with actin dynamics. I-BAR shares its function between actin bundling and membrane binding but it is still obscured what molecular mechanisms are responsible for these functions. The aim of our project is to investigate the detailed membrane binding properties of the I-BAR of IRSp53 and its relations to the actin cytoskeleton. *In vitro* FRET experiments and fluorescence quenching studies were carried out between the I-BAR and liposomes made up from different lipid constructs. We have found that the I-BAR has preference to bind to the negatively charged lipids (K_d = 1-2 μM) however it can also bind to the uncharged lipids. The fluorescence quenching studies reflected that the accessibility of the I-BAR surface was higher toward the negatively charged lipids than for the uncharged ones. The I-BAR membrane interaction is controlled by the polimerization state of actin where filamentous actin stabilizes while the globular actin disrupts their interaction. The I-BAR domain influences the actin polymerization, the affect depends on the I-BAR concentration. Our results suggest that the I-BAR-actin interaction may have a crucial role in filopodia formation and regulation.

P1-07

The Drosophila formin dDAAM is required for axon growth in the adult brain

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In the developing nervous system growth cones have an essential role in guiding axons to their correct target sites. Directed growth cone motility in response to extracellular cues is produced by the coordinated regulation of peripheral F-actin and central microtubule networks. Key regulators of actin dynamics are the so called nucleation factors, such as formins, which promote actin assembly by associating with the fast-growing end of actin filaments, and facilitate the formation of unbranched filaments.

We have previously examined the function of the *Drosophila* formin dDAAM in the embryonic CNS, where this protein shows a strong accumulation in the developing neurites. Genetic analysis suggested that dDAAM plays a major role in the regulation of axonal growth by promoting filopodia formation in the growth cone. We noticed that *dDAAM* exhibits a strong expression in certain regions of the larval and adult brain as well. Specifically, in the developing mushroom body dDAAM is highly enriched in the newly born axons suggesting that dDAAM might be a general regulator of *Drosophila* axonal development. Consistently, by loss of function analysis we detected axonal projection defects in the mushroom body. To identify proteins that may act together with dDAAM in the regulation of axonal growth, we carried out a genetic interaction analysis. We demonstrated that dDAAM interacts with *Ena* and *profilin.* Moreover, we identified Rac as the most likely activator of dDAAM in the developing nervous system.

P1-08

Development of anti-tropomyosin compounds which target the actin cytoskeleton

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The actin cytoskeleton in animals is composed of two broad classes of actin filaments; those which contain tropomyosin and those which do not. There are over 40 tropomyosin isoforms. The isoforms are spatially segregated in cells and define the functional capacity of individual actin filaments. Currently available drugs which target the actin cytoskeleton have a poor ability to discriminate between different types of actin filaments. The availability of drugs which target actin filaments based on their tropomyosin isoform composition would provide powerful tools to manipulate the actin cytoskeleton. In addition, such drugs would provide the opportunity for clinical development as chemotherapeutics. In this study we have demonstrated that it is indeed possible to disrupt specific actin filament populations by targeting specific tropomyosin isoforms. Multiple classes of anti-tropomyosin compounds has been developed which preferentially disrupt the actin cytoskeleton. These compounds have the ability to discriminate between muscle and cytoskeletal tropomyosins and impair both tumour cell motility and viability. Our lead compound, TR100 is effective *in vitro* and *in vivo* in reducing tumour cell growth in neuroblastoma and melanoma models. Importantly, it shows no adverse impact on cardiac structure and function which is the major side effect of current anti-actin drugs. This proof of principle study demonstrates that it is possible to target specific actin filament populations based on their tropomyosin isoform composition. These drugs provide the ability to test the functional roles of subpopulations of actin filaments both in vitro and in vivo.

P1-09

Effect of cofilin on the polymerization of actin

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It is widely accepted that ADF/cofilin can enhance actin filament turnover by promoting the disassembly of actin filaments. It can be achieved via increasing the off-rate of actin monomers at the pointed end of filaments and stochastic fragmentation of the actin filaments.
Beside the role of cofilin in actin filament disruption, growing body of evidence supports a direct role of cofilin in the actin filament assembly as well. In vitro biochemical assays revealed that cofilin can enhance the actin polymerization in a concentration dependent manner.
In this current work the impact of cofilin on actin filament assembly was assessed by using fluorescence spectroscopy methods and TIRF microscopy. Bulk polymerization assays revealed that low concentration of cofilin (cofilin:actin<2:1) enhances the polymerisation of actin, while high concentration of that (cofilin:actin>2:1) impairs the assembly of actin filaments. The possible molecular explanation behind that can be the stabilization of actin dimers by cofilin at low concentration and the appearance of actin monomers with double bound cofilin at high cofilin concentration. The latter population is not able to assemble into filaments explaining the impaired polymerization ability of actin in this case. This hypothesis presumes that two distinct cofilin binding site exist on actin monomer. This assumption was tested by calculating the energy transfer between fluorescently labelled actin and cofilin. The result of the binding assay was correlated with the observation of bulk polymerization assays. The effect of cofilin on actin polymerization was investigated by TIRF microscopy as well.

P1-10

Binding Properties of the Myosin 16b Ankyrin Domain

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The unconventional myosin 16b is a member of the myosin motor protein superfamily. It can be found both in the nucleus and in the cytoplasm, and it may have a role in the regulation of the cell cycle and the cell proliferation. It consist of an ATP- and actin-binding motor domain, a short neck region containing the calmodulin light chain specific consesus IQ-motif, a tail domain and as a unique feature: an N-terminal domain preceding the motor: a nearly 400 amino acid long ankyrin repeat containing domain. The ankyrin repeats contain highly conserved helical structures and between them variable loops, which makes possible the proteins containing ankyrin repeats to take part in various protein-protein interactions eg. connecting the actin cytosceleton to the membrane proteins.
In our experiments we observed the recombinant ankyrin domain of the myosin 16b, expressed in *E. coli* and purified via affinity chromatography and gelfiltration. The interaction between the ankyrin domain and actin was examined with cosedimentation and polimerization assay.
During the cosedimentation we found that the ankyrin domain of the myosin 16b binds to actin with a K_d approximately 7 µM and a saturation ratio 1:3. Ankyrin does not influence the actin polimerization significantly. Further experiments are necessary to elucidate the proper function of the ankyrin domain, wether it can influence the motor function or the regulation of the myosin 16b, and wether it can facilitate processive movement or it has only anchoring function.

