A fluorescence microscopy image showing a dense network of neurons. The neurons are stained with two different dyes, resulting in red and green signals. The red signal highlights certain cell bodies and processes, while the green signal highlights others. The overall appearance is a complex, interconnected web of cellular structures.

Cell News

**Newsletter of the
German Society for Cell Biology**

Volume 37, 1/2011

Annual Meeting, Bonn
30 March – 2 April 2011

DGZ



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Please submit your best and favourite image of your cells for **"Cell of the month"**.
We will select and present your scientific artwork on the main page of the DGZ website and
put it into the gallery after one month. Please submit your images to dgz@dkfz.de

Cover image: Brain sections visualize the morphology and dendritic branching of hippocampal neurons in the CA1 region of n-cofilin mutant mice (Rust et al., EMBO J., (2010), Vol. 29, p1889-1902). Sporadic expression of GFP in about 10% of neurons in a Thy1-GFP transgenic background (Feng et al., Neuron, (2000), Vol. 28, p41-51) was used to reveal the detailed morphology of individual neurons. Nuclei were counterstained in red using propidium iodide.

Get ready for the Annual Meeting in Bonn

On strike

Cell biologists never strike, on the contrary – “self-exploitation” is the rule for life scientists. Going to Dresden last week, I found myself sitting there with poor prognosis for a timely return to Heidelberg. The locomotive driver union was on strike. However, the sun was shining and, after the job was done, I enjoyed walking the streets. Eventually I hit a second-hand bookstore. With my soft spot for dog eared books, I found a book on “Philosophische Gegenwartsfragen” (1933), by Hans Driesch (1867-1941), a prominent cell biologist originally, who did pioneering work on “developmental mechanics”. Later in his life he turned to philosophy and was criticized by his colleagues to have drifted into “neovitalism”. It is quite interesting to read “Studien über Ganzheit” from someone who has become famous for generating single embryo cells (“Schüttelversuche”) and showing that they were able to grow into viable organisms. As a sidemark, Hans Driesch was one of the first non-jewish German professors to be forced into retirement in 1933 by the national socialist’s regime, because of his socialist ideas (Ilse Jahn, ed.: Geschichte der Biologie. Nikol-Verlag, 2004).

Annual Meeting Affairs

The reason to go to Dresden was of course something else. In 2012, March 23rd to 24th, we will have the annual meeting in Dresden. Elisabeth Knust serves as the Congress President, and she will be supported by Ewa Paluch and Marino Zerial as members of her organization committee. The congress site is terrific and I hope that this future congress will be as successful as the coming one in Bonn promises to become.

For this years’ congress, taking place on **March 30th to April 2nd** at the **University of Bonn**, right in the centre and five minutes to walk from the main station, we received 309 abstracts in total. Of these, 42 are speaker’s abstracts, the remaining 267 abstracts will be presented as posters. This

means that we should witness a very good attendance and hence an excellent basis for scientific discussion. Moreover, as a consequence of this excellent attendance, we have to display the posters in two sessions: the first half of the posters will be presented on Wednesday evening and on Thursday, the second on Friday and Saturday (for details see the programme on p. 4-6).

This year we had, for the first time, the category *Late Abstracts*. We will keep this category for the next meeting, as it will enable us to set a fixed data for abstract submission that will not be prolonged (i.e. 31st of January) without losing contributions from those who submit later. However, late subscribers are not considered for the selection of poster talks and will have to present under General Subjects. An overview of the meeting program is presented on p. 4 to 7. The five plenary sessions and the fifteen symposia cover a wide range of subjects that promise to both fulfil the expectations for people’s special subjects as well as ample opportunity to get insight into new research areas by leaders in the field. Moreover, for the fifteen symposia, 30 speakers have been selected from the abstracts to guarantee intense contribution from the community. This year, we will have these symposia in the morning. We hope that the morning talks, covering material displayed in the poster session, will attract many of the attendees to the poster sessions, which take place during the *Welcome Reception* on Wednesday evening and at noon on Thursday and Friday. Like every year, we will have a poster committee which selects a number of posters to be awarded prizes by the DGZ, which will be presented at Saturday noon.

Last but not least, it is a pleasure to announce that this year the Carl-Zeiss Lecture will be given by Ueli Aebi, Biozentrum, University of Basel. His talk will deal with the nuclear pore complex and nucleo-cytoplasmic transport as revealed by light, electron and atomic force microscopy.

Meetings This Year

The “**International Meeting**” of the DGZ on the many function of microtubules is at the web site. Please do not wait to register. As the number of participants is indeed limited, we suggest to register early. The deadline is April 22nd, 2011. Along these lines, in order to reach members for special information, it is important for us to have your email address. If you never received announcements from the DGZ, send a mail stating that you are a member and that you would like to receive essential information. Knowing about all the spam we get every day, I can promise that you will not get more than six mails per year, but these mails will really matter. Of course, we will not give your address to anybody. Our address can be found on the DGZ- website, and it is in English on demand by clicking the Union Jack

<http://www.zellbiologie.de>

The next “**Young Scientist Meeting**” will be held in Jena, September 8th to 10th. Sven Diederichs and Dirk Grimm are assembling an exciting program on “**RNA and Disease**”. Among the speakers that accepted to come are foreign guests, in particular **Frank Slack** (Yale University, New Haven) **George Calin** (MD Anderson Cancer Center, Houston) and **Brian D. Brown** (Mount Sinai School of Medicine, New York). All master and PhD students as well as Post-Docs in their early phase, please note the date and get prepared. The registration will be opened in early summer. For DGZ members there will be no registration fee, and food and lodging will be covered by the DGZ. Hence, do not hesitate to become a member as your membership will support this and further events. And – as we were asked at the “Actin Dynamics” meeting, you do not need a German passport to become a member. “Foreigners” are welcome. Recently, we welcomed a scientist from India. Any passport will suffice, and we will not even ask for it. The commitment to serious science is all we ask for.

The **Summer School on Actin Dynamics** is limited to 30 participants. It will take place

PREFACE

in Regensburg, September 17th to 23rd. An impressive crew of ten tutors is advertised, Andreas Bausch to Petra Schwille, hence you better hurry to go online (www.zellbiologie.de). We congratulate Eugen Kerkhoff, Klemens Rottner and colleagues for bringing up this enterprise.

Last but not least, for early October this year we plan to have a **“Special Interest Meeting”** focussed on “Physical Oncology”, organized by Josef Käs (Leipzig) and Sarah Köster (Göttingen). It is meant to bring together cell biologists, physicists, oncologists and material scientists for intensive discussion about the biophysical aspects of cancer cells. The next issue of Cell News will provide further details.

Our Advisory Board

At the board meeting during our last annual meeting in Regensburg, the members decided to expand the number of members to twelve. We assembled the board nearly com-

pletely, and you might have recognized that we had a distinct change in the members (p. 1, *Cell News*). This process has now been completed, since Anne Spang (Biozentrum, University of Basel) and Sylvia Erhardt (ZMBH, Heidelberg) have accepted our invitation to join the Advisory Board. We thank all of the new members for taking an active role in our society and, in particular, cordially welcome these new members.

Cell News, accelerated

The newsletter is taking on speed. We have a series of highly interesting articles, including two contributions classified as “Off the bench”. One of the articles was advocated by a mentor, the other one was sent in by a PhD student (after reconciliation with the supervisor). We hope that in the future more of these reports will be submitted in addition to the Research News. These latter articles are, by-and-large, invited by us, but nevertheless we do recommend to contact

us if you think you are working in a field that needs to receive more attention by the community and if you are willing to provide an interesting article. We want to emphasize at this point that the copyright for all of the material stays with the authors and, hence, there should not arise any problems in case the authors wish to use this material for later publications. Moreover, the *Perspectives* series is meant for contributions from scientists with “oversight”. In this issue you will read about actin, from a perspective that textbooks hardly will be able to provide. Cora-Ann Schoenenberger, Hans Georg Mannherz and Brigitte Jockusch bring together more expertise than most of us can even consider. Their article “The many faces of actin” may be a “mindblower” for everybody who is thinking: “yes, this guy sticks around everywhere”. No, it is much more complex than you ever wished it to be. But here we get the harvest of three life-long research careers, combined. So enjoy!!!

Harald Herrmann

34th DGZ Annual Meeting, 30 March – 2 April 2011, Bonn

Conference Chairs:

Prof. Dr. Dieter O. Fürst and
Prof. Dr. Walter Witke

Venue:

University of Bonn, Main building
Regina-Pacis-Weg 3
53113 Bonn (Germany)

Information and Registration:

www.dgz2011.de

DGZ-members

Regular: 185 EUR, Students*: 90 EUR

DGZ non-members

Regular: 245 EUR, Students*: 155 EUR

Social events

Welcome reception, included in the registration fee
Get together: 20 EUR

Day tickets

Member or non-member: 85 EUR, Students*: 55 EUR

* Please provide confirmation

DGZ Member Meeting 2011

We are inviting all members to attend our next member meeting that will take place on

Thursday, 31 March 2011, 19:00 (room HS 1)

at the University of Bonn, main building, Regina-Pacis-Weg 3.

Agenda: 1. Confirmation of the minutes of the last year's DGZ member meeting 2010 2. The president's annual report 3. Financial report 4. The auditors' report 5. Approval of the executive board 6. “Other”

Scientific Programme

Wednesday, 30 March 2011

13⁰⁰–13¹⁵ Opening Ceremony

Welcome speech by the chairmen:
D.O. Fürst, W. Witke (Bonn)
Welcome speech by the president of the DGZ:
H. Herrmann-Lerdon (Heidelberg)

13¹⁵–17⁰⁰ Plenary Session 1: Cell polarity

Chairs: E. Knust (Dresden), J. Ahringer (Cambridge/GB)
J. Ahringer (Cambridge/GB): Genetic interaction RNAi screening for a c. elegans cell polarity network
F. Bradke (Martinsried): Intracellular mechanisms of neuronal polarization and regeneration
E. Knust (Dresden): Drosophila crumbs from epithelial cell polarity to retinal degeneration
M. Piel (Paris/F): Effects of external constraints on dividing cells
E. Rodriguez-Boulan (New York/USA): Polarized trafficking in epithelia

17⁰⁰–17³⁰ Break

17³⁰–18³⁰ DGZ Award Ceremony

Walther Flemming Medal
P. Meraldi (Zurich/CH): The metaphase plate – still full of surprises
Binder Innovation Prize
C. Behl (Mainz): Aging makes a difference: Implications for protein homeostasis and neurodegeneration
Werner Risau Prize
S. Sawamiphak (San Francisco/USA): Controlling vessel sprouting: a team effort of VEGFR2 and ephrinB2

18³⁰–19³⁰ Carl Zeiss Lecture

U. Aebi (Basel/CH): From nuclear pore complex structure to nucleocytoplasmic transport

19³⁰ Welcome Reception / Poster Session I

CCC - Cell cycle and cancer, CN - Cellular neurobiology, CM - Cellular mechanics, CP - Cell polarity, FM - Frontiers in microscopy, GS - General subjects, IN - Inflammation, MCO - Muscle cell organisation, NEN - Nuclear envelope and NPCs, SRG - Signaling and Rho GTPases, VT - Vesicle trafficking

Thursday, 31 March 2011

09⁰⁰–12⁰⁰ Symposium 1: Frontiers in microscopy

Chairs: R. Pepperkok (Heidelberg),
W. Kühlbrandt (Frankfurt/M)

J. Briggs (Heidelberg): Correlated fluorescence and 3D electron microscopy
S. Jakobs (Göttingen): Focusing on mitochondria with STED-microscopy
W. Kühlbrandt (Frankfurt/M): Cryo-EM bridges the gap: from atomic structure to in situ arrangement of membrane proteins
R. Pepperkok (Heidelberg): High content microscopy for systems biology and speakers selected from the abstracts

09⁰⁰–12⁰⁰ Symposium 2: Signaling and Rho GTPases

Chairs: A. Pfeifer (Bonn), G. Schmidt (Freiburg/Br)
C. Brakebusch (København/DK): Rac1 function in skin disease
O. Pertz (Basel/CH): Spatio-temporal Rho GTPase signaling during neurite outgrowth
A. Pfeifer (Bonn): Regulation of small G proteins by the cGMP pathway
G. Schmidt (Freiburg/Br): Molecular strategies of pathogenic bacteria – toxins control Rho GTPases and speakers selected from the abstracts

09⁰⁰–12¹⁵ Symposium 3: Nuclear envelope and NPCs

Chairs: A. Noegel (Köln), B. Fahrenkrog (Gosselies/B)
B. Fahrenkrog (Gosselies/B): Dissecting the functional role of nucleoporin Nup153 in mitosis
R. Foisner (Vienna/A): Regulation of A-type lamins in tissue progenitor cells
M. Hetzer (La Jolla/USA): Cell cycle dependent differences in NPC assembly
S. Neumann (Köln): Nesprins as keeper of nuclear and centrosomal organization and signal transduction
B. van Steensel (Amsterdam/NL): Genome-nuclear lamina interactions and speakers selected from the abstracts

09⁰⁰–12⁰⁰ Symposium 4: Inflammation

Chairs: M. Pasparakis (Köln), J. Tschopp (Epalinges/CH)
F.R. Greten (München): Anti-inflammatory functions of NF- κ B
M. Pasparakis (Köln): Mechanisms regulating epithelial immune homeostasis and inflammation
J. Tschopp (Epalinges/CH): The inflammasomes
F. Zipp (Mainz): Th17 mediated neurodegeneration and speakers selected from the abstracts

12⁰⁰–15³⁰ Poster Session I (continued)

12⁰⁰–13⁰⁰ Lunch Symposium*: Carl Zeiss MicroImaging GmbH

12⁰⁰–13⁰⁰ Lunch Symposium*: IBA GmbH and Dualsystems Biotech AG

* Snacks and beverages will be served free of charge in these symposia.

34th DGZ ANNUAL MEETING

15³⁰–19⁰⁰ **Plenary Session 2: Cellular mechanics**
Chairs: J. Lammerding (Cambridge/USA), S. Köster (Göttingen)
C. Chen (Philadelphia/USA): Regulation of cell function by the mechanics of cell-material interactions
P. Friedl (Würzburg): Physical limits of cell migration
J. Guck (Cambridge/GB): Optical rheology of cells – from microscopic mechanisms to biological relevance
S. Köster (Göttingen): Mechanics of intracellular protein networks
J. Lammerding (Cambridge/USA): Intra cellular mechanics in physiology and disease

15³⁰–19⁰⁰ **Plenary Session 3: Cell cycle and cancer**
Chairs: I. Hoffmann (Heidelberg), Z. Storchova (Martinsried)
H. Bastians (Göttingen): The Chk2 Brca1 tumor suppressor pathway ensures chromosomal stability
I. Hoffmann (Heidelberg): Regulation of centrosome duplication in normal and malignant cells
G. Kops (Utrecht/NL): Maintaining genomic stability: how mitotic kinases regulate chromosome segregation
Thomas U. Mayer (Konstanz): Mitosis: the challenge to equally distribute the genome
Z. Storchova (Martinsried): Limiting the propagation of polyploid cells

19⁰⁰ **DGZ MEMBER MEETING**

Friday, 1 April 2011

09⁰⁰–12⁰⁰ **Symposium 5: Host pathogen interactions**
Chairs: T. Stradal (Münster), A. Haas (Bonn)
S. Backert (Dublin/IR): Virulence factors and signal transduction mechanisms in infection with *Helicobacter pylori*
A. Haas (Bonn): Greasing the virulence machinery – *Rhodococcus equi* and phagosome biogenesis
H. Hilbi (München): Virulence and trafficking of the vacuolar pathogen *Legionella*
T. Stradal (Münster): *Salmonella*: 50 ways (plus one) to enter the host
and speakers selected from the abstracts

09⁰⁰–12⁰⁰ **Symposium 6: Muscle cell organisation**
Chairs: D.O. Fürst (Bonn), F. Schnorrer (Martinsried)
D.O. Fürst (Bonn): tba
M. Gautel (London/GB): Links from the sarcomere to mechanical strain sensing and muscle protein turnover control
C. Jagla (Clermont Ferrand/F): Muscle-type regulation of the fusion process
F. Schnorrer (Martinsried): Spalt mediates an evolutionary conserved switch to fibrillar muscle fate and speakers selected from the abstracts

09⁰⁰–12⁰⁰ **Symposium 7: Cellular neurobiology**
Chairs: C. Steinhäuser (Bonn), A. Acker-Palmer (Frankfurt/M)
A. Acker-Palmer (Frankfurt/M): Neuro and vascular guidance in health and disease
E. Audinat (Paris/F): Multiple states and functions of brain microglia
E.M. Schumann (Frankfurt/M): Local control of synaptic function
C. Steinhäuser (Bonn): Neuron-NG2 glia synapses in the brain
and speakers selected from the abstracts

09⁰⁰–12⁰⁰ **Symposium 8: Vesicle trafficking**
Chairs: R. Jahn (Göttingen), P. Saftig (Kiel)
W. Annaert (Leuven/B): Presenilins and endosomal trafficking: relevance for Alzheimer's disease
S. Höning (Köln): Functional regulation of the clathrin adapter AP2
R. Jahn (Göttingen): Membrane fusion in the secretory pathway
P. Saftig (Kiel): Lysosomal membrane proteins: role in lysosomal biogenesis, autophagy, phagocytosis and cholesterol homeostasis
and speakers selected from the abstracts

12⁰⁰–15³⁰ **Poster Session II**
CA - Cell adhesion, CBA - Cell biology of addiction, CI - Cellular immunity, CD - Cytoskeletal dynamics, HPI - Host pathogen interactions, IF - Intermediate filaments: novel functions, MMA - Molecular mechanisms of ageing, NCR - Non-coding RNAs, PS - Proteostasis, SC - Stem cells

12⁰⁰–13⁰⁰ **Lunch Symposium***: Leica Microsystems
*Snacks and beverages will be served free of charge in this symposium.

12⁰⁰–13⁰⁰ **Lunch Symposium**: ibidi GmbH

15³⁰–19⁰⁰ **Plenary Session 4: Cytoskeletal dynamics**
Chairs: W. Witke (Bonn), R. Grosse (Marburg)
R. Grosse (Marburg): Regulation of MAL and SRF through formin-mediated actin assembly
M. Schuh (Cambridge/GB): Oocyte + Actin: old love – new affairs
T. Surrey (London/GB): Mechanisms of microtubule – cytoskeleton dynamics studied in vitro
M. Vartiainen (Helsinki/Fl): Dynamics of nuclear actin
W. Witke (Bonn): Functions of the actin cytoskeleton in neuron physiology

15³⁰–19⁰⁰ **Plenary Session 5: Stem cells**
Chairs: B.K. Fleischmann (Bonn), M. Zenke (Aachen)
O. Brüstle (Bonn): From pluripotent stem cells to neurological disease modeling
M.A.G. Essers (Heidelberg): Activation of dormant hematopoietic stem cells by IFN α or the endotoxin LPS

34th DGZ ANNUAL MEETING

B.K. Fleischmann (Bonn): Potential and pitfalls of cellular replacement approaches in the heart
H. Schorle (Bonn): Factors regulating early lineage specification; programming (and reprogramming) murine trophoblast cells
M. Zenke (Aachen): Pluripotency and stem cell differentiation

from 19³⁰ **Get Together**

Saturday, 2 April 2011

08³⁰–11³⁰ **Symposium 9: Cellular immunity**
Chairs: W. Kolanus (Bonn), K. Rajewsky (Boston/USA)
W. Kolanus (Bonn): ARF-GEFs and immune functions
T. Mempel (Charlestown/USA): Cellular interactions in the adaptive immune system
K. Rajewsky (Boston/USA): Physiological and viral signals controlling survival, proliferation and transformation of antibody forming cells
M. Reth (Freiburg/Br): Islands on the membrane: How to keep the B cell antigen receptor silent? and speakers selected from the abstracts

08³⁰–11³⁰ **Symposium 10: Cell biology of addiction**
Chairs: A. Zimmer (Bonn), B.L. Kieffer (Illkirch/F)
B.L. Kieffer (Illkirch/F): Protracted abstinence from drugs of abuse
I. Kitchen (Gilford/GB): mGluR5 receptors and addiction neurobiology
R. Maldonado López (Barcelona/E): Endocannabinoid system and drug addiction
A. Zimmer (Bonn): Transcriptional profiles after acquisition of nicotine operant behaviors: separating drug effects from the anticipation of its effects and speakers selected from the abstracts

08³⁰–11³⁰ **Symposium 11: Proteostasis**
Chairs: J. Höhfeld (Bonn), F. Melchior (Heidelberg)
Z. Elazar (Rehovot/IL): Mechanism of autophagosome biogenesis
R. Hampton (La Jolla/USA): Protein quality control in cellular regulation and stress management
J. Höhfeld (Bonn): Chaperone-assisted degradation – degrade to maintain
E. Krüger (Berlin): Regulation of degradation capacities of the proteasome in response to changing proteolytic requirements and speakers selected from the abstracts

08³⁰–11³⁰ **Symposium 12: Cell adhesion**
Chairs: R. Fässler (Martinsried), J. Ivaska (Turku/FI)
R. Fässler (Martinsried): Analysis of integrin signaling using mouse genetics
C. Grashoff (Charlottesville/USA): Analysing the spatiotemporal regulation of integrin-dependent force transduction
J. Ivaska (Turku/FI): Negative regulation of cell adhesion
G. Serini (Candiolo/I): Regulation of integrin function by semaphorin receptors and speakers selected from the abstracts

11³⁰ **Lunch / Poster Session II** (continued)

11³⁰–12⁰⁰ **DGZ Poster Awards**

12³⁰–15³⁰ **Symposium 13: Molecular mechanisms of ageing**
Chairs: M. Hoch (Bonn), C. Englert (Jena)
D. Bohmann (Rochester/USA): Oxidative stress defense, aging and stem cells
C. Englert (Jena): A short-lived fish as a new model of age research
M. Hoch (Bonn): Cross regulation of metabolism, innate immunity and aging
J. Lingner (Epalinges/CH): Telomeric repeat containing RNA (TERRA) and the regulation of telomerase and speakers selected from the abstracts

12³⁰–15³⁰ **Symposium 14: Non-coding RNAs**
Chairs: G. Schratt (Marburg), E. Izaurralde (Tübingen)
E. Izaurralde (Tübingen): Mechanisms of miRNA-mediated silencing
K.S. Kosik (Santa Barbara/USA): miRNA and the diversification of cellular phenotypes
G. Meister (Martinsried): Regulation of small RNA-guided gene silencing
G. Schratt (Marburg): MicroRNA function in neurons and speakers selected from the abstracts

12³⁰–15³⁰ **Symposium 15: Intermediate filaments: novel functions**
Chairs: T. Magin (Leipzig), R. Leube (Aachen)
M. Labouesse (Illkirch/F): Extrinsic mechanical tension promotes hemidesmosome maturation
R. Leube (Aachen): Cross talk between cell adhesion and cytoskeletal filaments in cell and tissue function
T. Magin (Leipzig): Cytoskeletal control of cell architecture, signaling and immune regulation
C. Stewart (Singapore/SG): Lamins in development, disease and aging

Detailed scientific programme: www.dgz2011.de

General Information

The main train station in Bonn is located right next to the venue and the campus and can be reached within 10 minutes walking.