P1-11

Effect of neuronal tropomyosin isoforms on actin dynamics

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Tropomyosins (TM) are elongated, actin-binding dimer proteins that polymerise at their N- and C-termini and attach to the actin filament along its longitudinal groove. In mammals more than 25 products from 4 genes have been described. The significance and division of labour of the diverse tropomyosin isoforms is poorly known yet. It is partially due to the limited research on the fundamental biophysico-chemical properties of the individual isoforms. As in the most cell types, also in neurons several tropomyosin isoforms can be found, whose localisation is different and is under a strict spatial and developmental regulation.
Three tropomyosin proteins specific to neural cells were identified: TMBr-1, TMBr-2 and TMBr-3. During the embryonic development the TM5NM2 variant is present in the axon of the mammalian neurons that later in the foetal period is replaced by the TMBr-3 isoform within a short time. The TMBr-1 protein appears also in the last third of the intrauterine development. The strictly regulated change of the expression patterns may be related to what role the individual TM variants in the cell play to orchestrate the microfilament dynamics and the neural process growth.
We aimed to produce native tag-free proteins from coding sequences cloned into pET28a vector and to investigate the effects of TMBr-1 and TMBr-3 isoforms on the kinetic parameters of the actin filament assembly and on its stability against depolymerising agents. In our experiments we applied cosedimentation assay, fluorimetric and stopped-flow measurements.

P1-12

The effect of mouse twinfilin-1 on the structure and dynamics of actin

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The actin cytoskeleton of eukaryotic cells plays a key role in many processes. The structure and dynamics of the cytoskeleton are regulated by a large number of proteins that interact with monomeric and/or filamentous actin. Twinfilin is a 37-40 kDa actin-binding protein composed of two ADF-homologous domains connected by a short linker.
The effects of the mouse twinfilin-1 (TWF1) on the monomeric actin were studied by biophysical techniques. The affinity of TWF1 to the ATP-actin monomer was determined by fluorescence anisotropy measurements (K_D = 0.015μM). The fluorescence of the actin bound ε-ATP was quenched by acrylamide in the presence and absence of TWF1. Twinfilin reduced the accessibility of the bound ε-ATP which indicates that the nucleotide binding cleft shifted toward a closed conformational state. Stopped-flow experiments confirmed that the kinetics of nucleotide exchange of actin decreased in the presence of TWF1. The thermodynamic properties of actin monomer were also investigated with differential scanning calorimetry in the presence of TWF1. The results indicate that TWF1 stabilized the structure of the monomeric actin.
These results can help to understand in more details the regulation of G- and F-actin by actin binding proteins.

P1-13

Signal transduction pathways leading to L-plastin Ser5 phosphorylation in breast cancer cells

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Tumor cell migration and invasion are greatly dependent on actin cytoskeleton modulation, orchestrated by a large variety of actin-binding proteins. Among these, the leukocyte-specific actin crosslinker L-plastin is ectopically expressed in several solid human cancers and is often considered as a metastatic marker.
L-plastin has been reported to be phosphorylated *in vitro* and *in vivo* with residue serine 5 (Ser5) being the major phosphorylation site. L-plastin Ser5 phosphorylation increases its F-actin binding and -bundling activity and regulates actin turn-over, and, in cancer cells, Ser5 phosphorylation is required for cell invasion. In addition, we have provided evidence that L-plastin associates with a protein complex comprising the Src-kinase substrate cortactin, a regulator of actin dynamics.
Our recent results highlight a correlation between the Ser5 L-plastin phosphorylation level and the invasive capacity of cancer cells as we observed a high baseline L-plastin phosphorylation in the invasive breast cancer cell lines 1001, BT-20 and MDA-MB-435S in contrast with regulated L-plastin phosphorylation in the non-invasive breast cancer cell lines MCF7 and SK-BR-3. Distinct protein kinases seem to be responsible for L-plastin phosphorylation depending on the cell type and environment. Indeed, PKA has been shown to be able to phosphorylate L-plastin *in vivo* and *in vitro*. Interestingly, our results indicate that L-plastin Ser5 phosphorylation appears to be also mediated by PKCδ and/or a PKCδ-regulated kinase and they suggest a cross-talk between PKA- and PKCδ-dependent pathways in L-plastin phosphorylation.
Our study aims to pave the way for the establishment of L-plastin phosphorylation as a molecular indicator for deregulated signalling associated to carcinoma progression.

P1-14

Identification of the molecular interaction partners of the formin dDAAM

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The formin proteins are an important and evolutionarily well conserved class of actin binding proteins with essential biological functions, including cell division, cell migration and organelle transport. In these processes the best understood molecular role of formins is to promote the nucleation and elongation of unbranched actin filaments, although some formins have also been implicated in the regulation of microtubules. We have previously shown that the single Drosophila DAAM ortholog, dDAAM, is involved in multiple aspects of trachea development and axonal growth regulation, however the molecular mechanisms underlying these morphogenetic functions remain to be uncovered. To gain a better understanding of the molecular functions of dDAAM, we aim to identify the protein interaction partners of dDAAM with biochemical and genetic methods. The biochemical interaction partners will be identified by affinity chromatography. To this end, we will use a dDAAM-Flag fusion protein by tagging the dDAAM gene *in situ* by site specific mutagenesis. To complement the biochemical approach, interaction partners will also be identified by a genetic interaction screen using the hypomorphic $dDAAM^{EX1}$ allele. The results of these efforts will be presented on my poster.