In cooperation with Deutsche Bahn (DB) the Conventus Congressmanagement & Marketing GmbH offers special conditions in context with the DGZ. More detailed information is indicated on the conference website www.dgz2011.de.

Public Transport: Tram Routes 16, 18, 63 and 66: Take the tram from station „Bonn Hbf“ (Main station) in direction of Bad Godesberg and exit at station Universität/Markt. Departs every 15 minutes.

Fare: Please note that the registration fee already includes free public transport within Bonn during the conference.

By car: The main building of the university and the city centre can be reached via the motorways 555, 562 and 565 as well as the highways 9 and 56.

Parking: Vehicles can be parked in the underground car park “Universität Bonn”. There will be tickets for congress participants at a reduced rate (9 EUR per day) which are “release tickets”. That means, when entering the entrance to the underground car park you will be asked to take a ticket from the ticket machine. You can discard the ticket right after. For leaving use the conference tickets, which are available at the Check In. More information to car parks in Bonn can be found on www.parkhaus.org.

Map of Bonn



Source: Kartengrundlage: Kataster- und Vermessungsamt der Bundesstadt Bonn, 2011

THE MANY FACES OF ACTIN

Cora-Ann Schoenenberger,
Hans Georg Mannherz and Brigitte M. Jockusch

Zusammenfassung

Dieser Aufsatz behandelt schwerpunktmäßig die vielfältigen Aktivitäten von Aktin. Ausgehend von historischen Befunden zur Aktin-Struktur betont er die enge Beziehung zwischen Struktur und Funktion. In diesem Kontext wird besonders auf unkonventionelle Aktin-Konformationen hingewiesen. Deren Existenz in lebenden Zellen ist bisher unvollständig dokumentiert, sie scheinen aber für supramolekulare Aktin-Assemblierungen mit besonderen physiologischen Funktionen bedeutsam zu sein, zum Beispiel für die di-

versen Aktivitäten von Aktin im Zellkern, die bisher noch weitgehend unverstanden sind.

Summary

This article presents the multiple activities of actin. Starting from some historical findings on the actin structure, it emphasizes the close relation between structure and function. In this context, we point to unconventional actin conformations. Their existence in living cells is not yet well documented, however, they seem to play a special role in

the supramolecular patterning that underlies some of the physiological functions of actin and may contribute to actin's diverse activities in the nucleus that are poorly understood so far.

Actin is a universal protein expressed in all organisms of the present day world. The prototype was originally identified in mammalian muscle. It is quite fascinating to read about the early observations by the physiologists Kuehne (1859) in Heidelberg and Halliburton in London (1887) on contractile "clots" obtained from muscle extracts that were then refined by the biochemists Straub and Szent-Györgyi and led 1942 to the identification of "actin" as the "substance" capable of activating the ATPase of myosin (reviewed in (Schleicher and Jockusch, 2008)). In the following years, many isoforms and homologues were detected and characterized in cells and tissues from all phyla, most recently in prokaryotes. According to their closely related structures, manifested in the "actin fold" (Kabsch and Holmes, 1995), actins and actin-related proteins (ARPs) are considered members of the large (and still growing) actin-like ATPase superfamily. Sequence homology of actin family members is primarily seen in their ATP-binding pocket that is also present in families of Hsp70 chaperones and sugar kinases, allowing for a hypothesis on the common evolution of all these proteins (Bork et al., 1992).

Looking at the history of actin's impact on biological and biomedical research one notices periods when it was more or less "fashionable" to study properties and functions of actin. Reminiscent of the geological periodicity of ice ages, there are heights of actin research separated by troughs, and this is reflected in the number of conferences, publications and successful grant applications. In the beginning, myosin was the more interesting protein for many years. Indeed, at the famous 1971 Cold Spring Harbor Symposium on "The mechanism of muscle contraction"

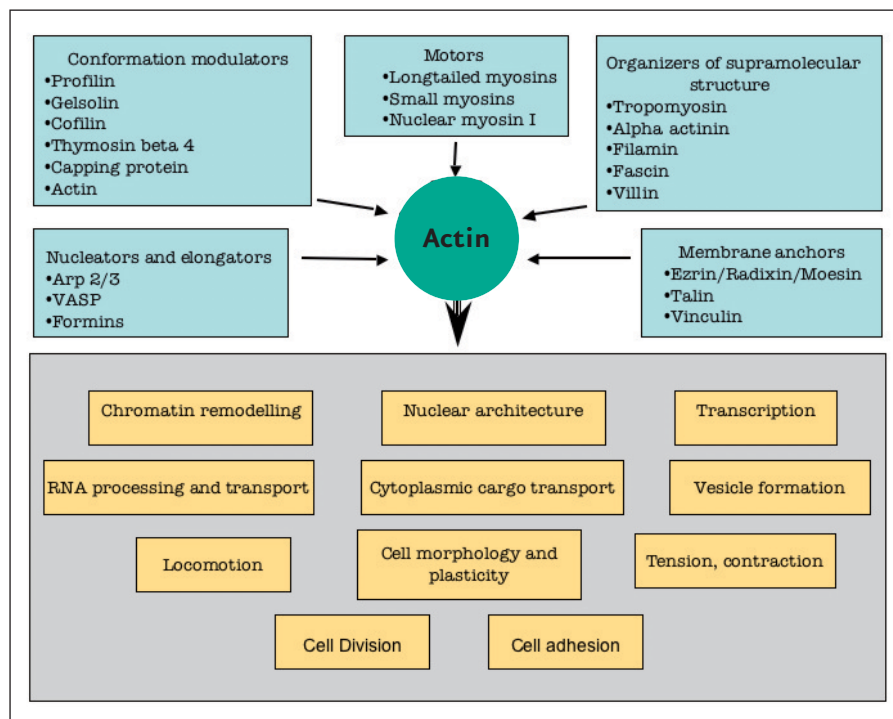


Figure 1: The many faces of actin.

Actin is indispensable for many different processes in the nucleus and in the cytoplasm of the eukaryotic cell (depicted in yellow). To perform this multitude of tasks it has to interact with a wide variety of actin-binding proteins (ABPs) – including itself - that regulate its conformation, polymerization and supramolecular structure in many ways. So far, approximately 150 ABPs have been identified of which only a selection is listed (blue boxes).

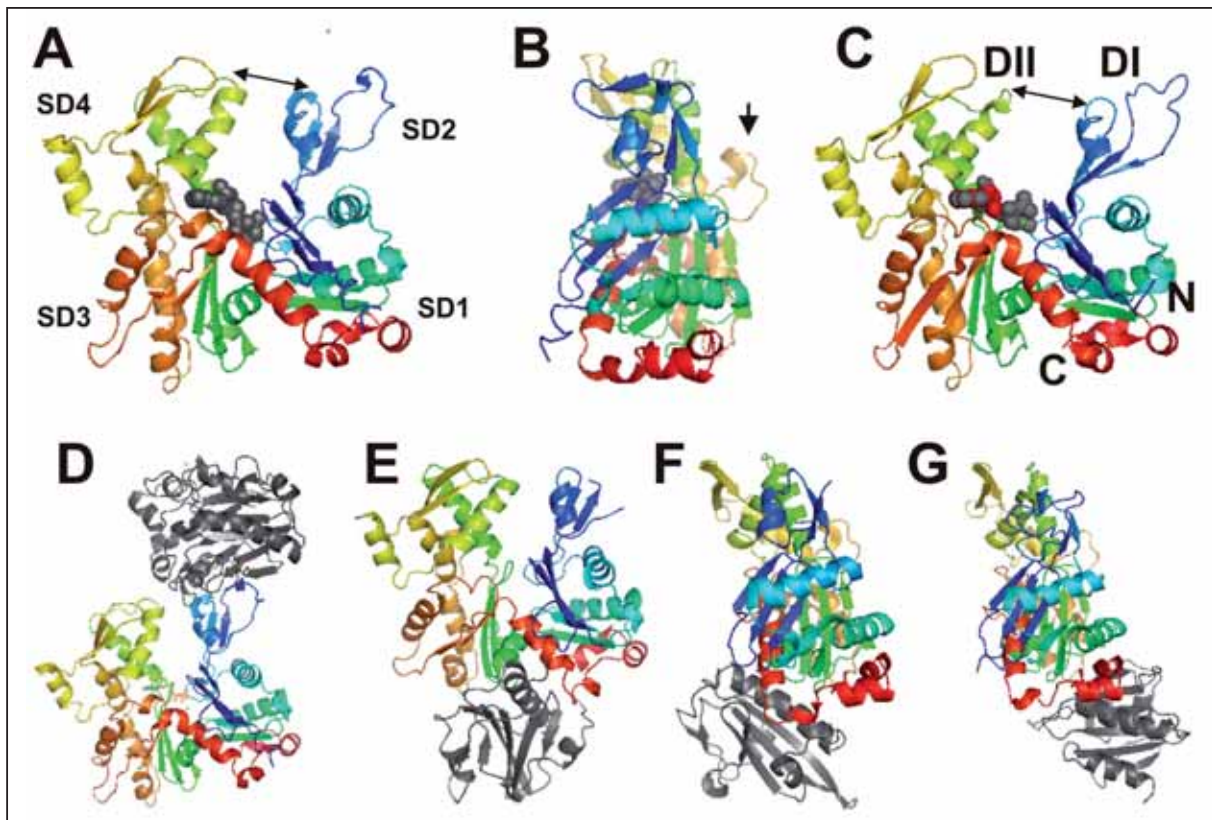


Figure 2: The atomic structure of actin and actin-ligand complexes.

(A). Standard view of actin derived from the actin:DNase I complex (Kabsch et al., 1990) in ribbon representation. The two main domains DI and DII (indicated in (C)) are divided into two subdomains, termed SD1 to SD4. N- and C-terminus (N and C, shown in (C)) are located in SD1. ATP is bound at the bottom of the cleft between the two main domains. The adenosine moiety is directed towards domain II and the phosphate chain towards domain I. (B) Gives a side view of actin with the front view (shown in A and C) pointing to left. Note the protrusion at the back representing the “hydrophobic plug” (arrow). (C) Standard view of actin derived from the actin:profilin complex (Chik et al., 1996), showing the more “open” conformation (compare the width of the cleft between (A) and (C), as indicated by the double headed arrow). (D - E) Atomic structures of actin complexed with DNase I (D), gelsolin segment 1 (McLaughlin et al., 1993) (E, front view and F, side view) and profilin (Schutt et al., 1993). (G, side view). The upper area of actin (the DNase I binding site) defines the surface area of the slow growing end in F-actin, the lower part, where gelsolin binds to, the fast growing end. The mode of gelsolin segment G₁ binding, i.e. placing a helix with hydrophobic residues into the hydrophobic region between SD1 and SD3 (E) is used by a number of other actin binding proteins like Vitamin D binding protein, thymosin β ₄ and possibly many of the WH2-domain proteins, like ciboulot and the actin nucleator protein Spire. Profilin attaches further to the rear (G).

there were only few contributions about actin, probably the most relevant being the complete sequence of muscle actin by Marshal Elzinga and John Collins (published in (Elzinga et al., 1973)). However, at this very meeting there was already a session held on “Contractile proteins in non-muscle tissue” with six contributions that heralded the subsequently rapidly evolving area of cytoskeletal research. Right now, we are experiencing a new height in actin research, as for example demonstrated by the fact that the “Deutsche Gesellschaft für Zellbiologie” orga-

nized a special International Meeting on “Actin dynamics” (Jena, September 2010). This conference was strongly focussed on the role of actin “nucleators” in the dynamics of actin supramolecular structures. More meetings on actin are announced for 2011 (in Stresa, Italy, again on “actin dynamics”, and in Stockholm, on “nuclear actin”).

It goes without saying that the actin world comprises significantly more than can be dealt with in a particular conference. In a eukaryotic cell, actin-based components govern vital processes in both, the cytoplasm

and the nucleus (see Figure 1). A plethora of different proteins that are still increasing in number orchestrate spatio-temporal control of these phenomena. We yet need to unravel how a rather small, compactly folded protein like actin manages to interact with so many different ligands. A simplified and by far not complete scheme of the different classes of actin binding proteins (ABPs) is presented in Figure 1. Structural studies have indicated that binding of partners to the surface of actin leads to conformational changes in actin itself and/or in polymeric actin assem-

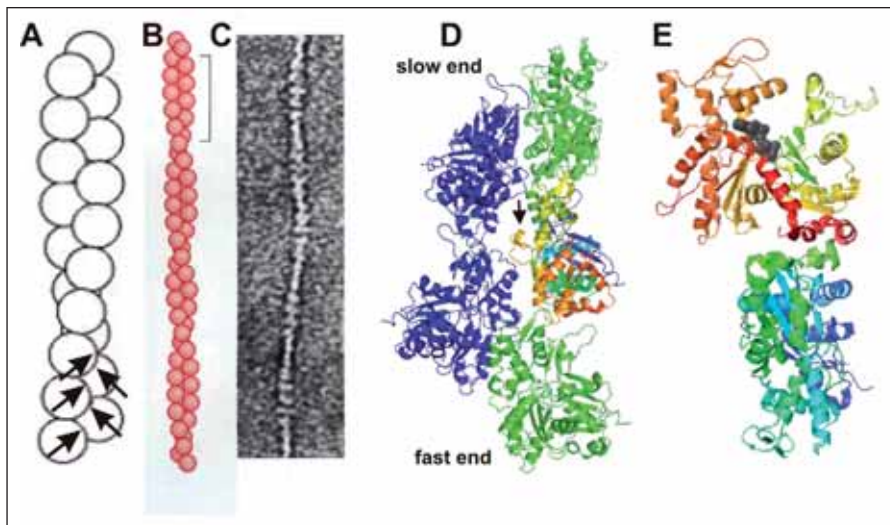


Figure 3: Polymeric and dimeric actin structures.

(A) Simple helical representation of F-actin with subunits shown as round spheres. The arrows indicate the directions of the one-start genetic helix. (B) Similar representation as in (A) with the half-pitch of about 360 Å indicated by the bracket. (C) Electron micrograph of F-actin after negative staining. (D) Atomic structure (Lorenz et al., 1993) of F-actin showing five subunits in ribbon representation with the central one coloured in spectral mode. The arrow points to the hydrophobic plug mediating the inter-strand contact. The orientation of the subunits towards a fast and slow growing end was defined by the DNase I and gelsolin segment 1 (G1) binding sites, respectively, see Fig.2. (E) Atomic structure of the bipolar LD complex. The ATP molecule in the cleft of the upper unit and the end of the polypeptide chain (c) are indicated. The subunits are oriented in such a manner that polar filament growth is not possible (Bubb et al., 2002).

blies. Further variability in the actin world is achieved by several actin isoforms that are developmentally regulated or expressed in different tissues, where they show differential affinity for the same ligand protein. In addition, actin may be posttranslationally modified, for example by Ser/Thr or Tyr phosphorylation, acetylation, acylation or ubiquitination. ADP-ribosylation of specific residues of the host actin is another strategy followed by several pathogenic bacteria to improve their survival (Lang et al., 2010). Actin shares its structural plasticity with another important, ubiquitously expressed protein, tubulin (see Manfred Schliwa and Ralph Gräf, *Cell News* 36/4, 2010). Under physiological conditions, tubulin and actin show a preference for one particular supra-molecular structure that is the product of a polymerization process. For actin, this is the “conventional” double helical filament

called F-actin. Based on its structure and its dynamic behaviour, F-actin is engaged in the majority of actin’s functions. However, there is now growing evidence that there are other actin conformations that may also be involved in important cellular processes. One approach to understanding actin’s action in different cellular functions started with elucidating its molecular anatomy.

Struggling with the structure

In the second half of the last century, cytoskeletal proteins could already be isolated in large quantities and reasonable purity, allowing many laboratories to attempt crystallisation for the 3D-structure determination. Unfortunately, actin turned out to be an extremely difficult candidate – at the salt condition needed for crystal growth, it eluded crystallization by rapidly polymerizing into filaments. The breakthrough came

from a fortuitous observation: it was Uno Lindberg who crystallized a protein complex from bovine spleen that was able to inhibit deoxyribonuclease I (DNase I; (Lindberg, 1967)), not knowing at that time that the proteinaceous inhibitor of DNase I would later turn out to be actin (Lazarides and Lindberg, 1974). The biological significance of the interaction of DNase I, a secreted enzyme, and the intracellular structural protein actin is still a mystery, although the extremely high affinity suggests functional importance. In addition to actin, the inhibitory complex isolated from spleen contained another protein termed profilin. Crystallization of the 1:1 complex of cytoplasmic actin and profilin was reported in 1976 (Carlsson et al., 1976). Both studies demonstrated that when prevented from polymerization by a protein ligand, actin can be forced to form crystals. Subsequently, crystallisation of actin complexed with bovine DNase I was reported (Mannherz et al., 1977). However, it took another fourteen years to arrive at a low resolution structure of the actin:DNase I complex that clearly defined the actin and DNase I moieties (Suck et al., 1981) and ten more years until the high resolution (2.8 Å) structure of the actin:DNase I complex was completed (Kabsch et al., 1990). Structure determinations of chemically modified actins, different isoforms, actin related proteins (ARPs) or bacterial homologues, mutated variants, or in complex with various actin binding proteins followed in rapid succession. Meanwhile the atomic structure of actin has been solved about 80 times. All structures determined so far basically confirmed the initially proposed structure of skeletal (alpha-) actin in complex with DNase I (Kabsch et al., 1990).

The molecular anatomy of G- and F-actin

It is undisputed that the diversity of actin’s physiological functions are based on its structure and thus we recapitulate some of

the basic facts. The actin molecule (G-actin) is composed of a single polypeptide chain of 375 residues that is folded into two almost equally sized main domains termed I and II. These are separated by a deep cleft that harbours a binding pocket for small molecules like nucleotides or phalloidin (see below). Each main domain can be further divided in two subdomains (SD), resulting in SD1 – SD4. Figure 2A shows the standard (“butterfly”) front view of actin. All subdomains contain a central beta-sheet surrounded by a varying number of alpha-helices. G-actin is a flat molecule as shown by the side-view in Fig. 2B, with dimensions of about 55 x 55 x 35 Å. The bound nucleotide is located at the bottom of the deep cleft (Figure 2A). Here the peptide chain crosses twice between the two main domains so that the N- and C-terminus are located in SD1. The link region at the base of the cleft forms a flexible hinge region, allowing rotational movements of the two main domains relative to each other.

DNase I mainly binds to the so-called D-loop in SD2 (Figure 2C), whereas gelsolin segment 1 (G1) and profilin attach to the bottom at the cleft between SD1 and SD3 (Figure 2E-G, respectively).

In muscle, most of the filamentous actin polymers (F-actin) are the major constituent of the thin filaments of the sarcomere and cyclically interact with myosin heads to produce contractile force (see for example Dieter Fürst et al., Cell News 36/3, 2010). Analogous actomyosin-based elements are present in all non-muscle cells. To understand the molecular basis of force production, and cellular motility, much effort has been undertaken to elucidate the orientation of the actin monomers within F-actin and to define structural alterations associated with filament formation. F-actin can be described in two ways: as a two-start right-handed double helix with a half-pitch of 360 Å or as a one-start left-handed genetic helix with a rotational translocation of 167° and an axial

rise of 27.5 Å (Figure 3A-D). Using these helical parameters and based on fibre diffraction data of orientated gels, Holmes et al. proposed a model for F-actin which was published back-to-back with the 3D-structure of the DNase I:actin complex (Holmes et al., 1990). In this initial model SD3 and SD4 were oriented towards the filament axis, whereas SD1 and SD2 were on the outside enabling in particular SD1 to interact with F-actin binding proteins. This model was then refined by allowing the subdomains to rotate individually in order to obtain the best calculated fit to the fibre diffraction pattern (Figure 3D, Lorenz et al., 1993)).

A recent study using high magnetic fields to obtain optimal alignment of F-actin filaments within the orientated gels has increased the resolution of the F-actin structure to about 4 Å (Oda et al., 2009). The main result of this work came as a surprise: apparently, there is a considerable conformational difference between the G- and F-state of actin

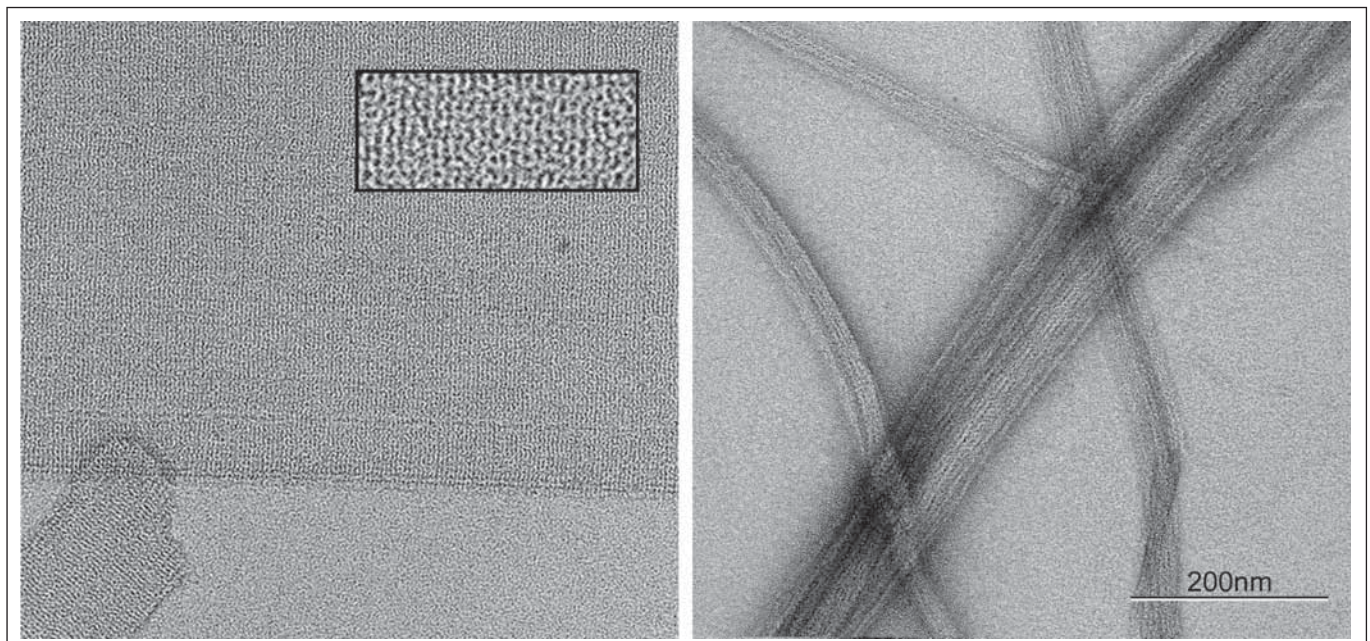


Figure 4: Ultrastructural analysis of actin polymorphism in vitro.

The electron micrograph on the left shows negatively stained, gadolinium-induced crystalline sheets prepared from rabbit skeletal muscle actin. The inset at higher magnification reveals the 2D crystal lattice of this type of sheet. In the crystal, contacts occur between actin subunits that are packed in an antiparallel orientation. Paracrystalline bundles (right panel) of laterally aligned F-actin filaments are formed by adding 10-50 mM MgCl₂ to G-actin. Similar to the packing in the sheets, subunits of interfilament dimers formed by neighboring filaments with opposite polarity are also in an antiparallel orientation.

subunits. So far the crystallographic analyses had suggested that the actin conformation persisted rather robustly. However, the new data indicated a 20° propeller-like rotation of the two main domains relative to each other making the molecule much flatter (Oda et al., 2009). This large conformational change clearly confirmed many of the previously assumed actin-actin contacts and supported the notion that the longitudinal intra-strand contacts are much stronger than those between the two helical strands. While the intra-strand contacts are formed by a number of loops, the contacts between both strands are mainly established by the so-called hydrophobic plug (as seen in the side view, Figure 2B, arrow).

Diffraction data might generate the impression that the described structures are static and fixed. Comparing the actin structures obtained by X-ray diffraction analyses did not indicate substantial conformational changes, although this might be due to crystallographic contacts, stabilizing a particular conformation. Nevertheless, it did not come as a great surprise when electron microscopy of negatively stained *in vitro* polymerized fi-

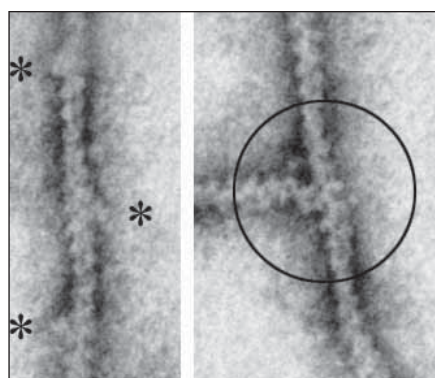


Figure 5: LD incorporation alters filament morphology. Scanning transmission electron microscopy analysis of negatively stained hybrid filaments from copolymerization of crosslinked LD with G-actin in the presence of phalloidin. The incorporation of LD leads to frequent lateral protrusions (asterisks) that extend to approximately half of the filament diameter. Hybrid filaments form interfaces (circled) that look like branching sites.

laments revealed that slight conformational changes exist within F-actin. The rotational translation between actin subunits (twist) and their tilt differed between individual filaments or varied even within a single filament, suggesting that multiple conformations can coexist within a single F-actin filament (Bremer et al., 1994; Galkin et al., 2010). Thermal fluctuations and the dynamic behaviour of the actin subunits, even when incorporated into the filament might allow these changes in orientation that can be recorded as “snapshots” of different states.

There is more than G- and F-actin

Already in the early days of actin structure determination, dos Remedios and Dickens described that in the presence of trivalent lanthanide cations (for example gadolinium), purified actin forms ordered assemblies as those shown in Figure 4 (left panel) that are not composed of F-actin (dos Remedios and Dickens, 1978). Besides providing a model of the actin monomer in projection to 15Å resolution, crystalline actin arrays revealed subunit contacts (Aebi et al., 1981; Steinmetz et al., 1998) that are distinct from those observed in mature filaments. Considering that contacts between molecules in the crystalline state could be fortuitous, the antiparallel arrangement of actin subunits found in the gadolinium-induced sheets may not relate to actin’s natural biological forms. Although at first sight, an antiparallel orientation of actin subunits appears to be inconsistent with the F-actin filament geometry, one has to keep in mind that filaments represent an ensemble of different states with quite some flexibility in terms of subunit interactions (Steinmetz et al., 1997b). In particular, the conformation of subunits at the filament ends or at branching sites remains an open question. Moreover, it is possible that such an arrangement exists in solution, despite its incompatibility with the F-actin structure.

Consistent with this notion, chemical cross-linking experiments have demonstrated the

transient formation of an antiparallel actin dimer at the onset of polymerization *in vitro* (Millonig et al., 1988). Although absent in F-actin filaments at steady-state, this so-called ‘lower dimer’ (LD) incorporates into growing filaments and has been correlated with the ragged and branched filament morphology at initial stages of polymerization (Steinmetz et al., 1997a). Consistently, co-polymerization of isolated LD and G-actin produced hybrid filaments with prominent lateral protrusions (Figure 5). Intriguingly, ultrastructural analysis revealed a high incidence of filament ends interfacing with other filaments like one would expect at branching sites. Antiparallel actin dimers have also been found in association with actin-binding proteins (ABPs). For example, in the presence of calcium gelsolin forms a ternary complex with two actin monomers that nucleates actin polymerization and caps the barbed end of filaments (Yin, 1987). Chemical crosslinking of this ternary complex yielded a single dimeric actin species with an electrophoretic mobility indistinguishable from that of LD (Hesterkamp et al., 1993). Consistently, isolated crosslinked LD bound to gelsolin in 1:1 stoichiometry, but this complex did not nucleate filament assembly. Binding of the toxin latrunculin to actin inhibits filament polymerization but does not prevent the formation of LD and has been used to accumulate this species for structural analysis (Bubb et al., 2002). LD-like contacts are also present in experimentally obtained actin assemblies such as crystals and paracrystalline actin bundles shown in Figure 4 (right panel) where neighbouring filaments have opposite polarities (Steinmetz et al., 1997b). Taken together, these observations strongly argue for a role of the LD in supramolecular patterning of actin into bundles and networks (Schoenenberger et al., 2002).

Although reports on antiparallel actin dimers appear recurrently, it still leads a marginal existence. One of the occasions where it comes into view is when the mushroom toxin

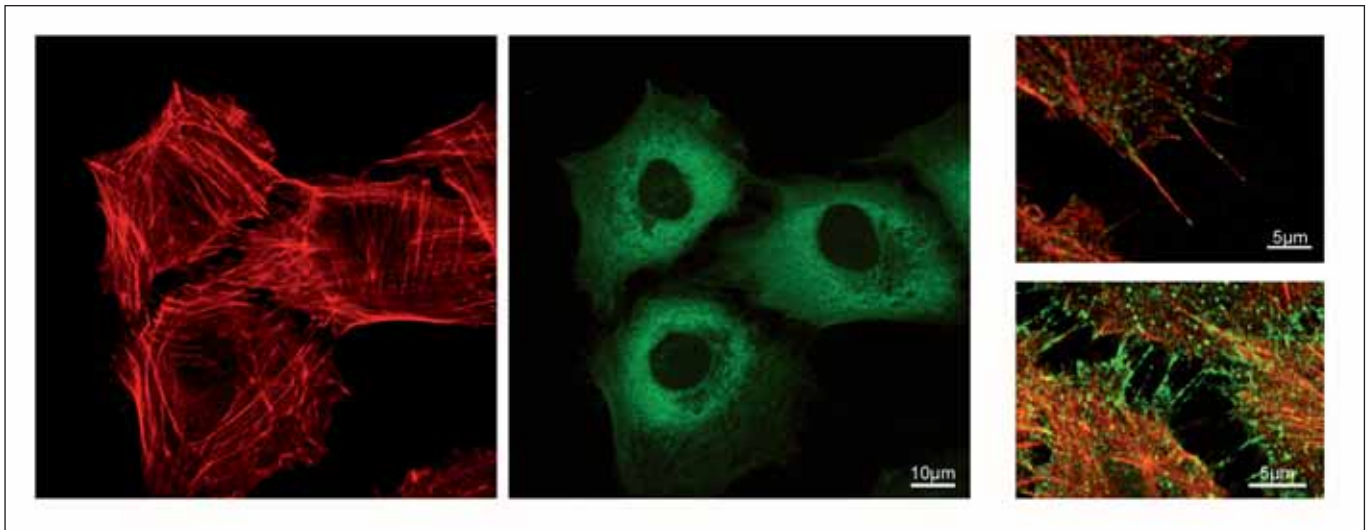


Figure 6: Multiple structural states of actin exist in the cytoplasm.

The mushroom toxin phalloidin, which specifically binds to filamentous F-actin, reveals a prominent actin stress fiber network (red) throughout the cytoplasm of rat fibroblasts. Simultaneous immunofluorescence labeling with the monoclonal antibody 1C7 (green), which only binds to non-filamentous actin (Schoenenberger et al., 2005) shows that a significant amount of the cellular actin is in this state. Morphological features, such as filopodial extensions exhibit a specific supramolecular pattern of actin in different structural states.

phalloidin fails to reveal the structural identity of a cellular actin species. The most prominent example is the conformation of nuclear actin (see below). Although the involvement of actin in diverse nuclear processes such as chromatin remodelling, transcription regulation and long-range chromatin organization impose its presence in the nucleus, as was proposed more than thirty years ago (see for example (Scheer et al., 1984)), the structural states underlying these functions remain enigmatic. Uncommon forms of actin such as LD have been suggested as candidates that could possibly fill this gap (Jockusch et al., 2006; Pederson and Aebi, 2002). Indirect support for the notion that an LD-related actin might be present in the nucleus was provided by the treatment of mast cells with latrunculin that led to an accumulation of different conformational states of actin in the nucleus (Pendleton et al., 2003). However, the transient nature of LD has hampered studying its functional significance in a complex cellular environment.

Actin dynamics rely on two different mechanisms.

Undoubtedly, the prevalent form of actin in the eukaryotic cytoplasm is F-actin. Hence, this form was studied most extensively and thereby revealed a highly dynamic behaviour. Two synergistic components are essential for this property: the intrinsic asymmetry of the actin subunit and the interactions with actin binding proteins.

The dynamic nature of F-actin is not confined to the rather subtle changes described above. The structural asymmetry of the subunits (see Figure 2) and the rapid polymerization of ATP-charged subunits combined with the rather slow ATPase activity of actin lead to filaments with a fast growing and a slow growing end. Consumption of ATP-actin at the fast growing end results in a critical level of subunits where further ATP-actin addition is balanced by the loss of ADP-actin at the slow growing end. This leads to filaments of constant length but with a dynamic behaviour: the subunits travel from the fast to the slow growing end. This “treadmilling” was discovered in the test tube by Albrecht

Wegner thirty five years ago (Wegner, 1976) and is a property also of the other major cytoskeletal element in cells, the microtubules (see Manfred Schliwa and Ralph Gräf, Cell News 36/4, 2010).

In cells, F-actin treadmilling is tightly controlled by protein ligands. The ability of actin to interact with so many different proteins (about 150!) must have imposed strong evolutionary pressure to conserve its interacting surfaces. Protein ligands can bind to either end of the filament, they may provide subunit assemblies for the initiation of polymerization (nuclei), and they can facilitate or inhibit filament elongation and stability (see Figure 1). It is not within the frame of this article to describe all the different ligands specific for either G- or F-actin, but a few examples may highlight the great variability and stunning complexity of the consequences of their binding to actin.

Changes of the twist of F-actin has been noticed after binding of proteins of the ADF/cofilin family (McGough et al., 1997) or thymosin beta 4 (Ballweber et al., 2002). This is in line with the observation that these

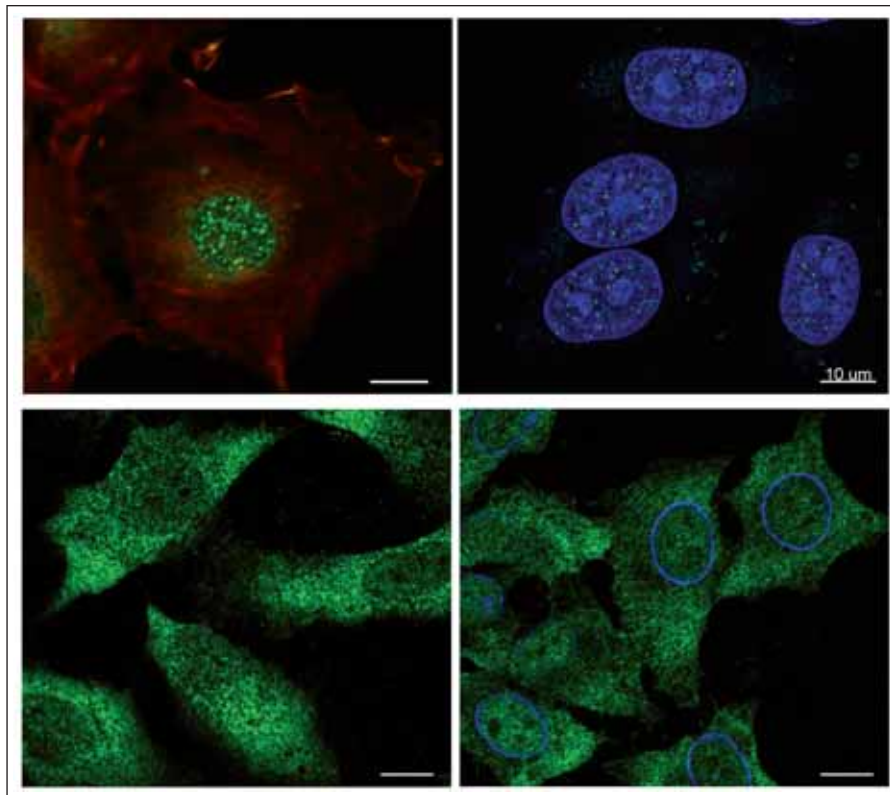


Figure 7: Conformation-specific antibodies reveal different forms of actin in the nucleus of mammalian cells.

The monoclonal antibody 2G2, which was raised against a profilin-actin complex (Gonsior et al., 1999) shows a discrete dot-like labeling pattern in the nucleus of HeLa cells (top), whereas the staining with 1C7, a monoclonal produced against cross-linked lower dimer actin (Schoenenberger et al., 2005), is fine punctate and disperse (bottom). The image shows confocal sections through the plane of the nuclei that are indicated in blue by Draq5 DNA dye (top) or by fluorescent labeling of the inner nuclear membrane protein emerin (bottom). 568-phalloidin reveals filamentous F-actin (red) in the cytoplasm.

proteins alter the conformation of the subunit: they induce a narrowing of the cleft between domains I and II in G-actin that harbours the nucleotide-binding pocket. In contrast, profilin widens this cleft. Hence, the differential binding of these proteins leads to a more “open” or more “closed” configuration (compare Figure 2A with C), which may influence the ATP exchange rate and the nucleotide hydrolysis, and thus the stability of the filaments (Paavilainen et al., 2008). In this context, profilin plays a special role as a multitasking ligand: it binds to actin, phospholipids and to the polyproline stretch contained in many nucleator/elongator proteins

(Jockusch et al., 2007). Therefore, it can deliver and concentrate polymerization-competent actin close to the plasma membrane for the growth of new actin filaments. However, growth can also be negatively regulated by blocking the fast growing end with specific capping proteins (Cooper and Sept, 2008). In summary, the intrinsic asymmetry of actin and the known interactions between actin and different ligand proteins explain to a large extent the behaviour of F-actin in cells. However, so far nothing is known about the consequences of the binding of ligand proteins like gelsolin to unconventional actin forms like LD.

Actin localization is a collectors' item

Ten years after the 1971 Cold Spring Harbor Symposium on “The mechanism of muscle contraction” the focus on actin had shifted to non-muscle cells. At the 1981 Cold Spring Harbor Symposium on “Organization of the cytoplasm” exciting new findings on the presence, organisation and physiological relevance of the cytoskeleton were reported. Research on the actin cytoskeleton had been spurred by actin-specific antibodies (Lazarides and Weber, 1974). In the following decades, well-characterized polyclonal and monoclonal antibodies against actin became available, although their production and characterisation was - and still is - far from trivial since actin, as a highly conserved protein, is a notoriously weak immunogen. These antibodies, used in indirect immunofluorescence and microinjection revealed the presence of filamentous actin and specific actin assemblies (“patches”) in single cells from yeast and amoebae, and in cells cultured from insects, vertebrates and plants. In addition, the advent of fluorescently labelled phalloidin (Wulf et al., 1979) further aided F-actin visualisation in cells as demonstrated in Figure 6. Later on, transfection of cells with fluorescent actin fusion proteins, video microscopy and immuno electron microscopy confirmed and refined this view. In the cytoplasm of the eukaryotic cell, filamentous actin is present in cortical webs underlining motile cells, it exists in bundles engaged in cell adhesion, it forms the structural core of cell appendices in vertebrate filopodia, microvilli and neurites, and in germinating plant pollen, it coats endocytic and phagocytic vesicles and it is a prominent component of the contractile ring in mitotic and meiotic cells.

In the nuclear compartment, antibodies showed actin at the lining of the inner nuclear membrane, as part of the topographical scaffold of chromatin, as a component of the gigantic enzyme complexes involved in

chromatin remodelling and of all three RNA polymerases during transcription (reviewed in (Jockusch et al., 2006) and in (Schleicher and Jockusch, 2008), see also Figure 1).

Unravelling actin's functional diversity

The enormous amount of information on the location of actin in eukaryotic cells raises some general questions: is actin's function at all these sites linked to contractile phenomena? If so, is the site-specificity of different contractile processes defined solely by different motors, i.e. different myosins, or are different sets of actin ligands involved? Does actin have functional abilities beyond its contractile property that await discovery?

In the cytoplasm, actin can create motility by two different mechanisms. In cellular protrusions like filopodia, microvilli and pollen tubes, the polarized actin filaments are oriented with their fast growing end towards the plasma membrane. Powered by the addition of polymerization-competent actin subunits they generate sufficient tensile strength to push the plasma membrane forward, while myosins, the classical actin motors, are absent. The velocity of actin filament growth observed in these locations, and also in the fine webs existing in the distal part of the sheet-like extensions of motile cells, the lamellipodium, exceeds that calculated for pure treadmilling (Lai et al., 2008). In contrast, various myosins contribute to force production and motility in the more proximal part of the lamellipodium, in the actin bundles comprising the contractile ring, in the apical belts of epithelial cells and in the adhesive structures of adherent and tissue-forming cells, in membrane invaginations during phagocytic or endocytic cup formation. In both types of actin-based motility, proteins of the nucleator/elongator families (see Figure 1) deliver polymerization-competent subunits to the growing filament. Moreover, they link the actin system to the outside world, as they are also

down-stream effectors of various signalling pathways. Furthermore, they are themselves subject to activity regulation by posttranslational modifications or other proteins (see for example (Stradal et al., 2004)) before they can stimulate F-actin growth. Since the identification of Arp2/3, a heptameric complex containing two actin-related proteins (ARPs; (Machesky et al., 1994)) and VASP (Reinhard et al., 1992) that act primarily as nucleator and elongator, respectively, many different proteins with analogous functions have been identified (Chesarone and Goode, 2009).

What about actin in the nucleus? Here, our knowledge is very limited. There are numerous reports showing that nuclear actin optimizes the performance of all three RNA polymerases and actin's presence in chromatin remodelling complexes, at the inner nuclear membrane, in filaments traversing the nucleus must be important, since it persists throughout evolution. However, the conformational state of nuclear actin is unknown and thus the relation between structure and function, as described above for actin in the cytoplasm. Staining with fluorescent phalloidin, a specific and undisputed reagent to identify F-actin, has so far not revealed conventional actin filaments in nuclei under physiological conditions. This does not a priori exclude the existence of F-actin in the nucleus, as it is known that binding of proteins that change the twist of the filament, like cofilin, can interfere with phalloidin binding. Cofilin belongs to the actin binding proteins that have been identified in the cytoplasm and the nucleus. Furthermore, the identification of a specific nuclear myosin (NM1) that is expressed from yeast to man, and like actin, was found to be associated with transcription (Grummt, 2006), suggested that contractile forces between F-actin and myosin also play a role in nuclear functions of actin. However, some observations argue against this assumption. First, NM1 is a species of myosin that cannot form bipo-

lar filaments as would be required for actomyosin interactions leading to contraction. Second, NM1 has not always been found in the same nuclear protein complexes as nuclear actin.

Evidence that unusual conformations of actin are enriched in the nucleus and may be involved in at least some of its diverse nuclear functions evolved from studies with antibodies. Schoenenberger and collaborators have shown that conformation-specific antibodies are suitable tools to monitor distinct forms of actin in different cellular compartments (Schoenenberger et al., 2005). In contrast to phalloidin which exclusively binds to F-actin, the monoclonal antibody 1C7 recognizes predominantly non-filamentous forms of actin in the cytoplasm (Figure 6). It also detects a discrete form of actin in the nucleus that is clearly different from that recognized by 2G2 (Figure 7), a monoclonal antibody produced against profilin-actin complex (Gonsior et al., 1999). Similarly, subnuclear actin compartmentalization was also observed by these antibodies in plant cells (Cruz and Moreno Diaz de la Espina, 2009). Although cross-linked LD was used to generate 1C7, this antibody, whose epitope comprises a surface loop in SD4 of the actin molecule, may also react with other conformations where this epitope is exposed. Hence, the exact actin conformation detected by 1C7 in different compartments of the cell remains mysterious, and the questions raised above on the functional significance of actin's presence in so many diverse locations in the nucleus remain unanswered.

Outlook

The tasks for future researchers on actin are mainly twofold. First, it is obvious that we need to know more on the different supramolecular conformations actin adopts in cells, primarily in the nucleus, before we can understand its physiological functions at the mechanistic level, with all its consequences for cellular life. Second, a shift of our attention

from test tubes and cells to the actin world in organisms is imperative. This will include the detailed and profound functional analysis of actin and its regulatory proteins in tissues and during development of many different organisms. With the emergence of this area we will for sure be experiencing a few surprises: deleting specific ligands or tissue-specific isoforms of such ligands *in situ* may have different, unexpected consequences on the physiology.

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Uncovering the Secrets of VASP

Jan Faix

Background

The dynamic reorganization of the actin cytoskeleton allows the formation of highly specialized structures which mediate numerous cellular processes such as endocytosis, cytokinesis or cell migration [1]. The

movement of cells is for instance primarily powered by the insertional assembly of actin subunits at the barbed ends of actin filaments in the cell front and the simultaneous depolymerization at their pointed ends. The protrusive forces at the leading edge of the

cell generate a flat sheet-like extension with a densely branched actin array termed the lamellipodium and an unbranched lamellar array at the rear of the flat protrusion [2,3]. Lamellipodia that detach from the substratum frequently display a curly appearance and are referred to as membrane ruffles. The funnel-shaped phagocytic cups share many characteristic features with the lamellipodium and serve for the uptake of bacteria or other cells by professional phagocytes. Many cell types also form spiky finger-like protrusions called filopodia [4]. These highly dynamic structures contain compact bundles of several dozen of parallel actin filaments, typically measure a few micrometers in length, and are implicated in many important processes such as the guidance of neuronal growth cones, the zippering of epithelial sheets during morphogenesis and the adhesion to the substratum, other cells or even pathogens [5,6]. Contractile structures containing anti-parallel actin filaments are found in the lamellum, the cleavage furrow of dividing cells and stress fibers connecting focal adhesions [7]. Actin filaments also form a thin layer just underneath the plasma membrane called the cortical cytoskeleton to provide stiffness, structure, and shape to the membrane [8]. The major challenge we are facing in the actin field today is to identify the complete inventories of the participating proteins, determine their specific activities as well as their interplay with each other, and dissect associated signaling pathways in order to understand the molecular mechanisms underlying the formation of the more than 15 known cellular actin structures.