P1-15

The role of Drosophila DAAM in the development of the Indirect Flight Muscle

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In most cells the two major actin assembly factors are the formins and the Arp2/3 complex. Formins are producing long straight actin filaments, whereas the Arp2/3 complex promotes the formation of a branched actin network. Because the unbranched straight actin filament is the major form in striated muscle cells, it is possible that a formin family protein serves as the key regulator of actin dynamics in myofibrils. Recently, we found that the *Drosophila* formin DAAM (*dDAAM*) is highly enriched in the Indirect Flight Muscle (IFM) of the flies. Subsequently, with the use of muscle functional tests, immunohistochemistry and biophysical assays, we demonstrated that dDAAM plays role in thin filament formation and sarcomere assembly. The *dDAAM* loss of function mutants exhibit sarcomere length reduction, and in more extreme cases, severe sarcomere assembly defects. In addition, the *dDAAM* mutants exhibit a strong dominant genetic interaction with that of $Act88F^{km88}$, an IFM specific actin null mutant, and with that of the muscle specific *myosin* mutant.
All together, our findings suggested that sarcomeric actin assembly, and thus sarcomere organization in *Drosophila* is critically dependent on the formin dDAAM. Interestingly, the subsarcomeric localization of the dDAAM protein in Drosophila larval muscles and the localization of the mDaam1 protein in mouse muscles is very similar to each other, suggesting an evolutionary conservation of the DAAM muscle function. Based on our data, we propose a model for sarcomeric thin filament elongation.

P1-16

Actin-Binding WH2 Repeats in VopF, a Vibrio cholerae Effector Protein

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WASP Homology 2 (WH2) domains are intrinsically disordered actin binding modules of 30-40 residues, functionally evolved from the b-thymosin peptides that are present in a large number of proteins involved in motility processes. Their multiple functions in the regulation of actin assembly include G-actin sequestration, regulation of barbed end assembly and disassembly dynamics, filament nucleation and severing.
Bacterial and viral pathogens often target or use WH2 domains to harness the host actin cytoskeleton. For instance WASP proteins are activated by *Shigella* protein IcsA, or FBP11 of vaccinia virus, or effector EspFU of EHEC, or mimicked by *Listeria* ActA. *Vibrio cholerae* and *Vibrio parahaemolyticus* inject WH2 repeat-proteins VopF and VopL using a type III secretion system.
VopF is a 530 amino acids protein containing a N-terminal secretion and translocation domain, 2 proline rich regions, 3 WH2 followed by a C-terminal dimerization domain (VCD).
We set out to understand the mechanism of VopF mediated actin functions like nucleation, actin binding, sequestration and severing. We have characterized biochemical properties of individual and various WH2 repeats of VopF in monomeric and dimeric states. We show that individual WH2s only sequester G actin; WH2 domains facilitate the nucleation activity of the VCD; the 3 consecutives WH2s are required for severing; VopF remains bound to barbed ends following severing, preventing reannealing.

P1-17

Characterization of the function of the spectraplakin BPAG1 in skeletal muscle cells

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BPAG1 is a spectraplakin encoded by the dystonin gene. Alternative splicing of the dystonin transcripts results in several BPAG1 isoforms. BPAG1-a and BPAG1-b isoforms are expressed in myoblasts while BPAG1-b is the only isoform detected at the protein level in differentiated skeletal muscle. In muscle tissue, BPAG1-b is localized at the Z-disc and sarcolemmal regions. *dt* mice, carrying a disrupted dystonin gene, exhibit an intrinsic muscle weakness and present an altered skeletal muscle cytoarchitecture. BPAG1-a/b (600 and 800 kD) possess several structural domains among which are an actin-binding domain and a microtubule–binding domain. BPAG1-a/b are known to directly associate with p150^{Glued} subunit of dynactin, which mediates dynein-dependent retrograde transport of membrane organelles along microtubules. It was previously shown that disruption of BPAG1-a interaction with dynactin resulted in defected vesicular retrograde transport in neuronal axons. Our immunofluorescence studies in myoblasts showed that BPAG1-a/b are associated with microtubule plus ends and also have an asymmetric dot-like pattern in the cytoplasm. Study of the BPAG1-a/b knockdown effect on muscle cell migration, elongation, adhesion and cell spreading revealed that these proteins are necessary for myoblast migration, but not for the other processes. BPAG1-a/b knockdown had no effect on actin and microtubule network organization in myoblasts. We are currently investigating whether BPAG1-a/b have a role in vesicular transport, a critical process for cell migration.

P1-18

Studying the relationship between plant Golgi bodies and the cytoskeleton: "It's complicated"

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The Golgi apparatus is one of the main components of the secretory pathway and is involved in protein glycosylation, processing and sorting to different destinations. It is structured as a polarised stack of flattened membrane cisternae Fluorescent labelling with Golgi marker proteins fused to GFP or mRFP reveals that the plant Golgi apparatus is polydispersed and moving on the actin cytoskeleton (Boevink et al., 1998). While the relationship between Golgi and actin filaments has been extensively described, not much is known about the role of microtubules. Work by Crowell et al (2009) demonstrates that in *Arabidopsis* the insertion of the Cellulose Synthase Complex (CSC) into the plasma membrane is concomitant to Golgi bodies pausing on microtubules.
In the first year of this project, different approaches were used to study the role of actin and microtubules in Golgi movement in plant cells. Appropriate fluorescent marker proteins were tested to label the cytoskeleton and Golgi bodies in tobacco and *Arabidopsis* epidermal leaf cells; putative areas of interaction were then identified using confocal laser scanning microscopy. Golgi dynamics were studied in cells treated with microtubule or actin filament depolymerising agents, such as latrunculin B and oryzalin, and the time course of the depolymerization was recorded. Optical trapping experiments were performed on plants expressing green fluorescent markers to label actin and microtubule filaments and Golgi bodies. Initial data revealed that the expression of cytoskeleton markers affected Golgi movement. Treatment with depolymerizing chemicals showed the different roles of microtubules and actin filaments in Golgi dynamics.