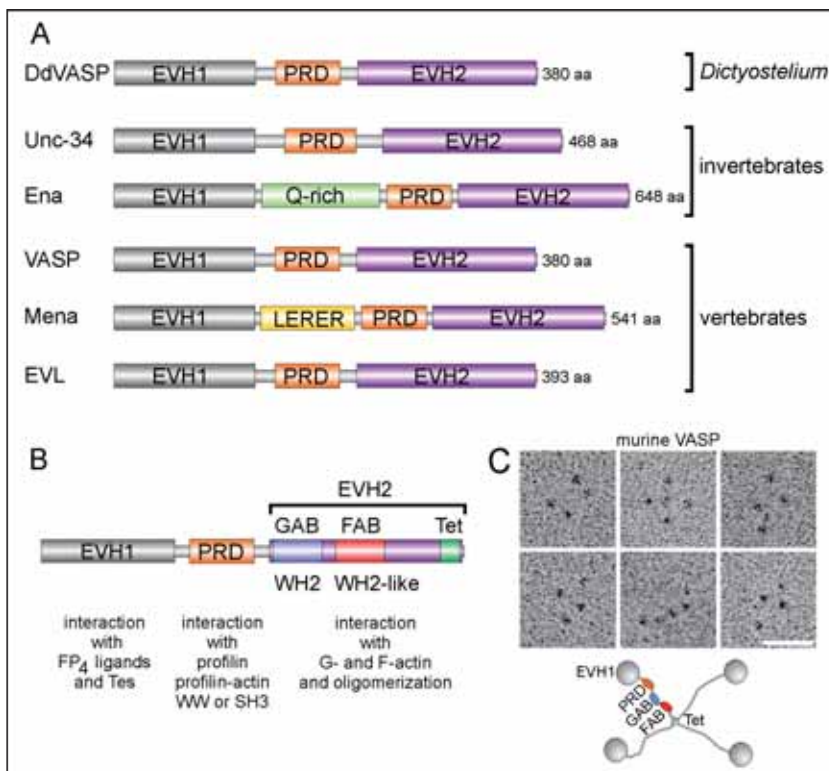


Fig. 1.: (A) The members of the best studied Ena/VASP proteins from different organisms including *D. discoideum* DdVASP, *C. elegans* Unc-34, and VASP or EVL (Ena/VASP-like) from vertebrates share a very similar domain organization encompassing an N-terminal EVH₁ (grey), a central proline-rich region (PRD, orange), and C-terminal EVH₂-domain (purple). *Drosophila* Ena (enabled) and Mena (mouse-Ena) additionally contain a Q-rich region (green) or LERER repeats (yellow) in between the EVH₁ and PRD, respectively. Numbers indicate amino-acid residues. (B) Structure/function relationship of Ena/VASP proteins. The globular EVH₁ region is required for subcellular targeting and can bind to proteins with FP₄-motif and also to LIM-motifs as found in the protein Tes. The central PRD binds profilin and profilin-actin complexes as well as SH₃- and WW-containing proteins. The EVH₂ domain encompasses a WASP-homology 2 domain (WH2) type G-actin binding (GAB) motif (blue), a WH₂-like F-actin binding module (FAB, red) followed by a tetramerization (Tet) domain (green), and is sufficient to drive actin assembly. (C) Electron microscopic images and derived cartoon of the highly flexible VASP tetramer. The VASP molecule consists of four globular domains, almost certainly representing the N-terminal EVH₁ domains. The PRD as well as the GAB and FAB motifs appear to be largely unstructured. Scale bar, 50 nm. Courtesy of John Victor Small and Guenther Resch.

Ena/VASP proteins are important regulators of actin assembly

Proteins of the Enabled/Vasodilator-stimulated phosphoprotein family (Ena/VASP) are key regulators of actin assembly and are ubiquitously expressed in motile eukaryotic cells. VASP (Vasodilator stimulated phosphoprotein) was initially identified as a PKA

substrate in platelets [9]. VASP, EVL (Ena/VASP-like) and Ena (Enabled) are grouped together in the conserved family of Ena/VASP proteins, which are found in vertebrates, invertebrates and Dictyostelium cells (Figure 1A). All members of this family share a conserved domain architecture: an N-terminal Ena/VASP homology 1 (EVH1) domain required for intracellular targeting followed by a central proline-rich domain (PRD), and finally a C-terminal EVH2 domain encompassing two WASP-homology domain (WH2) related actin-binding motifs, referred to as the G-actin-binding site (GAB) and the F-actin-binding site (FAB) as well as a tetramerization domain at the C-terminus (Figure 1B). The VASP tetramer contains long stretches of intrinsically disordered amino-acid sequences and is a very flexible molecule as assessed by electron microscopy (Figure 1C). The only known structured domains of the molecule are the globular N-terminal EVH1 domains and the tetramerization domain at the C-terminus of the protein, whereas the PRD as well as the GAB and FAB motifs are largely unstructured. Ena/VASP proteins accumulate in sites of active actin assembly such as tips of lamellipodia and filopodia or in focal adhesions (Figure 2). Filopodia fail to form in Dictyostelium mutants lacking the single VASP member and are markedly reduced in neuronal cells devoid all three Ena/VASP proteins [10,11], consistent with a conserved requirement of this protein family in filopodia formation in evolutionary distant organisms. Another issue of particular high interest is the involvement of Ena/VASP proteins in tumor progression and invasion. The Ena/VASP member Mena is highly unregulated in breast cancer while depletion of the same protein apparently inhibits metastasis and tumor progression [12,13]. Together, these results highlight the important role of these actin-binding proteins in the regulation of cell protrusion and motility, and are consistent with the direct correlation between VASP density and the protrusion rate of B16-F1 melanoma

cells [14]. Despite the relevance of Ena/VASP proteins for many additional actin-based processes including cell adhesion and the intracellular movement of pathogens such as *Listeria monocytogenes* [15], their molecular function remained under debate as different labs reported controversial activities of Ena/VASP proteins in vitro and in vivo (reviewed in [16]). Initially, VASP has been implicated to promote actin assembly by competing with the activity of capping protein, thus allowing spontaneous actin polymerization at the fast growing barbed ends [17-19]. This assumption was apparently supported by longer and less branched lamellipodial filaments in cells with excess Ena/VASP proteins, and shorter and highly branched filaments in cells lacking Mena and VASP [17]. Similar effects of VASP on filament length and branch spacing have also been reported for actin comet tail formation induced by *Listeria* or isolated proteins in biomimetic assays [20,21]. Although most of the above findings could

have been explained by a direct involvement of VASP in filament elongation, experimental proof of anti-capping versus direct elongation has been lacking. Additionally, other studies failed to detect a potential anti-capping activity of VASP [10,21]. To clarify this issue we have analyzed actin polymerization with trace amounts of fluorescently labelled actin monomers in the presence of VASP at the single filament level employing total internal reflection fluorescence (TIRF) microscopy. This sensitive method exploits evanescent wave excitation and allows the observation of fluorescent molecules in a thin region of up to ~200 nm above the cover slips with an excellent signal to noise ratio. Thus, a TIRF-based analysis is perfectly suited for the direct visualization of actin polymerization and polymer architecture near the coverslip-water interface [22]. Using this experimental setup with purified proteins, we could show that both mammalian and Dictyostelium Ena/VASP proteins actively drive filament

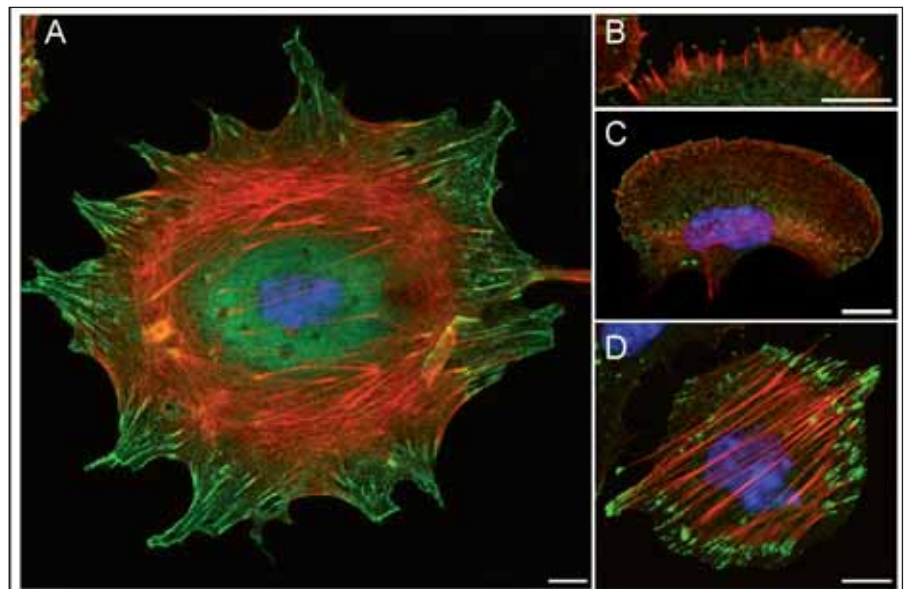


Fig. 2: Ena/VASP proteins accumulate in sites of active actin assembly. Localization of endogenous VASP in a NIH 3T3 fibroblast (A) and B16-F1 melanoma cells (C-D). The cells were fixed and stained for VASP with polyclonal antibodies (green). Filamentous actin was visualized with rhodamine-labelled phalloidin (red), and the DNA was stained with TO-PRO-3 (blue). Confocal sections are shown. Note prominent VASP accumulation in focal adhesions, the leading edge, and the tips of filopodia or microspikes which are filopodia-like structures that remain almost entirely embedded in the lamellipodium. Scale bars, 10 μ m.

elongation by delivery of actin monomers to the growing barbed end [23]. Interestingly, VASP from the highly motile amoeba *Dictyostelium* (DdVASP) accelerated filament elongation in vitro 7-fold as compared to the actin control with about 11 subunits/sec. Since human VASP (hVASP) accelerated filament growth only 2-fold (Figures 3A and B), DdVASP turned out to be well suited for analyzing the relevance of its different domains in actin assembly. Contrary to previous studies [24], we could show that both WH2-domain related motifs, namely GAB and FAB within EVH2 domain of VASP [10,25], could recruit and deliver actin monomers directly to filament barbed ends for efficient elongation [23]. During elongation in solution, Ena/VASP is not permanently associated with the growing end of the filament like a formin, but it only transiently binds to the

barbed end, transfers its bound actin subunits and subsequently either stays attached to the side of the growing filament or detaches from the filament [23,26,27]. Thus, filament elongation can be readily inhibited by low concentrations of capping protein under these conditions (Figure 3C).

Profilin and phosphorylation do not affect VASP-mediated filament elongation in vitro.

Previously, the PRD region of Ena/VASP proteins was shown to interact with profilin (Reinhard et al., 1995). Since profilin-actin complexes are believed to constitute the main pool of polymerization-competent actin in eukaryotic cells [28], we expected that, comparable to the proline-rich FH1 domain of formins [29], the PRD of VASP would recruit profilin-actin to promote ac-

tin assembly [30]. Surprisingly, however, neither of the three *Dictyostelium* profilin isoforms nor human profilin I accelerated VASP-mediated filament elongation in vitro [18,23,27]. Based on these findings we assume that profilin-actin can also bind directly to the GAB (or the FAB) for monomer delivery. This is in agreement with structural data showing profilin-actin in complex with GAB [30], and is further supported by a higher affinity of GAB of hVASP for profilin-actin than for actin alone [31]. The activity of Ena/VASP proteins was additionally proposed to be regulated by phosphorylation through cAMP and cGMP-dependent protein kinases [32,33]. Notably, VASP phosphorylation is also a useful medical indicator of individual responses to the drug clopidogrel preventing secondary atherothrombosis after ischemic stroke, myocardial infarction or in patients

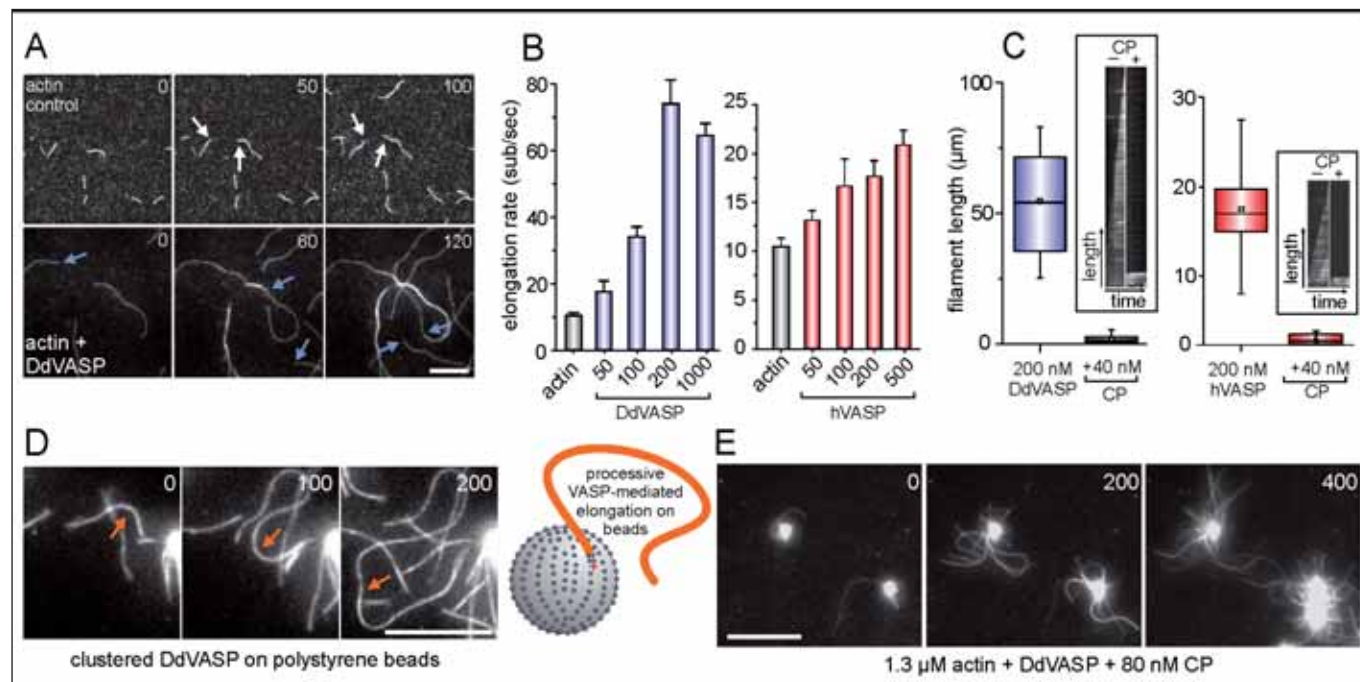


Fig. 3.: Different modes of VASP-mediated actin assembly and sensitivity to capping protein. (A) 1 μM ATP-actin and 0.3 μM Alexa-488 labelled ATP-actin was polymerized in the absence or presence of 200 nM DdVASP and visualized by in vitro TIRF microscopy. Time-lapse micrographs of the assembly reaction are shown and the time is indicated in seconds. Blue arrows mark barbed ends during filament elongation. (B) Concentration dependence of the elongation rates of DdVASP- or hVASP-mediated actin assembly. Note drastically different elongation rates mediated by these two orthologues. (C) Capping protein (CP) inhibits VASP-mediated filament elongation in solution. The length of actin filaments in the absence or presence of CP after 10 min was measured. Kymograph analysis in the insets illustrates the inhibitory effect of 40 nM CP on VASP-mediated actin assembly in solution. (D) Clustering of VASP on polystyrene beads promotes robust processive actin filament elongation. Orange arrows highlight buckling of a processively growing filament attached with its barbed end to the bead surface. Cartoon of a buckling filament is shown on the right. (E). Processive actin filament elongation on a bead surface becomes virtually insensitive to CP. Scale bars, 10 μm.

undergoing stent implantation [34,35]. Notwithstanding this, no effects on filament bundling or elongation using PKA-phosphorylated recombinant hVASP were observed in the TIRF assay [23]. Thus, the regulation of Ena/VASP proteins by phosphorylation remains elusive and needs to be investigated in future studies.

The clustering of VASP on a surface drastically affects its filament elongation properties

In the physiological context, Ena/VASP proteins operate at high density at membranes or the surface of bacterial pathogens. Thus, we analyzed the elongation properties of Ena/VASP proteins after coating of polystyrene beads. Upon clustering of VASP on functionalized beads, filament elongation became remarkably robust allowing the incorporation of thousands of actin subunits as indicated by the buckling filaments as exemplified in Figure 3D [23]. Most importantly, under these conditions VASP-mediated filament elongation becomes virtually insensitive to very high concentrations of capping protein (Figure 3E). Together, these data imply that clustered VASP tetramers not only shield growing filaments from capping proteins, but also suggest that VASP acts as a powerful, membrane-anchored filament elongator at the tips of lamellipodia and filopodia.

Molecular mechanism of Ena/VASP-mediated filament elongation

The finding that *Dictyostelium* DdVASP enhances filament elongation 7-fold, whereas the three mammalian orthologues VASP, Mena and EVL accelerates elongation maximally 2-fold, raised questions about the molecular determinants of rate control. To identify the molecular requirements for fast filament elongation, we designed chimeras by transplanting GAB and FAB of DdVASP either alone or in combination into the backbone of hVASP. The simultaneous insertion of the *Dictyostelium* GAB and FAB into hVASP

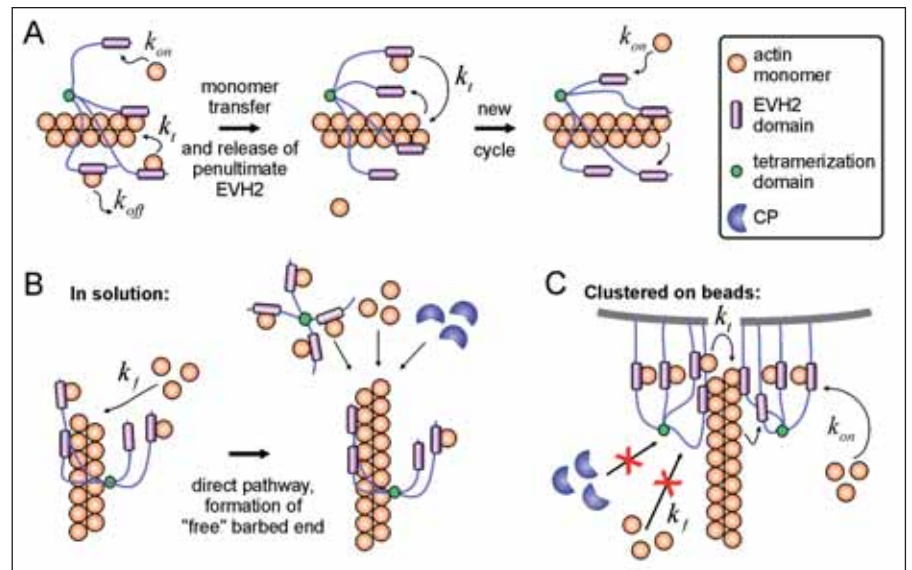


Fig. 4.: Molecular mechanism of VASP-mediated filament elongation. (A) The VASP tetramer is attached to the filament barbed end by at least one EVH2 domain during filament elongation. The free EVH2 domains capture actin monomers from solution with an on-rate constant k_{on} , and subsequently either transfer the subunit onto the barbed end with a transfer rate k_t or release the actin monomer back into solution with an off-rate k_{off} . The model also implies that upon binding of the GAB-associated monomer to the barbed end, the already bound GAB is instantly liberated from the barbed-end region so that it becomes available again to capture new monomer. By this mode of action, the VASP tetramer is able to processively track the elongating filament. (B) When VASP is free in solution, processive association of VASP with the barbed end is short-lived and therefore competes with the spontaneous addition of free actin monomers to the barbed end via a direct pathway with an independent transfer rate constant k_f . Free barbed ends in turn are accessible either for other VASP tetramers, actin monomers or capping proteins. The average elongation rate is therefore made up of the rate of VASP-mediated filament elongation plus the rate of the direct pathway. (C) The clustering of VASP on surfaces leads to processive association of VASP with the filament end, efficiently blocking access for capping protein and also inhibiting spontaneous addition of monomers via the direct pathway. Hence, under these conditions, filament elongation is exclusively driven by VASP. For details of the model see [26].

resulted in an equally high elongation rate as with DdVASP, whereby the WH2-like GAB motif turned out to have the most profound impact on filament elongation [26]. Subsequent spectroscopic analyses revealed that the *Dictyostelium* GAB binds to monomeric actin with an about 10-fold higher affinity than the human GAB, supporting the hypothesis that the filament elongation rate is directly correlated to the affinity of the GAB to G-actin. This hypothesis could be confirmed by the analyses of additional hVASP chimeras encompassing previously characterized WH2 motifs from the actin-binding proteins with different affinities for G-actin [26]. Given the high actin monomer concentration in cells, these findings suggest that Ena/VASP pro-

teins, independent of species, are fully saturated with actin and generally act as potent filament elongators. Based on these data and determination of the association rate constants of G-actin to human and *Dictyostelium* GAB a quantitative mathematical model of VASP-mediated filament elongation could be formulated [26]. It predicts that, at steady state, one polypeptide chain of the VASP tetramer tracks the barbed-end while the other three subunits can recruit actin monomer from solution and subsequently deliver it to the growing barbed end (Figure 4). Comparison of experimental data with the model further revealed that the clustering of VASP on surfaces efficiently blocks access for capping protein and also prevents spontaneous

addition of monomers during processive association of VASP with the filament [26]. Thus, contrary to the molecular ratchet model, which postulates passive incorporation of actin monomers to growing barbed ends due to thermal fluctuation between membranes and actin filaments, our data rather suggest that in cells filament elongation is actively controlled.

Perspective

Powered by classical biochemistry, molecular genetics and modern imaging techniques, the recent years yielded vast knowledge about the function of Ena/VASP proteins in different functions and cellular systems. Although we now also learned that Ena/VASP proteins mediate tethered processive filament elongation when clustered on a surface, the VASP molecule has not yet revealed all of its secrets, as many intriguing questions remain unanswered. For instance, it is elusive how VASP is recruited to the plasma membrane. Potential VASP ligands such as lamellipodin or PREL1/RIAM for instance are not expressed in *Dictyostelium*, still murine VASP accumulates in the tips of lamellipodia and filopodia after ectopic expression in *Dictyostelium* VASP-null cells. We also do not know how clustering of VASP is achieved, and it also remains to be seen how Ena/VASP proteins and formins, both of which can nucleate and elongate actin filaments, coordinate their activities at the tips of growing filopodia. Finally, it might be noteworthy to mention that *Dictyostelium*, sometimes denoted a bit condescendingly as a “lower” eukaryote, has actually turned out to be very useful to dissect and better understand the molecular activities of Ena/VASP proteins, including those of “higher” eukaryotes.

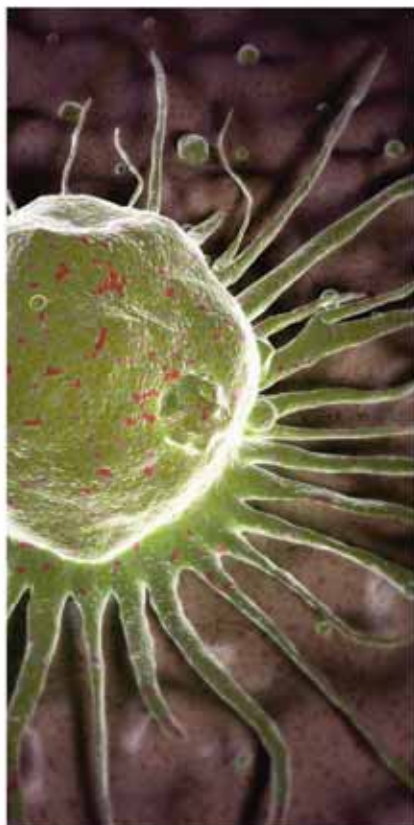
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Probing the biophysical properties of the cell nucleus and their relevance in health and disease

Philipp Isermann, Monika Zwerger, Jan Lammerding

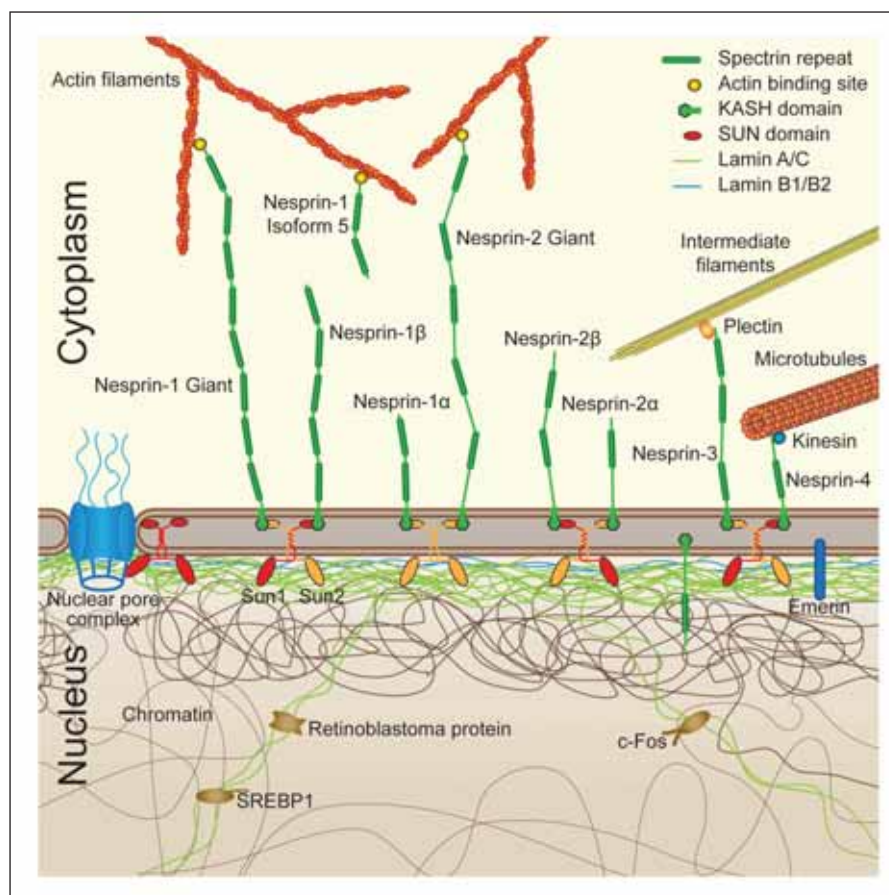


Figure 1: Structure of the nuclear envelope and its connection to the cytoskeleton. The nuclear envelope consists of the inner and outer nuclear membranes that enclose the perinuclear space. Lamins, which are the major constituent of the nuclear lamina, bind to chromatin and several transcriptional regulators and help anchor inner nuclear membrane proteins such as Emerin or SUN proteins (Sun1 and Sun2). Sun1 and Sun2 interact across the perinuclear space with the C-terminal KASH (Klarsicht/ANC-1/Syne-homology) domain of nesprins. The largest nesprin isoforms, Nesprin-1 Giant and -2 Giant, bind to actin filaments through their N-terminal actin binding site, thereby completing a physical connection between the nuclear interior and the cytoskeleton. Similarly, Nesprin-3 can bind to intermediate filaments via plectin, and Nesprin-4 connects to microtubules through Kinesin. Nesprin proteins are characterized by multiple splicing and initiation variants, resulting in a variable number of spectrin repeats, and not all nesprins contain an N-terminal cytoskeletal-binding site or the C-terminal KASH domain.

Background

Within the human body, cells live in a highly dynamic, mechanically stressful environment. For example, with each heartbeat, cells in the heart and many of the larger arteries are subjected to large deformations that result from contraction and subsequent extension of the ventricle and fluid shear stress and vessel strain in the major blood vessels. Similarly, any simple, every-day activity, such as reaching for a cup of coffee or sitting down, involves the coordinated contraction of numerous skeletal muscle groups and the relaxation and extension of the opposing muscles, again resulting in large scale cellular deformations within the muscles, connective tissue, and skin. Importantly, the concept of cellular force generation and resulting deformations is not restricted to mechanically active tissues. For example, many cells generate and apply forces to their environment to migrate. And unlike cells studied in a culture dish *in vitro*, *in vivo* cells extravasating from blood vessels or migrating within tissues must often squeeze through narrow spaces only a few micrometers in diameter, requiring substantial deformations of the cell body and the nucleus during the process.

The relationship between applied forces and the resulting deformations is governed by the mechanical properties of cells and their environment. While much research has been devoted to analyzing the impact of mechanical stress on the cytoskeleton and the contribution of diverse cytoskeletal structures, the biophysical properties of the nucleus have only recently begun to attract the interest of cell biologist and biophysicists. In this context, it is important to recognize that the nucleus is not simply a DNA containing compartment that floats around the cytoplasm; instead, it is tightly integrated into the cellular architecture and physically linked to diverse cytoskeletal structures such as actin filaments, intermediate filaments, and microtubules [1, 2] (Fig. 1). Therefore,

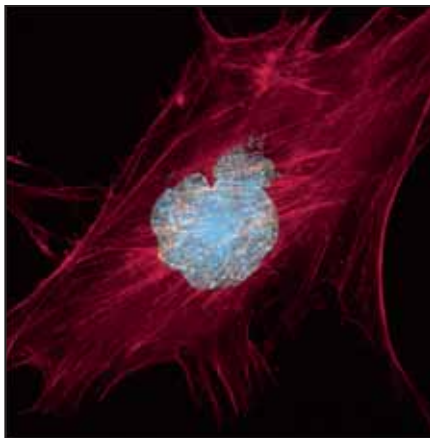


Figure 2: Abnormal nuclear shape in lamin A/C-deficient cells. Immunofluorescence image of an *Lmna*^{-/-} mouse embryonic fibroblast stained for DNA (Hoechst 33342, blue) and actin filaments (phalloidin, red).

forces applied to the outside of the cell or generated within the cell are ultimately transmitted to the nucleus, where they can result in nuclear deformations and possibly participate in the activation of mechanosensitive signaling pathways.

During the past decade, research on the biophysical properties of the nucleus and its constituents has gained an increased level of attention. One of the major reasons has been the discovery that mutations in nuclear lamins, particularly lamins A and C, which are encoded by the *LMNA* gene, are responsible for a large number of human diseases. These so called laminopathies include Emery-Dreifuss muscular dystrophy, limb-girdle muscular dystrophy, dilated cardiomyopathy, and Hutchinson-Gilford progeria syndrome [3]. Lamins are type V intermediate filament proteins and the major component of the nuclear lamina, a dense protein meshwork underlying the inner nuclear membrane. Importantly, lamins interact with numerous inner nuclear membrane proteins and soluble transcription factors, such as retinoblastoma protein (Rb), c-Fos, and SREBP-1 and are also found in the nuclear interior (Fig. 1). To date, more than 330 distinct *LMNA* mutations have been identified and linked to more than 10 human diseases

(<http://www.umd.be/LMNA/>). Despite significant progress in elucidating the diverse functions of lamins, a clear concept of how alterations in lamin proteins can cause such a plethora of diseases is still missing. One particular puzzling fact is that although lamins A and C are expressed in almost all differentiated cells, most of the mutations result in highly tissue-specific phenotypes, specifically affecting striated muscle and connective tissue. One potential hypothesis to explain these tissue-specific defects is that functional loss of lamins could render nuclei more fragile and result in progressive loss of cells in mechanically active tissues such as muscles and tendons, thereby contributing to the disease mechanism in muscular dystrophies and cardiomyopathies. Supporting this notion, cells from laminopathy patients often have abnormally shaped nuclei. Similarly, cells derived from lamin A/C-deficient (*Lmna*^{-/-}) mice, which develop severe muscular dystrophy and dilated cardiomyopathy [4], show striking defects in nuclear morphology (Fig. 2), suggesting ab-

normal nuclear mechanics. To put this 'structural hypothesis' to the test, our laboratory has designed and applied an array of biophysical assays to probe the mechanical properties of the nucleus and to explore the effect of specific lamin mutations. Importantly, while these techniques were developed in the context of exploring the pathobiology of laminopathies, they can also be applied to various other research areas, such as the biophysical characterization of cancer cells or to study the function of immune cells.

Tools to probe nuclear stiffness, fragility, and coupling to the cytoskeleton

Taking mechanical measurements of a material generally involves applying precisely controlled forces or deformations and measuring the corresponding deformations or responsive forces. A typical example is the determination of the elasticity (i.e., spring constant) of a rubber band by measuring the force necessary to achieve a certain extension. When measuring nuclear mechanics, one

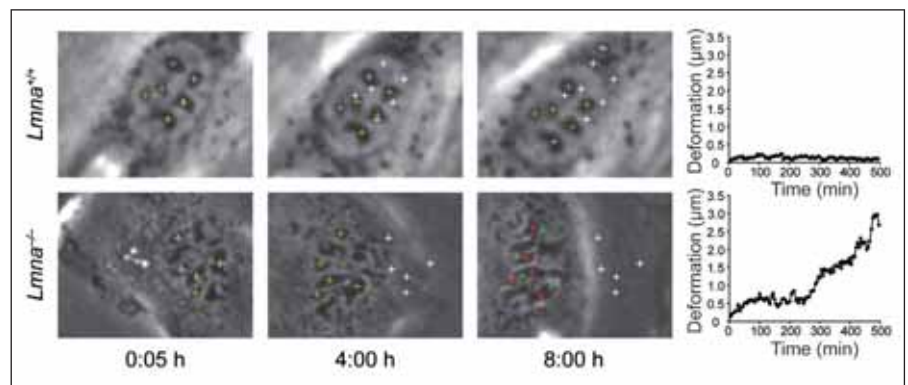


Figure 3: Quantitative analysis of nuclear deformation from time-lapse video microscopy sequences. Images of lamin A/C-deficient (*Lmna*^{-/-}) and wildtype mouse embryonic fibroblasts were acquired every 5 min for 8:20 h. Shown are representative snapshots at the 5 min, 4 h, and 8 h time points. White crosses denote the initial positions of nucleoli, which serve as reference points for nuclear shape; green crosses denote the positions according to the least-squares fit assuming linear-affine transformations, i.e., allowing for nuclear rotation, translation, and increase in size; red crosses mark the actual nucleoli positions. Deviations between the red and green positions indicate changes in nuclear shape independent of translation, rotation, or uniform changes in nuclear size. Wildtype nuclei (top row) appear very stable and undergo only minor deformations over time, whereas *Lmna*^{-/-} nuclei (bottom row) show large dynamic nuclear deformations. Plots on the right show the time-dependent average deviation between the actual nucleoli positions and the least-squares fit for the corresponding nucleus. ©Lammerding et al., 2005. Originally published in *The Journal of Cell Biology*. doi: 10.1083/jcb.200502148 [30].

challenge is presented by the fact that the nucleus is surrounded by the cytoskeleton, so that the mechanical stimulation can often be only indirectly applied. One possibility is to probe isolated nuclei, but due to the difficulty to match the physiological buffer conditions of the nuclear interior and the lack of cytoskeletal tension, the morpholo-

gy of isolated nuclei is typically quite distinct from that found inside the cell [5-7]. To avoid artefacts from nuclear isolation, the experiments in our laboratory are generally conducted on intact, living cells. The experimental techniques include quantitative analysis of time-lapse video microscopy sequences (Fig. 3), cellular strain application

(Fig. 4), nuclear microinjection, perfusion through narrow microfluidic channels, and microneedle manipulation to assess force transmission between the cytoskeleton and the nucleus (Fig. 5). Our initial studies have been based on mouse embryonic fibroblasts (MEFs) from animals lacking specific lamins to explore whether loss of lamins could affect nuclear shape, integrity and/or stiffness. The findings, described in detail below, highlight the importance of lamins A and C on nuclear structure and integrity and provide some interesting insights into possible disease-causing mechanisms underlying the wide range of laminopathies [8, 9].

Altered nuclear mechanics in lamin A/C-deficient cells

The first indication that loss of lamins A/C results in altered nuclear structure and stiffness came from immunofluorescence images of *Lmna*^{-/-} MEFs and time-lapse video microscopy studies. In contrast to the typical ovoid shape found in wildtype cells, *Lmna*^{-/-} MEFs often have extremely irregularly shaped nuclei (Fig. 2). Time-lapse sequences reveal dynamic changes in nuclear shape over an 8-hour period in *Lmna*^{-/-} MEFs (Fig. 3). By tracking the position of individual nucleoli relative to each other and comparing the results to a rigid body that can only rotate or translate but not change its shape, we have been able to quantify the time-dependent nuclear deformations. Whereas in wildtype cells, the nucleus only shows minor deviation from an idealized rigid body, *Lmna*^{-/-} nuclei show significantly larger deformations over time. Cells lacking lamin A but not lamin C had an intermediate phenotype, whereas cells deficient for lamin B1 were indistinguishable from wildtype cells [8].

One limitation of the time-lapse experiments is that they lack any information on the forces acting upon the nucleus. To probe nuclear mechanics in a more controlled fashion, we have designed a cellular strain assay in which cells are cultured on fibronec-

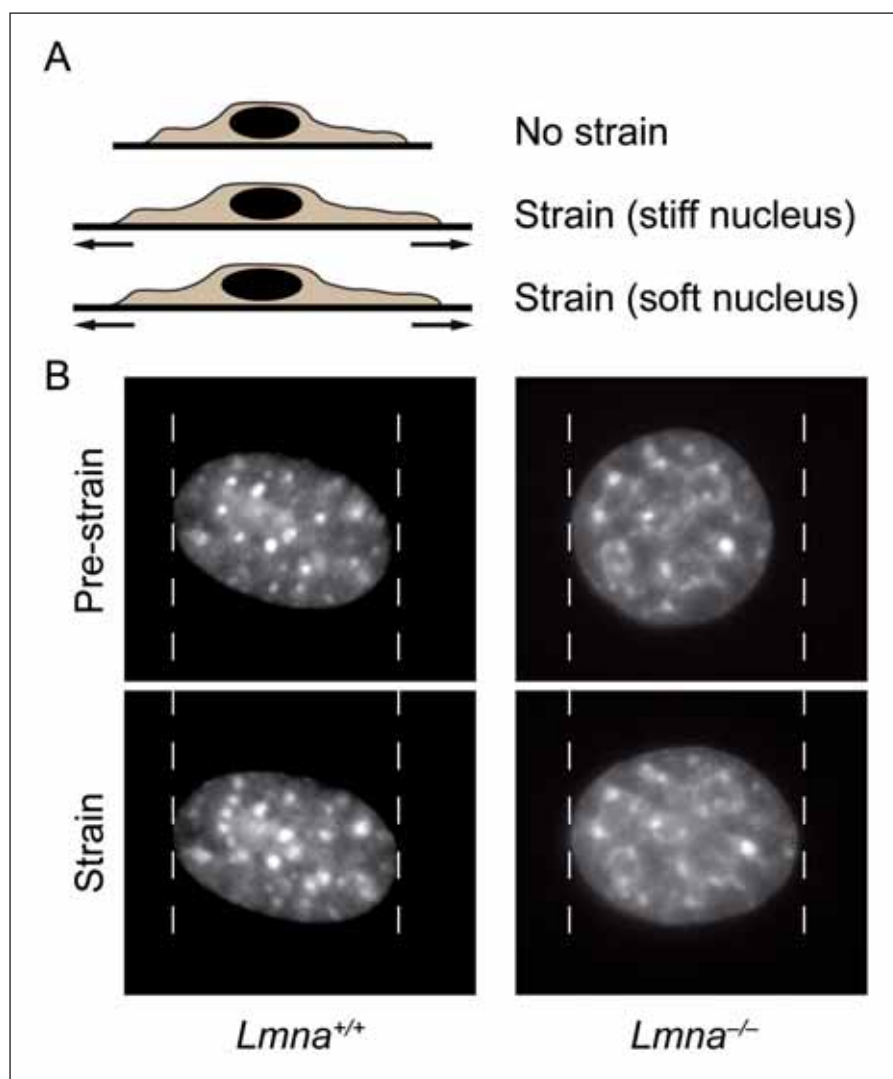


Figure 4: Cellular strain experiments to probe nuclear stiffness. (A) Schematic illustration of strain experiments on cells cultured on flexible silicone membranes. In normal cells, where the nucleus is significantly stiffer than the cytoskeleton, substrate strain application results in only small increases in nuclear size in the strain direction. In cells with compromised nuclear mechanics, for example after loss of lamins A/C, the softer nuclei are expected to deform more under the same cellular strain application. (B) Representative images of fluorescently labelled nuclei in wildtype (*Lmna*^{+/+}) and *Lmna*^{-/-} mouse embryonic fibroblasts before and during uniaxial strain application in the horizontal direction. The *Lmna*^{-/-} cells have significantly larger nuclear deformations, indicating reduced nuclear stiffness. Image adapted from [31]. Copyright © 2010 Elsevier.

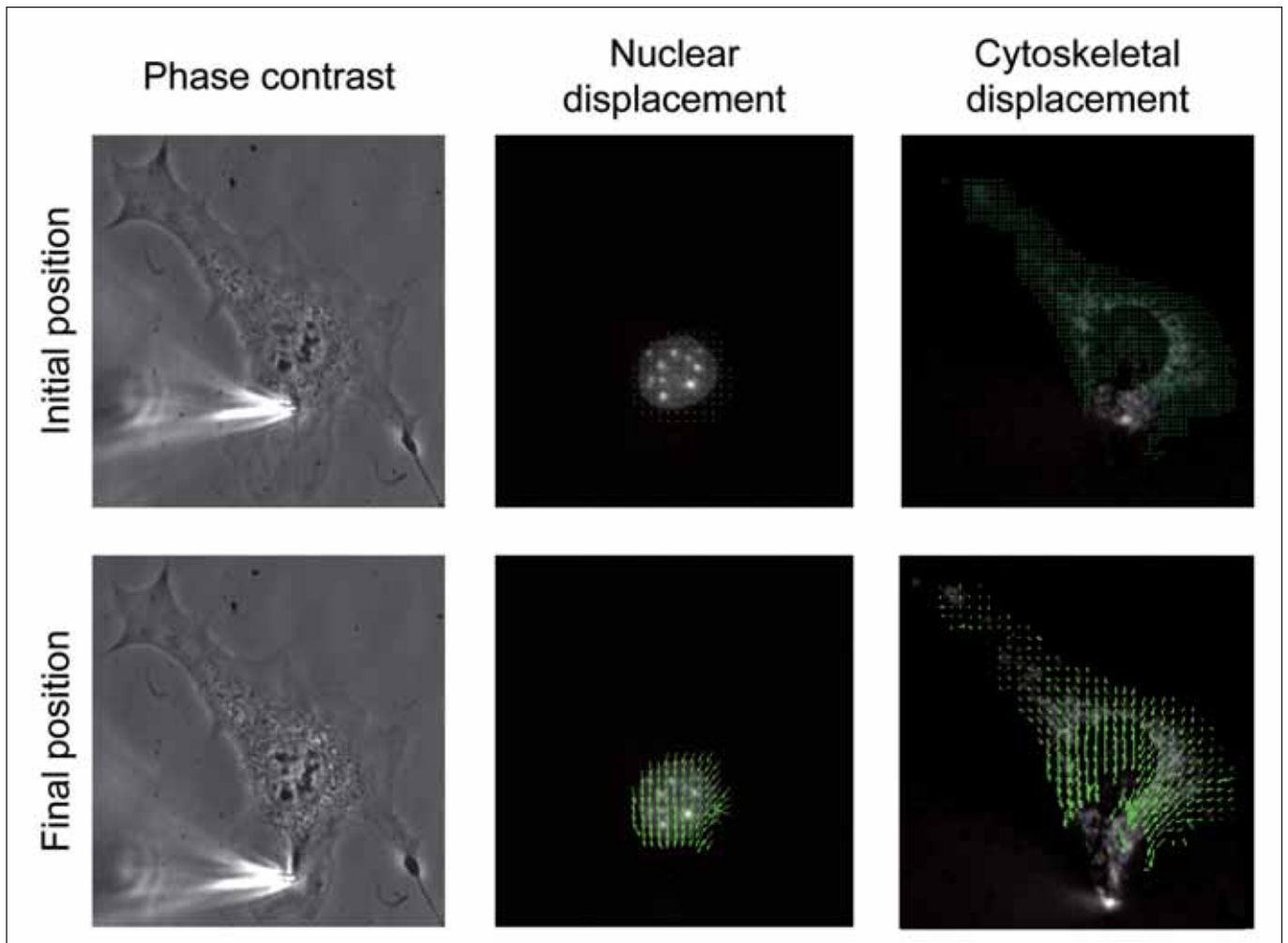


Figure 5: Microneedle manipulation assay to probe nucleo-cytoskeletal coupling. A microneedle is inserted into the cytoskeleton at $5\ \mu\text{m}$ from the nucleus and subsequently moved a fixed distance towards the cell periphery. Images of the fluorescently labelled cytoskeleton (or mitochondria) and nucleus are acquired before and during the microneedle manipulation and used to generate nuclear and cytoskeletal displacement maps. The panels show representative results from a wildtype mouse embryonic fibroblast stained for DNA (Hoechst 33342) and mitochondria (MitoTracker) before (top row) and during (bottom row) local strain application. The images illustrate that cytoskeletal forces are transmitted across the nucleus, resulting in induced nuclear deformations and cytoskeletal displacements away from the strain application site. These induced deformations are reduced in cells in which nucleo-cytoskeletal connections are disturbed, for example through loss of lamins A/C or dominant negative nesprin mutants (unpublished observation). Image adapted from [31]. Copyright © 2010 Elsevier.

tin-coated silicone membranes and subjected to well-defined uniaxial or biaxial strain. The cytoskeleton, which tightly connects to the substrate through focal adhesions, is subjected to the same strain as the underlying substrate and applies significant forces to the nucleus [10]. Normally, the nucleus is 2 to 10-times stiffer than the cytoskeleton [5, 10–14], resembling the situation of a stiff rubber ball surrounded by soft packaging material. Consequently, in wildtype cells,

the cytoskeleton absorbs most of the applied strain and the nucleus deforms very little (Fig. 4). In contrast, lamin A/C-deficient cells show significantly larger deformations, indicating reduced nuclear stiffness. Measurements of cytoskeletal stiffness confirmed that the increased nuclear deformation did not result from increased force transmission to the nucleus [9]. Consistent with the time-lapse experiments, cells lacking lamin A but not lamin C had intermediate nuclear stiff-

ness, while nuclear stiffness was normal in cells lacking lamin B1, indicating that lamins A and C are the main contributors to nuclear stiffness.