P1-19

Multifarious functions of TPPP/p25 on microtubule dynamics and organization

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Microtubules are major structural components in cells. Their dynamics and organization are central to their biological functions. Here I examined the effect of a new oligodendrocyte specific MAP, TPPP/p25, on the dynamics and organization of microtubules in order to identify its potential physiological role. Data obtained *in vitro* and with different cell models expressing TPPP/p25 either endo- or exogenously revealed that TPPP/p25 influences i) microtubule organization *via* its microtubule bundling activity; ii) microtubule dynamics both *via* its bundling activity enhancing microtubule stability and *via* its regulatory role on microtubule growth velocity due to inhibition of the activity of the tubulin deacetylase enzyme: HDAC6 iii) the acetylation level of microtubules *via* its inhibitory role on the activity of both tubulin deacetylase enzymes: HDAC6 and SIRT2; iv) cell motility. Finally, the role of TPPP/p25 is essential in oligodendrocytes, as the protein is strongly upregulated during oligodendrocyte differentiation while its down-regulation inhibited the maturation of oligodendrocyte progenitor cells into mature oligodendrocytes. Therefore, we suggests that the physiological role of TPPP/p25 in oligodendrocytes is the regulation of the acetylation level of microtubules, that post-translational modification promotes polarized microtubule-based trafficking essential for the maintenance and the development of the oligodendrocyte projections, while TPPP/p25-bundled and stabilized microtubules could serve as tracks for these trafficking events. Finally, the role of TPPP/p25 in the regulation of cell motility could have important impact when oligodendrocyte progenitors migrate to their final destination in the CNS. Any failure within these TPPP/p25-related processes could contribute to the development of different neurodegenerative diseases.

P1-20

SALS, a WH2-protein in sarcomeric actin assembly

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The morphology and dynamics of sarcomeric actin filaments are essential for proper muscle development and function. However, it is not completely understood how sarcomeric actin filaments are stereotyped in length and dynamics. Recently, SALS (*sa*rcomere *l*ength *s*hort) a WH2-domain-containing protein was identified in *Drosophila* as an important regulator of the assembly of sarcomeric actin structures. Disruption of *sals* by RNAi leads to lethality at the embryonic stages. This phenotype results from muscle defects caused by the improper organisation of sarcomeric actin filaments in SALS mutant embryos. Further loss-, and gain-of-function studies indicate that SALS influences sarcomere length by promoting actin filament assembly at the pointed ends and by antagonising with the pointed end-binding protein, tropomodulin. However, the exact role of SALS in muscle development has not been revealed.
To dissect the mechanism by which SALS contribute to the establishment of sarcomeric actin structures first we investigated the interaction of the WH2 domain containing fragment of SALS (SALS-WH2) with actin using biochemical and biophysical approaches.
We found that SALS-WH2 inhibits the assembly of actin monomers to filaments and enhances the disassembly of actin filaments even in the presence of tropomyosin. SALS-WH2 influences actin dynamics by shifting the monomer : filament equilibrium towards monomeric actin.
To reveal the biological role of SALS further studies are needed. The results can contribute to the understanding of the mechanisms underlying muscle function and the multifunctionality of WH2 domain proteins.

P1-21

Effects of filamentous actin bundling proteins revealed by EPR spectroscopy

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Eukaryotes have several highly conserved actin-binding proteins that crosslink filamentous actin (F-actin) into bundles present in cortical and cytoskeletal structures including microvilli, stereocilia and filopodia and are participating in processes as cell-matrix adhesion, cell motility, cell-cell interactions and sarcomere contraction. In our work we studied the changes in filamentous actin following the addition of fascin-1 and alfa-actinin using electron paramagnetic resonance spectroscopy (EPR). Spin labeled maleimide derivative was used to observe changes in hyperfine splitting and rotational correlation times due to interaction of actin and the bundling proteins. Evaluation of the EPR spectra suggests that fascin-1 and alfa-actinin induce detectable changes in the rotational freedom of the actin filaments. Interestingly, the two proteins exert different effects on the detected hyperfine splitting: alfa-actinin increases while fascin-1 decreases it. In line with the bundling phenomenon, the increase in the hyperfine splitting can be interpreted as a more rigid structure of longer rotational correlation time. The observed smaller hyperfine splitting can be thought as to be the consequence of shorter rotational correlation time or changes in the local microenvironment probed by the MSL caused by the fascin-1 binding. These interpretations can be in agreement with the known different physiological roles of the two proteins. Based on the structural differences of the proteins they can cause different way of actin filament binding that were reflected in different EPR signals.

P1-22

The effect of mDia1-FH2 on the ATPase activity of actin

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Formins are conservative proteins with important roles in the regulation of the actin based microfilamental system in eukaryotic cells. They have several domains including FH1, FH2, GPB and DAD. In the interaction between actin and formin the FH2 domain plays a key role. The 'mammalian Diaphanous-related 1' constitutes one of the subfamilies of the formins. These mDia1 formin fragments affect the conformation of the actin filaments in a concentration dependent manner. In the current work we have investigated whether the mDia1-FH2 affects the nucleotide exchange on the actin filaments. Steady-state fluorescence anisotropy and photometric coupled assay measurements showed that the ATP-ADP conversion was accelerated in the presence of formins, and the effect was stronger at greater formin concentrations. These observations indicate that there must be a tight coupling between the rate of nucleotide exchange on actin protomers and the conformational properties of the filaments.

P1-23

Tropomyosins regulate actin assembly factors in an isoform dependent manner

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In the cohesive structure of the cytoskeleton functionally distinct actin arrays orchestrate fundamental cell functions in a spatiotemporally controlled manner. Emerging evidences emphasize that protein isoforms are essential for the functional polymorphism of the actin cytoskeleton. The generation of diverse actin networks is catalyzed by different assembly factors, like formins and Arp2/3 complex. These actin arrays also exhibit qualitative and quantitative differences in the associated tropomyosin (Tm) isoforms. How the molecular composition and the function of actin networks are coupled is not completely understood.
We investigated the effects of different tropomyosin isoforms (skeletal muscle: sk, cytoskeletal 5NM1 and Br3) on the activity of mDia1 formin and Arp2/3 complex using biophysical and biochemical approaches.
The results revealed that different Tm isoforms have different effects on the mDia1-, and Arp2/3 complex-mediated actin assembly. The activity of the Arp2/3 complex is inhibited by skTm and Tm5NM1, whereas TmBr3 does not have any effect. All three Tm isoforms inhibited the activity of mDia1.
To dissect the mechanism underlying the Tm isoform specific regulation of assembly factors further studies are needed. The results can contribute to the understanding of these mechanisms by which Tm isoforms regulate the functional diversity of the actin cytoskeleton.