To probe whether decreased nuclear stiffness corresponds to increased nuclear fragility, we developed a microinjection assay in which fluorescently labelled dextran molecules are directly injected into the nucleus at increasing injection pressures. In wildtype cells, the 60-kD dextran molecules are trap-

ped inside the nucleus, since they are too large to passively diffuse through the nuclear pores, and nuclear envelope rupture and leakage of dextran into the cytoplasm only occurs at large injection pressures (>500 hPa). In contrast, even low injection pressures (<100 hPa) are sufficient to (transiently) disrupt the nuclear envelope in *Lmna*^{-/-} cells, indicating that loss of lamins A and C causes increased nuclear fragility, which renders these cells more susceptible to mechanically induced cell death when subjected to repetitive mechanical strain [9].

Interestingly, functional loss of lamins and other nuclear envelope proteins can have consequences outside the nucleus itself. Lamin A/C-deficient cells have decreased cytoskeletal stiffness [9, 15, 16] and defects in cytoskeletal organization [16, 17]. These findings suggest that lamins are involved in connecting the nucleus to the cytoskeleton, for example, by modulating the function of the SUN/nesprin complex, which links the nuclear interior to the cytoskeleton [1, 18]. To directly probe the role of nuclear envelope proteins on intracellular force transmission between the cytoskeleton and the nucleus, we have developed a microneedle manipulation assay that applies precisely controlled cytoskeletal strain at a defined distance from the nucleus while simultaneously imaging induced nuclear and cytoskeletal deformations (Fig. 5), advancing an approach pioneered by Maniotis and colleagues [19]. Cytoskeletal and nuclear displacements can be measured by tracking fluorescently labelled mitochondria, which serve as reliable fiducial markers for induced deformations of the actin and vimentin cytoskeleton (unpublished observation). Our custom-developed image analysis algorithms then compute detailed displacement maps of induced nuclear and cytoskeletal deformations (Fig. 5). Based on these displacement maps, we found that *Lmna*^{-/-} MEFs have significantly reduced nuclear and cytoskeletal displacements away from the strain application site, suggesting

defects in intracellular force transmission. These findings of impaired nucleo-cytoskeletal coupling could provide an explanation for the recent reports of impaired nuclear orientation and impaired cell migration in *Lmna*^{-/-} MEFs [16] and the misplacement of synaptic muscle nuclei from neuromuscular junctions in *Lmna*^{-/-} mice [20].

The effect of specific LMNA mutations on nuclear structure

Employing the techniques described above, we have shown that loss of lamins A and C decreases nuclear stiffness and increases nuclear fragility, as well as the rate of cell death under repetitive mechanical strain. These findings document the importance of lamins on nuclear—and even cellular—structure and integrity and provide a possible disease mechanism for laminopathies involving striated muscle, such as Emery-Dreifuss muscular dystrophy (EDMD) or dilated cardiomyopathy (DCM). However, while the majority of human LMNA mutations identified to date affect striated muscle tissue, other LMNA mutations result in diseases with only little or no muscle involvement, as in the case of familial partial lipodystrophy (FPLD) or the rare premature aging disorder Hutchinson Gilford progeria syndrome (HGPS). One potential explanation could be that only LMNA mutations resulting in muscle diseases cause a loss of structural function, whereas mutations associated with diseases that spare muscle tissue retain the structural function of lamins and instead alter protein-protein interactions and/or tissue-specific functions. To test this hypothesis, we used our biophysical assays to examine the effects of specific disease-causing mutations on nuclear structure and mechanics, comparing mutations associated with laminopathies affecting muscle tissue with those affecting other tissues. We found that skin fibroblasts from patients with EDMD had significantly softer nuclei than cells from healthy controls, whereas cells from FPLD patients had normal nuclear

stiffness, supporting this hypothesis. Since experiments with human patient fibroblasts are plagued by limited availability of samples and broad genetic heterogeneity, we modified *Lmna*^{-/-} and heterozygous *Lmna*^{+/-} MEFs to stably express a large panel of specific human lamin A mutations or wildtype lamin A at near-physiological levels in a uniform genetic background. Interestingly, we found that while several mutations associated with EDMD or DCM resulted in a loss of structural function, other EDMD and DCM mutants had surprisingly normal nuclear mechanics, and even subtle changes in the mutated amino acid position or the substituted residue could significantly affect the protein's structural “behavior” (unpublished observation). At the same time, we consistently found that mutations that cause FPLD did not affect the structural function of lamin A. Taken together, our data indicate that while loss of structural function may contribute to the muscular phenotype in some laminopathies, additional factors must influence the disease outcome. For example, certain mutations could affect the interaction of lamins with specific transcription factors, such as SREBP-1, while others could negatively impact nucleo-cytoskeletal coupling. This idea is supported by recent findings from the Gundersen laboratory reporting that lamin A mutations associated with EDMD and DCM, but not FPLD, resulted in defects in nuclear movement in a wound healing assay [21]. Clearly, more work is needed to elucidate and comprehend the structural roles of A-type lamins within the nucleus, and to integrate this knowledge with their numerous other functions, such as in nucleo-cytoskeletal coupling, signaling, gene expression, and DNA repair. One step we are currently taking in this direction is to explore the effect of lamin mutations *in situ*, i.e., measuring nuclear mechanics in muscle tissue preparations. These experiments will enable studying cells in their unperturbed physiological environment and may also reveal tissue specific defects.

Outlook

Exploring the disease mechanism underlying the diverse laminopathies is just one example of our work on nuclear biomechanics. Another currently underexplored research area is the role of nuclear mechanics in cell migration. The nucleus is the largest cell organelle and often occupies a large fraction of the total cell volume. Combined with the fact that the nucleus is significantly stiffer than the surrounding cytoskeleton, this suggests that deformation of the nucleus can constitute a rate limiting step for the passage of cells through narrow constrictions [22]. This idea is particularly relevant to the study of cancer spreading, as many cancers show significant changes in lamin expression. For example, silencing of lamins A/C was reported in leukemias and lymphomas [23], gastrointestinal cancer [24] and small cell lung cancer [25], and in skin cancer, reduced levels of lamin A correlated with rapid basal cell carcinoma growth [26]. Since lamins A and C are the major contributor to nuclear stiffness [8], future research should be aimed at investigating whether such changes in lamin expression are associated with decreased nuclear stiffness, and if increased nuclear deformability could in fact enhance the abilities of cells to squeeze through narrow tissue spaces or basement membranes, similar to the increased nuclear plasticity in terminally differentiated neutrophils, which downregulate lamin expression during granulopoiesis [27-29]. For these studies, the large cell-to-cell variability and the time consuming experimental procedures typically used for single cell measurements will present some of the major challenges in the biophysical characterization of cells. To address these challenges, we are currently developing high(er) throughput techniques that enable probing hundreds of cells per minute, for example, by measuring the deformability and transit time of single cells with microfluidic devices. Ultimately, such techniques could also lead to the identification of subgroups of cells

with specific biophysical characteristics, which could then be subjected to further functional studies. In the example of cancer cells, a high throughput screening approach might help detect particularly invasive cells or cancer stem cells and provide a novel diagnostic and prognostic tool to augment existing molecular biology based approaches.

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Centromere regulation: new players, new rules, new questions

Anne-Laure Pauleau and Sylvia Erhardt

Chromosome inheritance requires centromeres

Proper chromosome segregation is essential for maintaining normal chromosome number (euploidy) during mitosis and meiosis, and is therefore crucial for germ cell and zygote formation, differentiation and development of somatic tissues and organs. Aneuploidy—the loss or gain of chromosomes in a cell—is a hallmark of cancer. Knowledge of the basic biology of inheritance is essential if we are to understand the complex relationship between aneuploidy and human disease.

The region on every linear chromosome that is required for accurate chromosome segregation is termed the centromere. Functionally, the centromere is the minimal chromatin element that is sufficient to promote normal formation of the kinetochore, the proteinaceous structure that links microtubules to chromosomes¹. The kinetochore is also required for sensing errors in chromosome attachment to the spindle. The components of the Spindle Assembly Checkpoint (SAC) associate with the kinetochore and delay the metaphase to anaphase transition until all chromosomes have reached bipolar attachment to the spindle.

Centromeric regions are not conserved

Centromeres are usually located at a stably inherited site on each chromosome and are embedded into pericentric heterochromatin. Centromeric DNA sequences are extremely variable between species and even between different chromosomes in the same cell. For example, centromeres in *Drosophila melanogaster* consist of several islands of complex DNA embedded in long regions of repetitive DNA, whereas human centromeric DNA contains long arrays of tandemly repeated

alpha-satellite DNA that can stretch over mega bases². There is significant evidence that centromeric DNA and its surrounding pericentric heterochromatin are not necessary for the specification of centromere location (centromere identity). First, formation of fully functional neocentromeres has been reported on normally non-centromeric euchromatic regions suggesting that the DNA sequence is not necessary for centromere formation. Second, dicentric chromosomes exist in which a functional kinetochore forms only at one of the two centromeric regions, demonstrating that centromeric DNA is not sufficient for kinetochore formation². Finally, chromosome rearrangements are a hallmark of evolution and speciation, and are accompanied by centromere gains, losses, and movements, independent of underlying DNA sequences³.

Epigenetic mechanisms define centromere identity and inheritance

Centromere identity is regulated by epigenetic mechanisms in most eukaryotes, which means that specification of centromere location is inherited from one cell and organismal generation to the next independently of the underlying DNA sequence². The composition of centromeric chromatin is rather unique: instead of the canonical octameric nucleosome formed by two copies of H2A, H2B, H3 and H4, centromeric nucleosomes contain the histone H3 variant CENP-A (CENTromeric Protein A; CID for Centromere Identifier in *Drosophila*) in place of H3 only at functional centromeres. CENP-A is thought to be a key factor to regulate centromere identity epigenetically. CENP-A homologues have been identified from yeasts to mammals, demonstrating an evolutionary link between the centromeres of these organisms. The

importance of CENP-A for kinetochore function has been demonstrated by several gene depletion studies in different organisms. Elimination of mammalian CENP-A leads to failure to localize essential kinetochore proteins, chromosome segregation defects, and embryonic lethality as observed in mice null

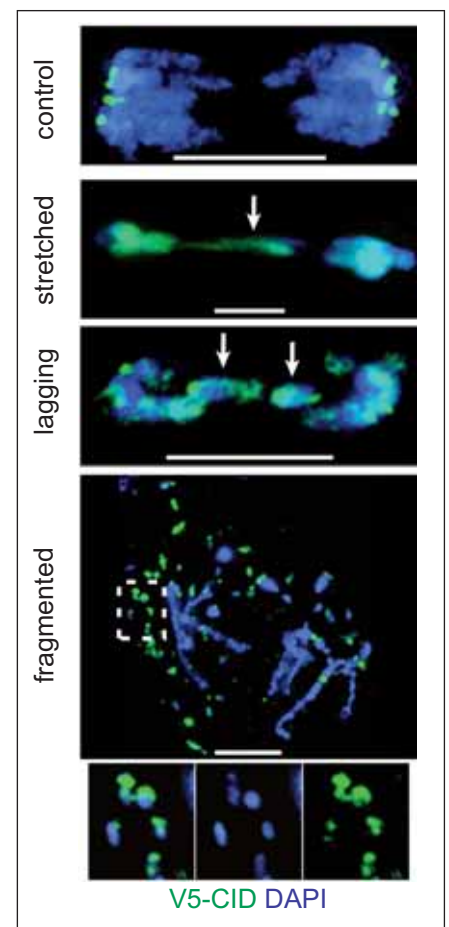


Figure 1: CID overexpression produces mitotic defects in *Drosophila*. Chromosome behavior in CIDV5 misexpressing larval discs and brains (blue=DAPI, green=CID). Low CID overexpression is associated with CID incorporation only at endogenous centromeres and normal progression through mitosis ('control'). High CID overexpression leads to ectopic CID incorporation into chromosome arms, which produces stretched, lagging, and fragmented chromosomes at anaphase. The white arrows mark the corresponding chromosome phenotype. Chromosome fragments within the white box are shown at higher magnification below. The fragments contain CID, and are most commonly located close to the spindle poles, suggesting that they were produced by abnormal spindle attachments at more than one chromosomal site. Scale bars, 5 μ m.

for CENP-A⁴. Similarly, *Drosophila* CID is also an essential protein, required for kinetochore formation and faithful chromosome segregation⁵. Interestingly, not only the absence but also the overexpression of CENP-A can lead to a high degree of chromosome missegregation and lethality. Overexpression can cause mislocalization of CENP-A to ectopic sites, as shown experimentally in human cells and *Drosophila*^{6,7,8}.

Strong CID overexpression in *Drosophila* leads to the formation of functional, ectopic kinetochores at some sites of CID incorporation into chromatin and causes massive segregation defects⁶ (Figure 1). Why only some of the ectopic sites form functional kinetochores that recruit and attach to microtubule fibers still needs to be investigated. A specific

chromatin environment consisting of certain histone modification marks, or the presence of repetitive elements in the vicinity may favor the functionality of certain sites. Importantly, CENP-A has also been shown to be massively upregulated in different tumors⁹. It is however unclear whether CENP-A overexpression in human cells is a cause or a consequence of tumorigenesis. The results from *Drosophila* indicate that CENP-A misregulation alone can cause chromosome segregation defects. While similar scenarios may occur during tumorigenesis, future studies will need to address this hypothesis.

Centromeric chromatin is different from euchromatin and heterochromatin

The importance of post-translational modifications of histones on gene regulation and chromatin integrity has become apparent in recent years, and new modifications as well as combinations of different modifications are constantly being identified¹⁰. Centromeric chromatin in flies and humans contains interspersed blocks of CENP-A and H3 nucleosomes. These domains may form a specialized three-dimensional structure during mitosis that serves as a unique platform for kinetochore formation². The histone H3-containing domains display a pattern of modifications that are distinct from both euchromatin and heterochromatin: histone acetylation and H3K9 methylation are mostly absent, whereas H3K4 dimethylation, K27 methylation and K36 methylation are pre-

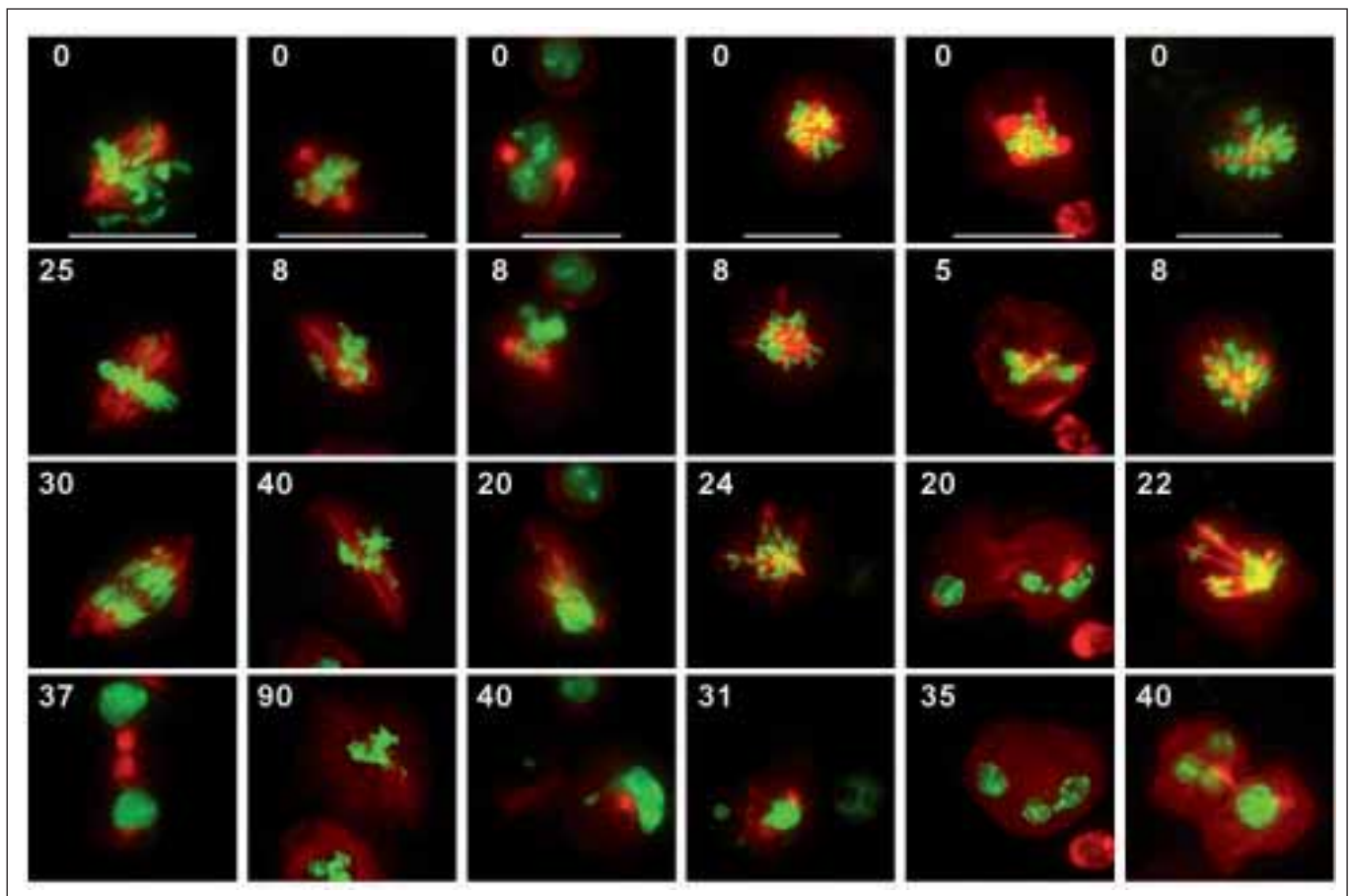


Figure 2: Kinetochore disruption causes mitotic defects in *Drosophila* S2 cells. Still frames from timelapse experiments of mitotic defects associated with RNAi knock down of CID, CENP-C, CAL1, CYCA, and RCA1 in cells expressing mCherry-tubulin and H2B-GFP. Control cells (left) displayed accurate and timely chromosome segregation. CID-, CENP-C-, and CAL1-depleted cells showed a dramatic mitotic delay, little to no chromosome movement, abnormally elongated and defective spindles, and chromosome missegregation; nevertheless, cytokinesis occurred. CYCA depletion caused defective spindles, missegregation of chromosomes, and cytokinesis defects. RCA1 depletion predominantly caused a cell cycle arrest. Times are minutes from the start of the video. Bars, 5 μ m.

sent^{11,2}. These distinct chromatin modifications at centromeres may be important for the correct assembly of the kinetochore, and may also contribute to centromere identity. For example, the distinct centromeric modification pattern could recruit a unique set of histone-binding proteins that influence the higher-order structure of the centromere in mitotic chromosomes. These proteins could also recruit newly synthesized CENP-A and specify centromere identity epigenetically. In accordance to this model, centromeric H3K4me2 has recently been shown to be required for long-term kinetochore maintenance as lack of H3K4me2 progressively leads to impaired loading of newly synthesized CENP-A¹¹. Even though all canonical histones are known to contain posttranslational modifications (PTMs), little is known about PTMs of CENP-A and how PTMs may influence different aspects of centromere biology, from loading of CID and recruiting components of the kinetochore to correct chromosome attachment and segregation.