P1-24

Actin-regulated feedback loop based on Phactr4, PP1 and cofilin maintains the actin monomer pool

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Phosphatase and Actin regulating proteins (Phactr proteins) bind actin and protein phosphatase 1 (PP1), and are linked processes ranging from angiogenesis to cell cycle regulation. Phactrs share a highly conserved RPEL domain with the Myocardin Related Transcription Factor (MRTF) family. The RPEL-domain of MAL/MRTF-A regulates both nuclear localization and transcription coactivator activity via actin binding. We show that in contrast to MRTF-A, the RPEL domain is not responsible for the subcellular localization of Phactr4. Instead, PP1 and monomeric actin seem to bind to the RPEL-domain in a competitive manner and actin seems to regulate PP1 activity through Phactr4. We noticed that at low actin monomer levels Phactr4 induces PP1, which affects to phosphorylation status of one of its downstream targets, cofilin. When dephosphorylated, cofilin is in its active form and can sever and depolymerize actin filaments, which will replenish the actin monomer pool. Therefore, our data revealed an important role of Phactr4 in a feedback loop, where actin monomers regulate their own amount via the activation of cofilin, which is one of the key regulators of actin dynamics. Depending on the protein context, the RPEL domain can thus elicit mechanistically different responses to maintain the cellular actin balance.

Abstracts (posters)

Poster session II

Monday, 5 November

P2-01

Cell Shape versus Cell Polarity in Guiding Mitotic Spindle Orientation

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In animal cells, the orientation of the mitotic spindle dictates daughter cell positioning, which has important consequences for cell fate in tissues. The Hertwig rule, or long axis rule, states that the spindle aligns with the long cell axis. Recent works involving single cell manipulation refined this rule in different contexts: micro-patterning adhesion proteins revealed that in cultured somatic cells, the mitotic spindle aligns with cortical cues defined by cell adhesion geometry. On the other hand, placing sea urchin eggs in micro-wells of defined geometries confirmed that cell shape has a crucial effect on spindle positioning. In somatic tissues, cells might have both cortical cues and anisotropic shape during mitosis, but no experimental system has so far been developed to study the competition or synergy between these two factors.

In this study, we propose a new device to address that question, combining micro-wells and micropatterning techniques: cells were plated on micro-patterned adhesive lines and topped with a PDMS chamber containing micro-channels, so that the interphase cells were not in contact with the channels, but mitotic cells, when rounding up, would contact and enter the micro-channels. Micro-patterns control adhesion geometry, thus dictating cortical polarity while micro-channels impose a non-rounded shape to the mitotic cell body. Time-lapse microscopy of dividing cells was used to record the orientation of the mitotic spindle all along mitosis. We also assessed, using confocal microscopy, the 3D shape of the cell, as well as the distribution of cortical actin and microtubules.

P2-02

Myosin VI-stimulation-dependent accumulation in the nuclei: association with the nuclear components and transcription sites

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Myosin VI (MVI) is the only myosin that moves towards the minus end of actin filaments, suggesting its unique biological functions. Recently, we have shown that in neurosecretory PC12 cells (derived from rat pheochromocytoma) MVI is present both in the cytoplasm and in the nucleus, and that depletion of MVI inhibits cell migration and proliferation. Here, we found that upon PC12 cell stimulation with 56 mM KCl and treatment with leptomycin B (inhibitor of nuclear export) MVI (both endogenous and GFP-tagged) translocates to the nucleus, where it colocalizes not only with RNA polymerase II complex but also with transcriptionally active regions and several nuclear compartments such as PML bodies, nuclear speckles, transcription factor Sp1 and heterogeneous nuclear ribonucleoprotein U (hnRNP U). After stimulation MVI was recruited to transcription sites as tested with the BrUTP incorporation assay. Moreover mass spectrometry analysis of the PC12 cell extract obtained from a pull-down experiment with the recombinant MVI C-terminal tail cargo-binding domain (used as a “bait”) revealed a number of new potential MVI-binding partners. These proteins are known to be involved in nuclear trafficking and gene transcription.
These data indicate that stimulation-dependent increase of MVI in the nucleus is associated with the increase of transcriptional activity, thus supporting a notion that MVI could plays role(s) in the regulation of gene expression.

P2-03

Cofilin decorated bundle formation in different cell lines

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It has been established long time that cofilin plays a prominent role in the dynamic reorganization of actin network beneath the protruding cell edge. The dynamic remodelling of actin cytoskeleton involves the disassembly of "old" actin structures via depolymerisation and fragmentation of actin filaments. Because cofilin can enhance both processes, it is considered to be the most important "dynamizing" factor of the actin network in protruding events.
In this current work the effect of cofilin on lamellipodia formation and maintenance was investigated in immobile NIH 3T3 fibroblast cells and highly mobile fish keratocytes by using live cell video microscopy. Lamellipodia formation in NIH 3T3 cells was initiated with the simultaneous microinjection of Alexa 568-labelled cofilin and constitutively active Rac.
The most striking observation of the microinjection experiments was the appearance of cofilin decorated bundles beneath the protruding cell edge of NIH 3T3 cells. The bundles show dynamic nature; they mature into larger bundles, then start to move retrograde, while they shrink and cease at the posterior part of lamella. They are mostly originated from fragmentation of pre-existing bundles of filopodia. The microinjection of cofilin into highly mobile fish keratocytes also leads to the generation of cofilin decorated bundles which highlight the movement of actin structures from the front to the rear of cells.
In the light of these observations the cofilin decorated bundles can be good candidates for investigating the connection between the actin network beneath the protruding cell edge and the cytoskeletal system behind the locomotive machinery.

P2-04

Content analysis of the myosin heavy chain isoforms in rat female and male hindlimb muscles: the difference concerns soleus muscle

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It is known that the variability of myosin heavy chain (MHC) isoforms results in molecular heterogeneity of muscle fibers, and the dominant MHC isoform is considered to be responsible for functional differences of skeletal muscles. The present study has addressed for the first time the dimorphism of the MHC isoform content in fast and slow hindlimb muscles of adult female and male rats. The analyses performed by means of gel electrophoresis as well as by the immunofluorescence confocal microscopy and by in situ muscle ATPase stainings have shown the difference in the MHC content within the slow soleus muscle. A significantly higher content of the fast MHC IIa isoform and higher percentage of fast fibers was found in soleus muscle of males compared to females. In contrast, no evident sex-dependent differences in the MHC isoform content were observed for fast muscles: flexor digitorum brevis, gastrocnemius medialis and tibialis anterior. Thus our study indicates that the dimorphic content of MHC isoforms in hindlimb muscles concerns predominantly slow muscles.