CID incorporation into the centromeres is replication-independent

Canonical histones assemble with the newly duplicated DNA as the replication fork progresses with the help of several loading complexes. In contrast, although CENP-A is produced in G₂, it is only incorporated into centromeric nucleosomes at the end of the following mitosis, during late telophase-early G₁ in the case of human CENP-A, and during anaphase for CID in the *Drosophila* embryo^{12,13}. Therefore CID incorporation into centromeric nucleosomes is replication-independent, potentially excluding CENP-A as a target of classical histones loading factors. Extensive studies are ongoing to identify CENP-A loading factors in different species. In budding yeast, the non-histone protein Scm3 is required for CENP-A^{Cse4} centromeric localization. It was proposed that two Scm3 molecules form a hexameric nucleosome with two copies each of CENP-A^{Cse4} and histone H4¹⁴. However, in contradiction to those observations octameric nucleosomes containing two copies each of CENP-A^{Cse4}, H2B, H2B and H4 but not Scm3 could be re-

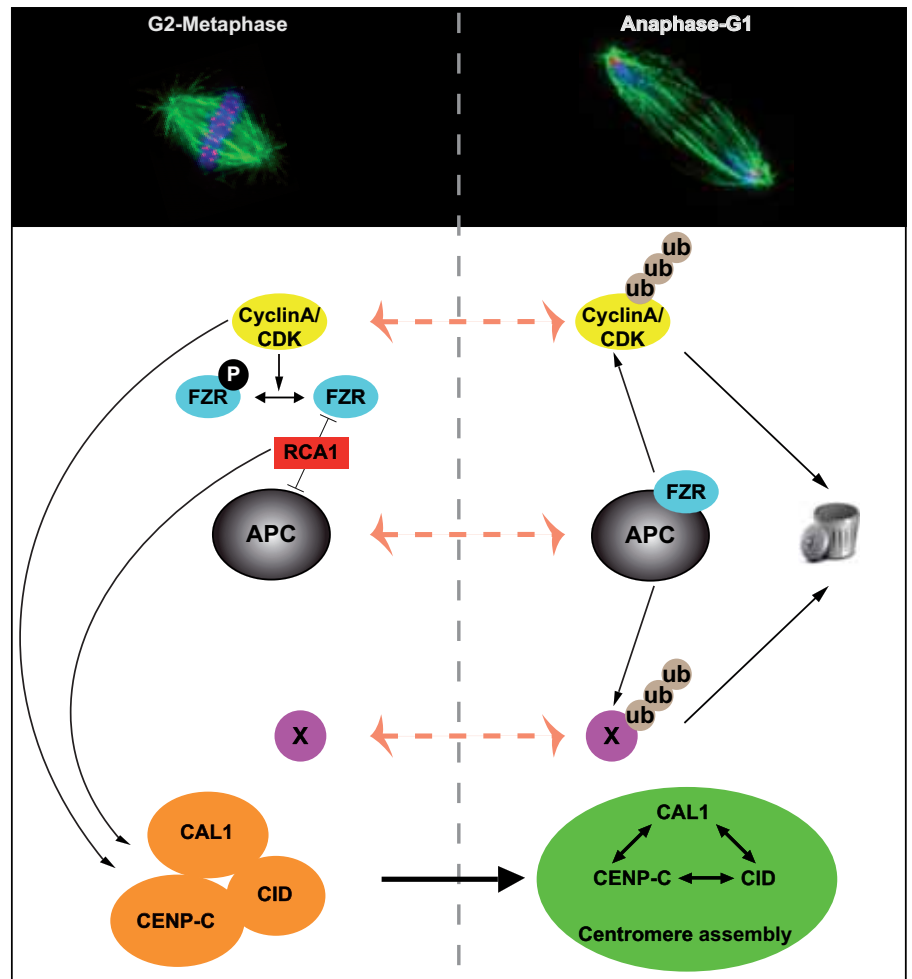


Figure 3: Model for cell cycle dependent centromere assembly in *Drosophila*. Orange indicates that the proteins are not competent for centromere assembly, the green background represents proteins incorporated into the centromere, and light red double arrowheads indicate that these proteins or complexes are required for CID-, CAL1-, and CENP-C- interdependent assembly. The APC substrate could be CYCA or CID, CAL1, or an unidentified substrate, X (see text for details).

constituted *in vitro*¹⁵. Controversies about the composition of centromeric nucleosomes are not restricted to yeast. It has been reported that fly and more recently mammalian centromeric nucleosomes may be tetrameric instead of octameric and that they introduce positive DNA supercoils^{3,16}. The newly solved crystal structure of human CENP-A-H4 dimers however, strongly suggests that chromatin changes caused by CENP-A-containing nucleosomes come from within the folded histone core domain and that CENP-A containing nucleosomes are octameric and with conventional left-handed DNA wrapped around it¹⁷. Recently, a distant human ortho-

log of Scm3 called HJURP has been identified as a chaperon of human CENP-A¹⁸. HJURP is transiently recruited to centromeric chromatin at the time of CENP-A loading at late telophase to early G₁ and is therefore an excellent candidate of a CENP-A-specific loading factor. The loading of CENP-A by HJURP depends on the CENP-A targeting domain (CATD) that is necessary and sufficient for replication-independent CENP-A localization to centromeric chromatin¹⁹.

CID localization to centromeres is highly regulated

In *Drosophila*, genome-wide RNA interfe-

rence screens recently showed that the conserved centromeric protein CENP-C and the newly identified protein CAL1 (Chromosome alignment defect 1) are required for CID localization at the centromere and correct centromere function^{20,21} (Figure 2). Depletion of either protein affects the localization of all known *Drosophila* centromeric proteins tested so far, making them core components of the kinetochore^{20,21}.

CENP-C is a component of the inner kinetochore plate that is incorporated concomitantly with CID at the centromere²³. Despite the fact that CENP-C is conserved from yeast to mammals, its requirement for proper kinetochore assembly varies between species. In contrast to mammalian CENP-C, *Drosophila* CENP-C plays a fundamental role as it is required for centromere maintenance, kinetochore assembly, microtubule attachment and SAC signaling²². One explanation for the differences in CENP-C function probably lies in the divergent composition of the inner kinetochore. Up to now, about 100 proteins have been found that form the vertebrate kinetochore¹. Of those, a large number of proteins known as the Constitutive Centromere-Associated Network (CCAN) are involved in the organization of the inner plate of the kinetochore. Besides CID, the only inner kinetochore protein identified so far in *Drosophila* is CENP-C. CENP-C is present more externally than CID and therefore was suggested to fulfill the structural roles of the human CCAN proteins that do not have clear homologues in *Drosophila*²².

Homologues of CAL1 are only present in drosophilids with no homologues identified in other eukaryotes²⁰. CAL1 is an essential and constitutive kinetochore protein, only expressed in proliferating cells. In addition to its centromeric localization, CAL1 also accumulates in the nucleolus similar to the human CENP-A loading factor HJURP¹⁸.

Interestingly CAL1 depletion not only affects CID localization, but also leads to a reduction in CID protein levels. Whether CID gene transcription, translation or protein stability is affected remains to be investigated. At the same time CID depletion also results in loss of CAL1 protein suggesting a reciprocal regulation at the protein level. Precise ana-

lysis of CAL1 synthesis and stability could provide new insight into how CID protein levels are regulated. CAL1 is also required for CENP-C centromeric localization but has no influence on its protein levels. CAL1 can be immunoprecipitated together with CID and CENP-C; the N-terminal domain of CAL1 interacts with CID, while its C-terminal region binds CENP-C²³. It was therefore proposed that CAL1 functions as a bridging factor between CID and CENP-C. However, it has not been excluded that CENP-C can directly interact with CID-containing nucleosomes in *Drosophila*, similarly to what has been observed with human CENP-C²⁴. Despite these considerations, CAL1 could provide a scaffold to stabilize CID-CENP-C complexes. Quantitative measurements of CAL1 levels at the centromere revealed that CAL1's centromeric amount is lower than that of CID and CENP-C leading to the hypothesis that CAL1 is the rate limiting factor for the incorporation of CID and CENP-C at the centromere²³. Simultaneous overexpression of CID, CENP-C and CAL1 led to increased centromeric levels of all three proteins and caused severe mitotic defects without ectopic kinetochore formation. Therefore centromeric incorporation of CID could be controlled by limiting amounts of CAL1.

Cell cycle regulation of CID protein

In *Drosophila* embryos, CID is incorporated in a replication-independent fashion during anaphase²³. This incorporation is independent of pulling forces generated by the mitotic spindle, but strictly coupled to mitotic progression. The mitotic Cyclin A (CYCA) and the Regulator of Cyclin A (RCA1) were recently shown to be required for CID centromeric localization and correct cell division²⁰ (Figure 2). Both CYCA and RCA1 play a central role in cell cycle control through the inhibition of the activity of the Anaphase promoting complex (APC)²⁵. CYCA in complex with cyclin dependent kinases (CDK) directly phosphorylates and inactivates the APC activator FZR/CDH1, while RCA1 binds to FZR/CDH1 and prevents the interaction of the APC^{FZR/CDH1} with APC substrates²⁶. CYCA is enriched at centromeres after entry into mitosis and decreases at anaphase

onset when it is degraded²⁰. RCA1 does not accumulate at centromeres or chromatin, is degraded at metaphase and newly visible in early G1. Depletion of FZR/CDH1 compensates the depletion phenotype of RCA1 or CYCA and leads to the correct localization of CID at the centromeres. These results indicate firstly that the APC is involved in CID localization at the centromere and secondly that the phosphorylation activity of CYCA/CDK has probably no direct role in this process. This also suggests that one or more APC^{FZR/CDH1} substrate(s) control centromere identity by coupling centromeric chromatin assembly to the cell division cycle (Figure 3). Whether the localization of CYCA at centromeres, which is dependent on CID, CENP-C and CAL1, is important for this process remains to be investigated. Moreover, previous studies in yeast and flies showed that proteolysis facilitates correct centromere formation by degrading non-centromeric CENP-A/CID, while CENP-A located at the centromeres is protected from proteolysis^{27,28}. The loading of CID occurs after anaphase initiation when APC^{FZR/CDH1} activity is high and mitotic CDK activity is low, meaning that an APC^{FZR/CDH1} substrate that controls centromere assembly would likely be ubiquitinated. Since premature APC activation results in the failure of centromere assembly this suggests that an APC^{FZR/CDH1} substrate acts between G2 and metaphase prior to APC^{FZR/CDH1} activity to make centromeres competent for assembly during anaphase – G1 (Figure 3). CAL1 levels at centromeres decline between metaphase and late anaphase and increase again in the absence of FZR. Moreover, depletion of FZR cannot rescue the reduction of CID protein levels due to CAL1 depletion, indicating that CAL1 acts downstream of APC. Thus the levels of CID and CAL1 are regulated by APC^{FZR/CDH1}, and one or both of them might be potential APC^{FZR/CDH1} substrates. One could therefore speculate that CAL1 is a licensing factor for CID incorporation under the control of APC^{FZR/CDH1}.

Concluding remarks

Recent evidence suggests that instead of a hierarchical process directed by CENP-A, centromere assembly appears to be a

co-dependent process requiring different constitutive kinetochore components^{20,29}. Several mechanisms appear to cooperate to ensure that CENP-A is loaded specifically at centromeres during a very limited time window. First, limiting factors may control the amount of CENP-A that is incorporated into centromeric chromatin. Then wrongly localized CENP-A nucleosomes are eliminated through a proteasome-dependent process. How this is accomplished at the molecular level remains to be characterized. Detailed functional analysis of the regulators and their partners as well as determining the importance of CYCA centromeric pool and the search for centromeric targets of the APC^{FZR/CDH1} should provide new insights in how CENP-A loading and centromere assembly is coupled to the cell division cycle.

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Vital functions of profilin 1 for late cytokinesis in differentiated mammalian cells

Ralph T. Böttcher and Mercedes Costell

Introduction

Profilins are key factors for dynamic rearrangements of the actin cytoskeleton. Most of our understanding about profilin function *in vivo* comes from experiments in yeast and gene deletion studies in early embryos which have suggested that profilins are essential housekeeping genes critically required for cell division and F/G-actin homeostasis. However, the vital functions of profilins in differentiated mammalian cells are uncertain because complete profilin deficiency is lethal in early embryos of higher eukaryotes.

Profilins as actin monomer-binding proteins

Actin is one of the most abundant proteins in eukaryotic cells that polymerize from its monomeric form (G-actin) into a filamentous state (F-actin). Actin directly regulates a variety of cellular functions, ranging from sensing and transducing external forces to the maintenance of cell shape and polarity, actin-based cell motility, membrane trafficking, cytokinesis, the regulation of nuclear transport and transcription. More than 100 actin-binding proteins exist in eukaryotic cells

that regulate the pool of monomeric actin within the cell, control actin nucleation, the elongation and severing of filamentous actin as well as its cross-linking and connection to the microtubule and intermediate filament network (Pollard & Cooper, 2009).

Profilin was identified over 30 years ago and since then has proven to be a versatile actin monomer-binding protein with unique properties. Profilins are small proteins with an approximate molecular mass of 15 kDa. They are among the most highly expressed (10–150 μM) cytoplasmic proteins and are distributed throughout the cytoplasm. Profilins are well conserved during evolution and are present in all eukaryotic cells from *Dicystostelium* to mammals. Mammals have four profilin family members: while mouse profilin 1 is ubiquitously expressed throughout all embryonic stages and in all tissues except skeletal muscle, profilin 2 expression is high in the central nervous system and profilin 3 and 4 are testis-specific. Genetic and cell biological studies have implicated profilins in many cellular processes such as cell migration, cytokinesis, endocytosis, transcriptional regulation as well as neuronal differentiation and synaptic plasticity.

Initially, profilin was identified as a G-actin sequestering molecule causing actin depolymerization *in vitro* (Carlsson et al, 1977). However, the view of profilin as pure actin-sequestering molecule changed over the years as later studies revealed that profilin enhances the rate of actin polymerization through different molecular mechanisms (Figure 1). Profilin-bound ADP-G-actin is rapidly recharged with ATP promoting turnover of the monomers so that they are available for faster polymerization (Goldschmidt-Clermont et al, 1992; Mockrin & Korn, 1980). In addition, profilin-ATP-actin complexes can be formed from the non-polymerizable thymosin β_4 -actin pool to associate productively with free barbed ends of F-actin (Pantaloni & Carlier, 1993). This led to the mo-

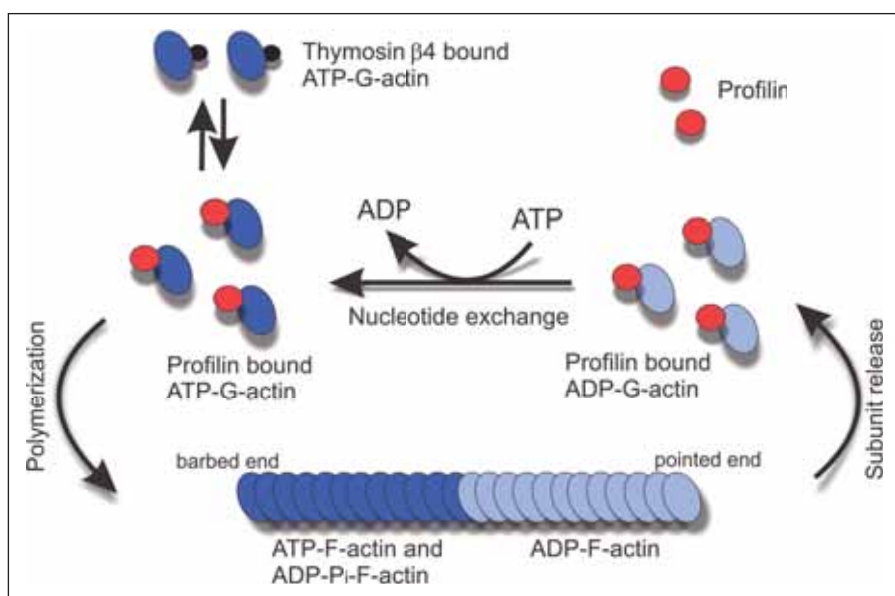


Figure 1: Role of profilin in actin treadmilling. ADP-G-actin is bound by profilin which enhances filament assembly by promoting ADP to ATP exchange on actin and by directing actin monomers to the barbed end of filaments. In addition, profilin displaces the actin sequestering protein thymosin β_4 from ATP-G-actin making ATP-actin available for polymerization. Within the actin filaments the ATP bound to actin is then hydrolyzed to ADP and actin depolymerizing factors cause the release of ADP-actin subunits from the pointed end.

del that profilin acts as a promoter of actin barbed end elongation by adding ATP-actin monomers to the growing end of the actin filament.

Besides actin, profilin 1 binds to phosphoinositides (mainly phosphatidylinositol-4,5-bisphosphate (PIP₂) and phosphatidylinositol-3,4,5-triphosphate (PIP₃)) and an increasing number of proline-rich proteins ranging from those participating in cytoskeletal to transcriptional control in cells (Witke, 2004). PIP₂ competes with actin and partly, polyproline binding of profilin 1 (Lambrechts et al, 2002) suggesting that PIP₂ could potentially act as a negative regulator of the actin-binding function of profilin *in vivo*. The binding of profilin to poly-proline-rich proteins could indicate cross-points of different processes with actin cytoskeletal dynamics or the regulation of such proteins by profilin in an actin-independent manner.

Roles of profilin in mouse development

Although the role of profilins in actin treadmilling has been established *in vitro*, the functional implications of loss of profilins in actin-dependent processes *in vivo* are less clear. Profilin 1 depletions in flies, worms and mice are embryonic lethal (Severson et al, 2002; Verheyen & Cooley, 1994; Witke et al, 2001). The complete knock-out of the ubiquitously expressed profilin 1 in mice leads to embryonic lethality as early as the 2-cell stage due to defects in cytokinesis (Witke et al, 2001).

Mice lacking brain-specific profilin 2 are viable but hyperactive and show increased novelty-seeking behavior which correlates with hyper-excitability and higher synaptic vesicle release probability in knockout neurons (Pilo Boyl et al, 2007). Although profilin 1 and profilin 2 are expressed in the brain, only profilin 2 is required for actin polymerization in the synapse, possibly through a pathway that involves the WAVE-complex indicating that the two profilin family members

have redundant but also specific functions in regulating actin polymerization in neurons.

To overcome the early developmental lethality and to examine the role of profilin 1 in somatic mammalian cells *in vivo*, a conditional profilin 1 knock-out mouse strain with a profilin 1-deficient cartilage has been generated (*Col2pfn1*) (Böttcher et al, 2009). Profilin 1 is the only family member expressed in the cartilage, and no other profilin isoform becomes upregulated after the *profilin 1* gene deletion, demonstrating that the *Col2pfn1* cartilage is a profilin-free tissue. The use of cartilage for studying profilin function has additional advantages: the cartilage is composed of only one cell type which can be readily isolated, cultured and studied *in vitro*. Furthermore, although chondrocytes are sessile cells embedded in a thick extracellular matrix, dynamic F-actin reorganisation plays an essential role to arrange chondrocytes into characteristic stacks or columns. The formation of chondrocyte columns, which is

indispensable for the longitudinal growth of long bones, starts when round-shaped resting chondrocytes enter the cell cycle. Upon cell division, the two daughter cells adopt a flattened shape, migrate rotationally to form the column and become postmitotic to finally enlarge and differentiate into so-called hypertrophic chondrocytes that are responsible for the calcification of the extracellular matrix (Morales, 2007).

Col2pfn1 mice are born at expected Mendelian ratio and most mice survive in the complete absence of profilins in the cartilage. Defective chondrocyte proliferation and disorganization of the growth plate columns are the salient features of profilin deficiency and lead to chondrodysplasia in *Col2pfn1* mice. While chondrocyte survival and differentiation proceed normally, functions associated with rapid actin cytoskeleton rearrangements are severely impaired. Proliferation rates in *Col2pfn1* growth plates decrease in the course of the first four weeks and are accompanied with frequent binucleation of

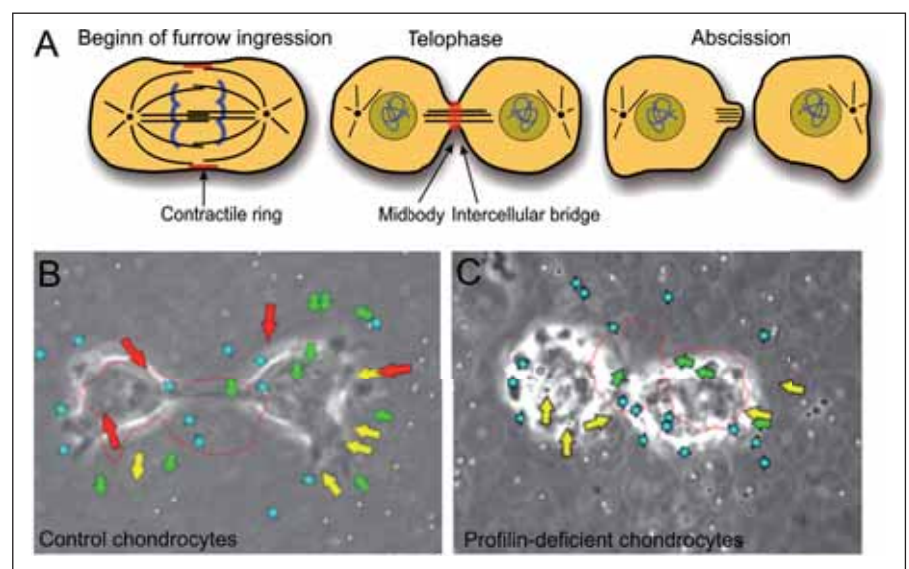


Figure 2: Abscission defects in profilin-deficient chondrocytes. (A) Schematic representation of different stages of cytokinesis. The actomyosin ring is depicted in red, chromatin in blue. (B, C) Traction force measurements of representative mitotic control and profilin-deficient chondrocytes on a flexible polyacrylamide substrate. Arrow direction and colour indicate deformation direction and magnitude (blue<green<yellow<red). The red line shows the location of the mitotic cells at the beginning of the measurement.

chondrocytes due to cytokinesis defects. In addition to alterations in proliferation, mutant chondrocytes exhibit an inability to arrange proper longitudinal columns of disc-shaped cells in the proliferative zone. Proliferative and hypertrophic chondrocytes in *Col2pf1* growth plates display changes in cell shape and size which could be the result of defects in actin cytoskeleton organization. Thus, the conditional deletion of profilin 1 in chondrocytes supports the view that profilins are critically required for rapid actin rearrangements, and also for maintaining cortical actin in differentiated mammalian cells.

Role of profilin in cytokinesis

The depletion of profilin 1 in mouse chondrocytes reveals an important role for profilin in late cytokinesis while actomyosin ring formation and ingression is unaffected (Böttcher et al, 2009). This is surprising as profilins are generally considered to be linked with their ligand formin in contractile ring formation (Glotzer, 2005). In mitosis, the newly forming sister cells are separated by a process named cytokinesis (Figure 2A). A contractile actin-myosin ring causes ingression of the cleavage furrow until the sister cells are only connected by an intercellular bridge. Abscission finally completes cytokinesis by the fission of this intercellular bridge. Genetic studies in yeast and in higher eukaryotes established an essential role of profilins during cytokinesis (Balasubramanian et al, 1994; Severson et al, 2002; Verheyen & Cooley, 1994; Witke et al, 2001). In many cases defects were shown to occur during early cytokinesis and were associated with malforming or non-constricting actomyosin rings. However, a closer and careful inspection reveals that the actual cytokinetic phenotypes of *profilin* gene deletions show a more heterogeneous picture. *Cdc3*, the *Saccharomyces pombe* profilin, is absolutely essential for contractile ring formation and cytokinesis (Balasubramanian et al, 1994). In contrast, profilin-deficient *Tetrahymena ther-*

mophila ciliates form and constrict contractile rings (Wilkes & Otto, 2003). *Dicystostelium* amoebae can bypass profilin deficiency by a furrowless, actomyosin-independent cell division (Haugwitz et al, 1994). A similar phenotype has been described for mammalian cells treated with high concentrations of the myosin II inhibitor blebbistatin (Kanada et al, 2005).

In animal cells contractile ring assembly is mediated by the GTPase RhoA and its effector proteins, formins and Rho-associated kinase (ROCK), which regulate actin nucleation and myosin activation, respectively (Glotzer, 2005). Formins nucleate actin filaments and associate with the barbed ends of actin. Profilins bind to proline-rich motifs of formins and thereby increase the actin nucleation rates of formins (Kovar et al, 2006; Romero et al, 2004). However, formins (besides fission yeast *Cdc12p*) can elongate actin filaments without profilin *in vitro* and rely on profilins for accelerating elongation rates (Kovar et al, 2006; Romero et al, 2004). In some profilin-deficient cells, including *Col2pf1* chondrocytes, the basal filament elongation rate of formins without profilin might be sufficient to generate a functional actomyosin ring. Besides *de novo* nucleation of actin filaments by formins, the recruitment of existing F-actin contributes to actomyosin ring formation in certain cells (Wang, 2005). Hypothetically, profilin deficiency may be compensated by an increased recruitment of pre-existing filaments to the cleavage furrow in some cell types, including mouse chondrocytes.