P2-05

ATR-mediated sensing of nuclear envelope dynamics

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Nuclear Pore Complexes (NPCs) traditionally regarded as transport gateways, have emerged as specialized hubs involved in organizing genome architecture, influencing DNA topology and modulating DNA repair. Our group had recently identified the mechanism by which checkpoint proteins can assist DNA synthesis across transcribed genes by relieving the mechanical tension caused by transcribed chromatin from NPCs (through phosphorylation of nucleoporins) using budding yeast as a model organism (Bermejo et al., Cell, 2011). The checkpoint mediated control of chromatin-nuclear envelope tethering is likely crucial in an oncogenic context in which chromosome replication has to face massive deregulated transcription. Hence, we extended our studies to vertebrates and examined checkpoint-dependent mechanisms sensing nuclear and plasma membrane tensions. Our preliminary results showed ATR/ATRIP localize at the nuclear envelope and it can be further stimulated upon mechanical stress. These observations suggest a conserved phenomenon in S. Cervesiae and vertebrates; where, ATR/ATRIP might be positioned high up in the hierarchy of variety of cellular defense mechanisms that might form a cascade/ network to control genomic instability.

P2-06

Role of Rac1 GTPases in the regulation of Dictyostelium cell migration

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Rac proteins are the only canonical Rho family GTPases in *Dictyostelium*, where they act as key regulators of the actin cytoskeleton. We monitored the dynamics of activated Rac1 in *Dictyostelium* cells using fluorescent probes that specifically bind to GTP-bound form of Rac1. The probes were based on the GTPase-binding domain (GBD) from PAK1 kinase and the IQGAP-related protein DGAP1. In moving *Dictyostelium* cells, GBD probe was strongly enriched at the leading edge where it co-localized with F-actin, whereas DGAP1 probe was localized to the trailing, retracting regions of migrating cells. As assessed by latrunculin B treatment, cortical localization of PAK1_GBD strictly depends on the integrity of the actin cytoskeleton, whereas cortical localization of DGAP1 does not. Taken together, these results imply that Rac1 GTPases play a dual role, both at the front and in the back, in migrating *Dictyostelium* cells. In order to further characterize the regulation of cell migration by Rac1 proteins it is necessary to compare characteristics of cells with upregulated and downregulated GTPases in more detail. Whereas level of Rac1 GTPases is increased in Rac1A-overexpressing cells, cells with reduced level of these proteins will be generated using a multigene silencing approach. Using these cells, we plan to analyze localization and dynamics of the fluorescent probes in Rac1-enriched and Rac1-deprived backgrounds, and to compare migration behavior of these mutant cells within the framework of a generalized Langevin model of cell locomotion.

P2-07

Spatial structure of cytoskeleton associated with nuclear envelope

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The cytoskeletal structures were observed in detail under electron microscope, in spite of restriction of observation area. The images of whole cell in fluorescent light microscopy did not show an individual actin filament, but mainly stress fibers. In order to detect spatial structure of cytoskeleton, high voltage TEM (1000 KV) and high resolution SEM were applied to unroofed cells. Our methods showed that cell contained more abundant single actin filaments besides stress fibers than in fluorescent microscopy. These filaments extended in all directions with aggregation and dispersion to form meshwork, and divided cytoplasmic space into many domains. Both non-bundled actin filaments and stress fibers attached to the nuclear envelope at many locations while associating with intermediate filaments that were identified as vimentin filaments by immunolabeling technique. Many vimentin filaments formed a layer and covered a part of the nuclear envelope. Microtubules were found within stress fibers and also contacted with the nuclear envelope. However, it has been unclear how actin filaments interact with the nuclear envelope. Immunolabeling with anti-nesprin-1 showed that nesprin-1 was located on the nuclear envelope, which was expected as an anchorage of actin filaments and nucleus.
In the process of cytokinesis, the mechanism of disassembly of the nuclear envelope has not been revealed. On closing to the metaphase, stress fibers were disappeared together with the nuclear membrane. The stress fibers which directly interact with the nuclear envelope seem to play an important role to form and disperse the nuclear envelope in cytokinesis.

P2-08

The actin cytoskeleton is involved in the endocytic process of diphtheria toxin transport

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The actin, basic component of the microfilament structure in eukaryotic cells regulates also signaling pathways by interacting with actin-binding proteins. It has been observed in our previous studies that the depolymerization of the filamentous actin (F-actin) occurs right after the infection of the endothelial cells with either diphtheria toxin (DT) or its mutant form CRM197 via the interaction between fragment A (FA) and F-actin. Besides the inhibitory effect of FA on protein synthesis through the ADP-ribosylation of eukaryotic elongation factor 2, the intracellular transport of DT has not been fully elucidated yet. In this study it is aimed to visualize the FA following the administration of 15 min to the human umbilical vein endothelial cells (HUVECs) under the treatment of jasplakinolide. Filamentous actin, early endosomes and FA were detected by immunofluorescence microscopy. The administration of jasplakinolide (0,1 μM) for 60 min has been observed to prevent the FA transport to the perinuclear area contradictory to the administration of the same concentration for 30 min. The preliminary data show that the dynamic structure of the actin filaments has been considered necessary for the endocytic process of diphtheria toxin transport.

P2-09

Does arginylation of β-actin regulates cell motility and adhesion: Effects of arginine deprivation

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Posttranslational protein arginylation has been found to be critical for mouse embryogenesis and angiogenesis [Kashina et al. (2006) Science 313, 192-196]. Interestingly, from over forty proteins that in fibroblasts have been found to be arginylated *in vivo* more than one third is involved in cytoskeleton organization and its regulation, including β-actin [Wong et al. (2007) PLoS Bio.. 5(10):e258]. In this report, we have tested whether arginine deprivation affects human U251MG glioma cells as well as primary culture rat glia cells.
After 48 hours of arginine starvation such cell morphology was dramatically changed. The cell adhesion (assayed as adhesive complex formation and homotypic adhesion on soft agar) and cytoskeleton organization (measured as F-:G-actin ratio and lamellipodia formation) were significantly reduced in comparison to cells grown on complete medium and a medium deprived of lysine (deprivation control cells). Also, the arginine-deprived cells, unlike the deprivation control cells, became less motile (random motility and wound healing tests) and invasive (Matrigel test on Transwell system). While proliferation seemed to be decreased, the viability of arginine-deprived cells remained unaffected. The observed changes were found in glioma but not in glia cells indicative of different arginine metabolism in transformed and phyiological cells.
We believe that these changes in arginine-deprived cells are caused by deficit in actin arginylation as 2D-gel electrophoresis revealed lack of positively-charged β-actin isoforms.

P2-10

MVa is involved in focal adhesion disassembly by affecting FAK localization at these sites

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Cell migration is a dynamic process that involves the continuous formation, maturation and turnover of matrix-cell adhesion sites, named focal adhesions (FA). The processes involving FA dynamics are complex and require a regulated interaction of numerous proteins and mechanisms for targeting these proteins to these sites, including the microtubule and actin cytoskeleton and their modulators and motors. Here we reported that myosin-Va could affect FAK distribution to FA sites and its implications in FA dynamics. Using *MYO5A* null fibroblasts derived from a Griscelli syndrome patient we observed no differences in FA formation between MYO5A$^{-/-}$ cells and control ones. However, intense staining for FAKpY397 was detected associated to FA of control cells, but not in *MYO5A-/-* cells. Tranfection with FAK c-myc enable localization of FAK in FA associated to stress fibers in human melanoma cells but not in MYO5A knocked down cells. FAK has previously been shown to be crucial for FA disassembly. Interestingly, FA disassembled synchronously with microtubule regrowth in control fibroblasts but in myosin-Va null fibroblasts FA persist after microtubules. We suggest that loss of function of myosin-Va in fibroblasts impaired focal adhesion disassembly by affecting FAK localization at focal adhesion sites.
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P2-11

Structural and nanomechanical impacts of a Gly245Asp actin mutation on fibroblast spheroids

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Tumor initiation and progression are accompanied by complex structural changes in the extra-cellular matrix and cytoarchitecture that are anticipated to develop differentiable mechanical responses. At the cellular level, changes in the nanomechnical response involve remodeling of the cytoskeleton. We employed a combination of light/fluorescence, scanning electron (SEM) and atomic force microscopy (AFM) to examine the impact of a Gly245Asp mutant actin (actin-Asp) on the cytoskeletal structure and nanomechanical properties of fibroblasts in relation to tumorigenic transformation. To approximate complex cell-cell and cell-matrix interactions that occur in tumors, and might modulate the effects of actin-Asp, we cultured parental Rat-2 and stably transfected Rat-2-sm9 cells as scaffold-free 3D spheroids. Bright field microscopy and SEM show that they share the principles of spheroid formation and compaction. However, Rat-2-sm9 and Rat-2 spheroids exhibit distinct phenotypes including differences in surface structure and size. Based on the enrichment of actin-Asp in membrane ruffles and its interference with actin polymerization in vitro, we attribute the alterations of Rat-2-sm9 spheroid structure to the mutant actin. Besides structural differences between normal and transformed spheroids, cross-sectional AFM stiffness profiles reveal a significantly softer core in Rat-2-sm9 spheroids after 3 days of culture under normal oxygen conditions. Overall, they exhibit gradual stiffening towards the periphery, which is a characteristic of cancer tissues (Plodinec et al., 2012).

P2-12

The formation of actin waves during regeneration after axonal lesion is enhanced by BDNF

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During development, the axons of neurons in the mammalian central nervous system lose their ability to regenerate. To study the regeneration process, axons of mouse hippocampal neurons were partially damaged by an UVA laser dissector system. This leads to depolymerization of cytoskeletal filaments and disassembly of adhesion contacts. During axonal regeneration, the cell has to restore the disrupted structures, elongate the dissected neurite, initiate growth cone navigation, and re-establish the homeostatic compression-tension equilibrium in order to recover the functionality of the connection with its targets.

The possibility to deliver very low average power to the sample reduced the collateral thermal damage and allowed studying axonal regeneration of mouse neurons at early days *in vitro*. The reorganization and regeneration of the axon was documented by long-term live imaging.

Growth cone-like structures (so-called actin waves) originating at the soma have been described as a transport mechanism toward the tip of the neurite necessary to assist its growth, and the healing and re-growth of the injured axon. The axons of neurons in the first 3 DIV were lesioned to monitor the formation of the actin waves and the effect of BDNF on cytoskeletal dynamics. A BDNF signaling pathway, activated in response to axonal damage, appears to favor actin waves formation possibly by opposing the dispersion of molecules involved in these processes and by facilitating a rapid turnover of actin. Here we demonstrate that BDNF regulates neuronal adhesion and favors the formation of actin waves during regeneration after axonal lesion.

P2-13

Bidirectional cargo transport: assessing molecular motor coupling and obstacle encounter using a novel in vitro assay for microtubule-based mRNP transport

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Asymmetric localization of a subset of proteins within the cytoplasm is achieved by cytoskeletal transport of their mRNA transcripts. Studying transport mechanisms *in vivo* is challenging because individual microtubules (MTs), cytoskeletal motor components and small mRNA:protein complexes (mRNPs) cannot be visualized readily. Here, building on an *in vitro* transport assay previously developed in our lab, I describe a novel technique to study transport of bidirectional mRNPs assembled in *Drosophila* embryo extracts. We use static motors and microtubule associated proteins as obstacles to assay the effects on the dynamics of mRNPs. To the best of our knowledge this is the first attempt to assay the effect of obstacles on the dynamic properties of bidirectional motor complexes bound to a physiological cargo. Strikingly, whereas it has been shown previously that purified unidirectional motors detach frequently upon encountering obstacles, we find that bidirectional mRNPs often reverse direction. Thus, bidirectionality may be advantageous in preventing cargo:motor complexes for dissociating from microtubules in the crowded *in vivo* environment. Surprisingly, we also find that in the absence of obstacles individual mRNPs exhibit a very strong correlation between their mean minus-end and plus-end run lengths and velocity. There is a reduction in this correlation due to the presence of obstacles on MTs. Our findings lead to a new model for bidirectional transport of mRNAs in which the mechanisms controlling minus and plus end dynamics are tightly coupled. This coupling is presumably difficult to observe in the complex *in vivo* environment due to the influence of obstacles.

P2-14

Bending stiffness of bundled actin filaments during its sliding on heavy meromyosin

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Dynamic bundling of actin filaments (F-actin) via actin binding proteins is important in assembling and reorganizing the cytoskeletal network during cell growth and motility. Fascin, an actin-bundling protein in filopodia, cross-links F-actin to produce bundles where all filaments have the same polarity. Several actin-binding proteins, including myosin, may modify the mechanical properties of F-actin *per se*. To investigate combined effects of fascin and myosin on the mechanical properties of phalloidin-labeled actin bundles, we here measured the persistence length (Lp; proportional to flexural rigidity) from the path of bundles when propelled by heavy meromyosin (HMM) *in vitro*. Lp was obtained from the equation: <cos(theta(0)-theta(*s*))>=exp(-s/(2*Lp)), where theta(0) and theta(*s*) denote the tangent angle for sliding direction at the start and after distance *s* along a trajectory, respectively. For F-actin in motility assays we found Lp=11.4±0.4 µm compared to Lp=12.6±0.3 µm when measured from thermal fluctuations of filaments in solution. For fascin-mediated actin bundles, Lp was significantly larger and decreased with increased actin:fascin ratio: Lp was thus 81.2±4.3 µm (*N*=30), 44.8±1.6 µm (*N*=68) and 34.6±1.6 µm (*N*=33) at actin:fascin molar ratios of 1.2:1, 2:1 and 4:1 respectively. With Lp for actin-fascin bundles in solution of 100-300 µm, the result suggests qualitative similarity to the F-actin data with lower Lp in motility assay. The mechanisms underlying the results are discussed in relation to methodological issues and motor induced structural changes in actin filament structure.

P2-15

Salivary peptide histatin enhances wound healing via ERK- and GPCR-dependent cell migration

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Wounds in the oral cavity heal faster than at other sites in the human body, e.g. the skin. Several factors have been implicated in this phenomenon, including the presence of saliva, which in rodents is a reservoir of many growth factors, such as epidermal growth factor (EGF) and nerve growth factor (NGF). In humans the identity of the involved compounds has remained elusive, since the saliva concentration of growth factors is 1,000 to 100,000 times lower than in rodent saliva. The present study was aimed at the identification of the factor(s) responsible for the alleged wound healing power of saliva, and a first characterization of the cellular processes.
Using an *in vitro* scratch assay we found that human saliva is able to induce epithelial cell migration, without involvement of EGF. By testing protein fractions of human saliva obtained by HPLC, we identified salivary histatins as the main migration-inducing factors in saliva. The activity of histatin was confirmed in a tissue-engineered epidermal skin equivalent that closely resembles healthy human skin.
Histatin activity was abolished both by inhibition of mitogen activated protein kinases ERK1/2 and MEK, and by pertussis toxin, an inhibitor of G protein coupled receptors, suggesting the involvement of a GPCR-dependent ERK1/2 signaling pathway.
Conclusion: Our results emphasize the importance of histatin in human saliva for tissue protection and recovery, and establish the experimental basis for the development of synthetic histatins as novel wound-healing agents.

P2-16

Myosin 1b can extract membrane tubes cooperatively by using catch-bond property

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Myosin 1b (Myo1b), a vertebrate myosin-I isoform, is essential for the membrane tube formation from the trans-Golgi network, although the mechanism by which the single-headed, non-processive and membrane-associated motor controls the tube formation is unknown. Laakso, *et al.* demonstrate that Myo1b acts as a force sensor responding to a resisting force, *i,e,* displays catch-bond behavior. This property may enable Myo1b to control the dynamic changes of the morphology of cell organelles. To further elucidate the mechanism by which Myo1b controls the membrane tube formation, we develop a reconstituted *in vitro* system composed of purified full length Myo1b, giant unilamellar vesicles (GUVs) containing phosphatidylinositol -4,5- biphosphate (PIP_2), and actin-fascin bundles immobilized on a glass substrate. We demonstrate for the first time that Myo1b alone can pull membrane tubes out from a GUV along actin bundles. We successfully observe the dynamics of tube elongation, Myo1b distribution along the tube, and the correlation between Myo1b density and tube elongation velocity. These observations are in good agreement with our theoretical model that explains the stochastic pulling of Myo1b with the significance of the catch-bond property. Furthermore, Cryo-EM observations provide the first direct evidence at molecular resolution of the binding of Myo1b to lipid bilayers.

P2-17

Structural rearrangement after modification of individual cytoskeletal elements and plasma membrane in CHO cells

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The cytoskeleton and the plasma membrane (PM) are the main structural elements of cellular mechanical integrity. While many details of the structure and function of different cytoskeletal elements and the PM are worked out, the complete picture of the interplay between these elements remains rather obscure. In this work we systematically disrupted different structural elements in CHO cells and analyzed the influence of the disruption of one element on the others. In particular, we employed nocodazole, latrunculin A and acrylamide as disruptors of microtubules, actin filaments and vimentin, respectively, and methyl-β-cyclodextrin for cholesterol extraction from the PM. The effects of different substances on cytoskeletal elements were imaged with confocal microscopy, and laser optical tweezers were employed to pull plasma membrane tethers in order to assess the amount of accessible PM reservoir.
Our experiments show that the disruption of one part of the cytoskeleton influences the reorganization of all other structural elements. While the intact cells that were more spread had a larger membrane reservoir, the disruption of the cytoskeleton resulted in a reduced cell size and an increased membrane reservoir. This indicates the existence of distinctive and independent sources of membrane reservoir. Interestingly, the largest increase of the membrane reservoir was observed after mictoubule disruption even though the microtubules do not explicitly take part in the sub-membrane cortical cytoskeleton.

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