The analysis of profilin-deficient chondrocytes also revealed that profilins are essential for late stages of cytokinesis, in particular abscission. One mechanism of abscission suggests that postmitotic sister cells can crawl apart to generate traction forces which contribute to thinning of the intercellular bridge and midbody cleavage. Such traction forces of dividing cells have been measured

in Swiss 3T3 cells (Burton & Taylor, 1997) and were lost in mutant amoebae devoid of conventional myosin (De Lozanne & Spudich, 1987). In profilin-deficient chondrocytes the abscission failure coincides with lower and less oriented traction forces in daughter cells (Figure 2B, C). Cell traction force is generated by actin polymerization at the leading edge that drives forward the leading front and by the cell's contractile apparatus consisting of an actin stress fibre system and myosins that contracts the cell body (Wang & Lin, 2007). The cellular tension is then relayed to the extracellular matrix (ECM) by integrins and integrin-associated proteins which connect the actin cytoskeleton with the ECM. Indeed, profilin-deficient chondrocytes display defects in all components of the traction force generating and relaying apparatus (Böttcher et al, 2009). First, the contractile apparatus formed by the RhoA/*mDia1*-induced actomyosin assembly of the stress fibre system is defective in the absence of profilin. *mDia1* is unable to efficiently elongate filaments in profilin-deficient chondrocytes, as evidenced by the failure to increase stress fibre assembly in cells expressing a constitutive-active form of *mDia1*. Second, chondrocytes lacking profilin exhibit a delay in re-spreading (and spreading in general) and fail to form lamellipodia. Third, the mutant cells display defects in transmission of the traction force to the substratum as focal adhesion assembly and maturation are defective in profilin-deficient chondrocytes. An impaired *mDia1* function could cause this defect in chondrocytes lacking profilin as the formin *mDia1* is critically involved in focal adhesion maturation in a Rho- and ROCK-independent manner (Riveline et al, 2001).

Conclusion and outlook

Even though profilins have been established as essential housekeeping genes critically required for cell division and F/G-actin homeostasis mouse chondrocytes can survive in the absence of profilins and it is intriguing

to speculate that other sessile, non-proliferating, differentiated mammalian cell types may also not require profilins to fulfil essential actin-based functions.

As profilin deficiency is lethal in early embryos of higher eukaryotes the use of conditional knockouts will help to study its involvement on functions that imply dynamic F-actin remodelling and generation of force. Special interest has the immune system where highly migratory leukocytes have to extravasate and migrate within tissues as well as cancer cells that rely on force generation during tissue invasion. This is particularly interesting in the light of recent studies that suggest a context-dependent role for profilin 1 in cell migration. While reduced profilin 1 levels increase the motility and aggressiveness of breast cancer cells (Zou et al, 2007) it has the opposite effects on endothelial cells (Ding et al, 2006).

Finally, one poorly addressed question is why some mammalian cells express additional profilin genes although profilin 1 is abundantly expressed at high concentrations in nearly all cells. One explanation could be that certain cell types might require higher profilin levels to regulate actin dynamics. However, other profilins might provide additional functions as indicated by different ligand binding properties of different profilins *in vitro* (Witke et al, 1998). The central nervous system will be an attractive model to elucidate specific and redundant functions of profilin 1 and profilin 2 as the phenotype of profilin 2 knock-out mice suggests that the two profilin family members function in different processes (Pilo Boyl et al, 2007).

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Parstatin, a novel PAR₁-derived peptide, with biological functions

Dimitris Zampatis and Nikos E. Tsopanoglou

Introduction

Protease-activated receptors (PARs) consists a new family of G protein coupled receptors (GPCRs), which are activated by proteases. Enzymes such as thrombin and trypsin bind to the receptor and then cleave its N-terminal extracellular domain. The newly unmasked amino terminus binds intramolecularly to the proximal transmembrane loop of the PAR, eliciting intracellular signaling and causing biological responses [1]. This unique intramolecular activation mechanism is termed “tethered ligand mechanism” [2].

PAR₁, the first member of this family to be cloned has been shown to binds with high affinity to thrombin. The proteolytic cleavage of the N-terminal region of human PAR₁ by thrombin, at the R₄₁/S₄₂ bond, results in the release of a 41-amino acid peptid and the generation of a new N-terminus with the characteristic sequence SFLLRN, which serves as a tethered ligand that activates the PAR₁ receptor itself. PAR₁ is expressed by a variety of cell types, including platelets, endothelial cells, vascular smooth muscle cells, cardiomyocytes, and a variety of epithelial cancer cell types, and participates in many pathophysiological processes. For example, PAR₁ has been proposed to play a pivotal role in haemostasis and vascular development [3]) and mediates the angiogenic activity of thrombin [3]. However, little attention has been paid to the 41-residue peptide fragment released during PAR₁ activation. Besides PAR₁ activation, it has recently been demonstrated that the above mentioned peptide could also exert biological effects. The name of “parstatin” has been coined for this peptide.

Parstatin causes platelet activation

Early studies have shown that parstatin promotes platelet aggregation and platelet-endothelial cell adhesion in vitro [4,5,6]. However, for most of these studies washed platelets were used in the absence of serum, which arises some concerns about the role of parstatin in platelet functions in vivo.

In more detail, it was shown that the cleaved peptide is a strong platelet agonist. Parstatin was more potent than TR₄₂₋₅₅ (TR-activating peptide) and almost as potent as thrombin to activate platelets. Treatment of platelets with the parstatin was followed by increased platelet surface expression of P-selectin, exposure of the fibrinogen binding site on the GPIIb-IIIa complex and the fibrinogen binding to the activated GPIIb-IIIa complex [5].

It could also be shown that the TR₁₋₄₁ is a more potent stimulus for platelet-endothelial cell adhesion since TR₁₋₄₁ was able to induce a threefold increase in platelet-endothelial cell adhesion and a 10-fold increase in the levels of fibrinogen bound to the platelet surface compared to a treatment with thrombin [6].

Parstatin is a potent inhibitor of endothelial cell functions and angiogenesis

Parstatin suppressed both basic angiogenesis and bFGF and VEGF stimulated angiogenesis in the chick chorioallantoic membrane (CAM) model and the rat aortic ring model. Parstatin also inhibited endothelial cell migration and capillary-like network formation in the Matrigel and fibrin angiogenesis models in vitro [7]. Treatment of endothelial cells with parstatin resulted in inhibition of cell growth by inhibiting ERK_{1/2} phosphorylation in a specific and reversible fashion and by promoting cell cycle arrest and apoptosis through a mechanism involving activation of caspases. The molecular mechanism by which parstatin could exert these effects

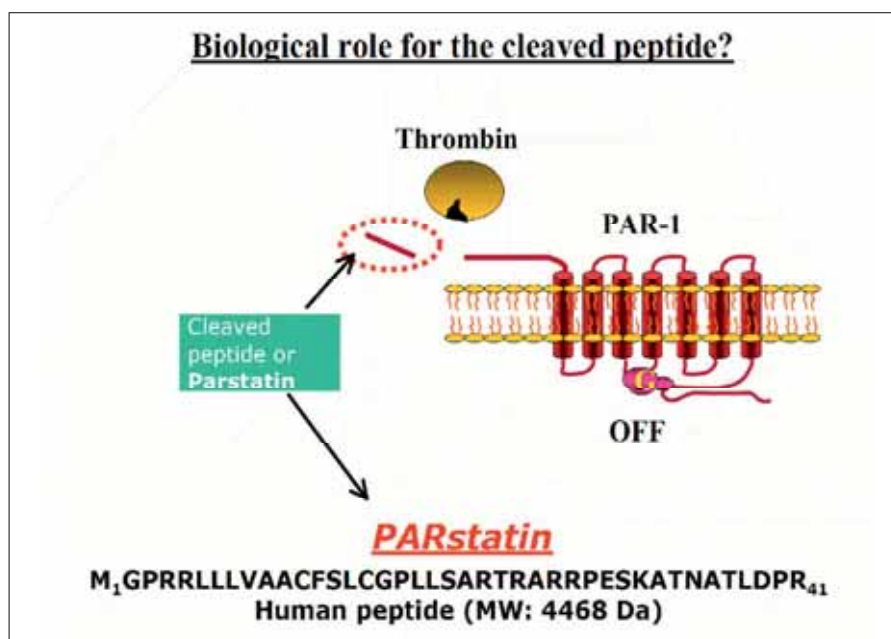


Figure 1: Putative generative mechanism of the Parstatin and its amino acid sequence

is under investigation. It is of interest that parstatin is able to cross the plasma membrane, indicating a crucial role of the hydrophobic domain of the peptide to exert its biological functions in endothelial cells [7]. Based on these findings, parstatin could be useful for treating angiogenesis-related diseases, such as angiogenesis-dependent cancer and ocular diseases.

A very recent study provides the first strong evidence that parstatin has a therapeutic potential in the treatment of neovascular ocular diseases [8]. Intraocular injection of parstatin strongly suppressed ischemia-induced retinal neovascularization in neonatal mice. Dose dependency with high potency and efficacy were observed in the ischemic retinopathy model in which maximal inhibition of about 60% was achieved with 3µg of parstatin. Similarly, intravitreal or subconjunctival administration of parstatin inhibited choroidal neovascularization at Bruch's membrane rupture sites in mice. Furthermore, it potently delayed the onset and progression of neovascularization in rat corneas with chemical burn-induced trauma. The anti-angiogenic effects of parstatin in these experimental models of ocular neovascularization are comparable to those of the most effective treatments currently known, such as treatment with anti-VEGF, anti-VEGF receptor-2, and anti-PlGF antibodies. The extent of the inhibition of ocular neovascularization by parstatin attainable in these experiments may be an underestimation of the inhibition to be expected in primates and humans. Parstatin peptide used in the abovementioned rodent models corresponds to the cleaved fragment of human PAR₁ which shares 63% and 67% homology to the mouse and rat parstatin, respectively. Despite of cross-species activity of parstatin, it a considerable species specificity could be demonstrated *in vitro* [7].

The anti-angiogenic therapy has the advantage that it preserves the function of retinal tissue by direct targeting of the neovascular

complexes. Current protein-based therapies for ocular angiogenesis inhibit only VEGF and, because of their large size, they have to be administered by repeated intraocular injections. Parstatin, which blocks both angiogenic activity of VEGF and bFGF, may provide greater efficacy for the treatment of ocular neovascularization than targeting of VEGF alone. In addition, agents that can be delivered by topical administration to the cornea offer substantial advantages (less invasive delivery mode, potential for a superior safety profile, and reduced systemic exposure). Parstatin as a cell penetrating peptide may be effective by topical application onto the eye.

Parstatin protects myocardium from ischemia and reperfusion injury

Furthermore, it was recently demonstrated [9] that parstatin is an effective agent for cardioprotection during ischemia and reperfusion. The protective role of parstatin was assessed in an *in vivo* and *in vitro* rat model of myocardial ischemia–reperfusion injury. Parstatin treatment before, during, and after ischemia decreased infarct size by 26%, 23%, and 18%, respectively. Parstatin treatment immediately before ischemia decreased infarct size by 65% and increased recovery in ventricular function by 23% in this *in vitro* model. The cardioprotective effects of parstatin were abolished by inhibition of nitric oxide synthase (NOS), ERK1/2, p38 MAPK, and KATP channels *in vitro*. Furthermore, parstatin increased coronary flow and decreased perfusion pressure in the isolated heart. The vasodilatory properties of parstatin were confirmed in rat coronary arterioles. These results provide the first strong evidence for a therapeutic potential of parstatin in the treatment of cardiac injury resulting from ischaemia and reperfusion. Interestingly, in an *in vivo* rat model of myocardial regional ischemia/reperfusion injury the parstatin fragment 1–26, which contains the functional domain of parstatin, has turned out to

be more potent in cardioprotection than full-length parstatin [10]. Parstatin (1–26) fragment was able to reduce infarct size by 78% and 62% when applied before or after reperfusion, respectively.

Conclusion

All the aforementioned experiments provide evidence about the ability of the synthetic parstatin peptide to act as a biological factor in cells. It is of great interest to further investigate whether the native peptide causes the same or any other biological effects.

How does parstatin exert its anti-proliferative effect? Is it acting as a competitive inhibitor of other ligand-receptor interactions? Does parstatin directly inhibit intracellular signaling pathways? Furthermore, is parstatin able to disrupt membrane integrity at the cell surface, nucleus or other cellular compartments?

Moreover, exogenous parstatin rapidly localizes to the cell surface, penetrates the cell membrane, and accumulates in the intracellular space [7]. The primary amino sequence of parstatin suggests that is constituted from two domains. The first 21 aa are highly hydrophobic while the remaining 20 aa are hydrophilic. The first part seems to be responsible for the ability of the peptide to penetrate the cell membrane due to its high hydrophobicity, since truncated forms of the peptide lacking this N-terminal sequence are ineffective.

Here it is important to mention that most GPCRs have a signal peptide sequence at their N-terminus. Signal peptides are targeting newly synthesized proteins to the endoplasmic reticulum, where the signal peptide it is cleaved off the protein, which is then guided through the secretory pathway to the plasma membrane. For the PAR₁ receptor it is not known until now, if it possesses a signal peptide or not. We only assume from its amino sequence that it contains a signal peptide which is cleaved off presumably at position 26. Since the PAR₁ receptor is activated by proteolytical cleavage of its N-terminus, it is of a great

importance to answer the question whether the PAR₁ receptor indeed contains a functional signal peptide and if this is cleavable or not. A further question is whether parstatin generation (coinciding with PAR₁ activation) serves to regulate and counterbalance signaling via its activated parent molecule. We will attempt to elucidate the exact length and the mechanism of action of native parstatin and how it penetrates the cell membrane and enters the cytosol. Furthermore we will try to identify further roles of PAR₁ in the numerous physiological and pathophysiological processes in which it is implicated.

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Signal Transduction – Receptors, Mediators and Genes *DGZ study group Meeting report*

Ralf Hass

The 14th Joint Meeting "Signal Transduction - Receptors, Mediators and Genes" was held at the Leonardo Hotel in Weimar in October 2010, and was organized by the Signal Transduction Society (STS) together with the study groups of the German Society for Cell Biology (DGZ), for Immunology (DGfI), for Biochemistry and Molecular Biology (GBM), and the special interest group "Chemical Biology" of the DECHEMA, DPhG, GBM and

GDCh. This meeting gathered about 200 participants with 11 invited keynote speakers and more than 120 scientific contributions for oral and poster presentations.

The meeting started with a workshop on "Immune Signaling" which was introduced by a keynote lecture of Thomas Hünig, Würzburg, on the T cell receptor co-stimulatory molecule CD28 and the corresponding fully humanized mouse anti-human CD28 mono-

clonal antibody (TGN1412) also called CD28 superantagonist.

A major topic of the meeting was dealing with "Drug Discovery and Design" whereby Toby Gibson, Heidelberg, discussed the possibilities of drugging cooperative signalling networks and Thomas Blundell, Cambridge focused on structural biology and fragment-based approaches to drug discovery. Moreover, Angela Russell, Oxford, reviewed chemical biology of stem cell modulation under the view to efficiently control and direct stem cell fate. Horst Kessler, München, introduced possible chemical modifications around the RGD peptide sequence motif which is recognized i.e. by integrins during cellular attachment to develop more selective integrin ligands. Finally, Chas Bountra, Oxford, demonstrated epigenetic factors involved in reversible chromatin modifications and in his keynote lecture he discussed if the reversion or at least an attenuation of chromatin modifications would enable the delivery of superior therapeutics i.e. for chronic diseases. Distinct DNA damage checkpoints upon telomere dysfunction-induced aging were summarized in the workshop "Tumor Biology" by Lenhard Rudolph, Ulm. Thus, Professor Rudolph presented in his keynote lecture different models for mechanisms of stem cell aging and demonstrated that a disrupted telomerase enzyme activity i.e. by down-modulation of the RNA-associated subunit TERC can induce premature aging.

The workshop "Cell Differentiation and Death" was introduced by Jochen Hühn, Braunschweig, on the role of the forkhead box transcription factor Foxp3 and epigenetic modifications within the Foxp3 gene during maturation and development of CD4+ regulatory T cells.

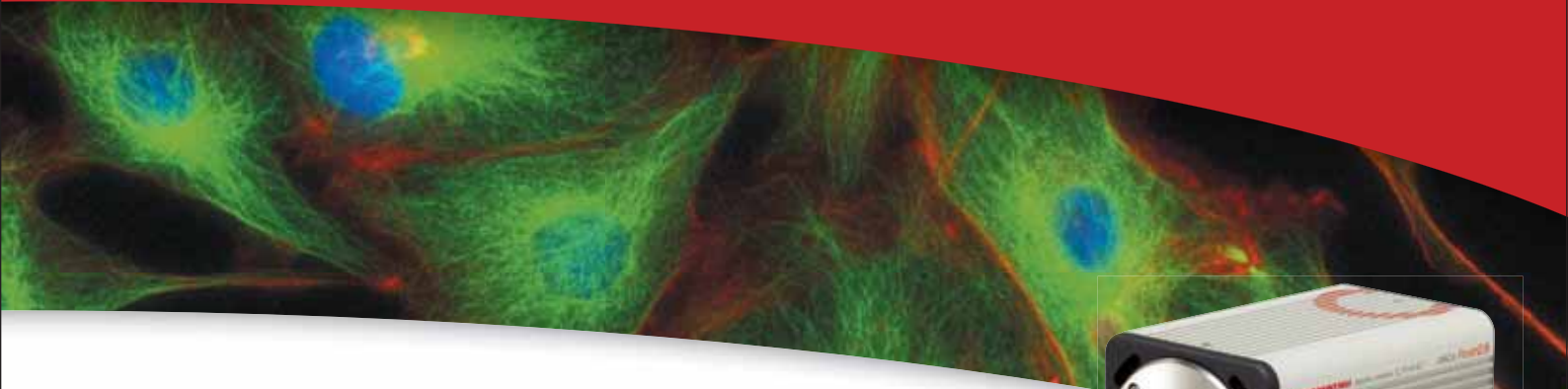
Eicke Latz, Bonn, was invited in the workshop "Pathogens and Disease" and his keynote talk described the function of the Nod-like receptor which belongs to the innate immune receptor family to recognize and respond to microbial infections.

The Honorary Medal 2010 of the Signal Transduction Society (STS) and its society journal CELL COMMUNICATION AND SIGNALING (CCS) was awarded to **Professor Anthony J. Pawson** for the discovery of protein interaction domains and elucidating their essential roles in the transmission of cellular signals. Prof. Pawson has been a tireless advocate and seminal contributor to this research field.



STS/CCS Honorary Medal 2010 to Prof. Anthony J. Pawson
http://www.lunenfeld.ca/about-us/news-and-media-stories/copy_of_dr_tony_pawson_receives_honorary_medal_from_the_signal_transduction_society
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The keynote lecture in the workshop "Growth factors, Cytokines and Chemokines" was given by Stefan Rose-John, Kiel, on pro- and anti-inflammatory activities of cytokines whereby he concentrated on the role of the metalloprotease ADAM 17 (also known as TACE (tumor necrosis- α -converting enzyme)) during inflammatory processes.

A special highlight of this joint meeting was the awarding of Tony Pawson (Toronto) with the first STS/CCS Honorary Medal. Dr. Pawson received this award of the STS and its Journal "Cell Communication and Signaling" (CCS) for his groundbreaking studies on conserved interaction domains, particularly src homology-2 (SH2) domains in the regulation of a variety of signal transduction pathways.

Concluding remarks

Once again, the STS Meeting has provided a state-of the-art overview of various areas

of signal transduction research in which progress is fast and discussion is lively. Therefore, within the last decade this joint effort of the STS with the support of the DGZ, DGfI, GBM and further scientific groups managed to establish an annual interdisciplinary Meeting on "Signal Transduction – Receptors, Mediators and Genes", which is well recognized as a most attractive opportunity to exchange results and ideas in the field. Accordingly, the organisation for this years meeting has already started which will be held again at the Leonardo Hotel in Weimar from November 7th to 9th. Together with the symposium on "Signaling in Immune Cells" this years workshop topics will include

- Receptor-triggered Pathways
- Tumor Biology and Cancer Therapy
- Signal Alterations Induced by Pathogens
- Adhesion, Motility, Morphology
- Stem Cell Development

- Senescence
- Cell Death
- Complex Signalling Systems/ Mathematical Models
- New Experimental Approaches

Further information about the program, abstract submission and registration of this years meeting can be obtained via the webpage of the DGZ (www.zellbiologie.de) or via the STS webpage at www.sigtrans.de.

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The Art of Trojan War – How Cellular Mechanisms Are Exploited by Viruses

Kay Grünewald¹ and Mario Schelhaas²

Meeting Report on the 9th Workshop 'Cell Biology of Viral Infections' of the German Society of Virology (GfV) in Deidesheim, September 29th – October 1st 2010.

Viruses are masters of disguise and subversion. They follow a Trojan Horse strategy to enter cells and use them to propagate. As intracellular parasites they have evolved over millions of years to take advantage of cellular mechanisms to support their life cycle as well as to avoid cellular defense mechanisms. In a way, they are THE experts on many cellular mechanisms such as endocytosis, nuclear import, transcription, replication, exocytosis, or apoptosis, signalling, and the innate immune system. Hence, studying virus life cycles implicitly contributes to our understanding of cellular mechanisms. Viruses provide a simple, limited component system that can be easily followed by the molecular biological, biochemical, and microscopical techniques.

To foster exchange and research at the crossroads of cell biology and virology, the German Society of Virology established the study group 'Cell Biology of Viral Infections' a decade ago. The main purpose of this study group is to bring together researchers of the

fields of virology and cell biology by means of informal workshops. Held again on the estates of the famous winery Basserman-Jordan (Deidesheim) in the palatinate region, this year's 9th annual workshop was as again generously co-sponsored by the German Society of Cell Biology. Four cell biological keynote speakers gave exciting insights into their particular research efforts.

Michael Way (Cancer Research UK, London) focussed on the various strategies that viruses use to hijack the cellular transport machinery. Using mainly the two poxviruses Vaccinia virus and Yaba-like disease virus as model systems and comparing them to e.g. retroviruses he exemplified the plethora of cytoskeleton interaction modes that are used by viruses at different stages in the life cycle. He showed that this does not only include a 'jump' from one cytoskeletal element to the next, but also that involvement of different motor proteins results in various speeds while using actin-based motility. This tale of actin and actin tails was then completed by spotlighting on some of the less known exit mechanisms that viruses use including the involvement of clathrin lattice sheets.

Wolfram Antonin from the Friedrich Miescher Institute in Tübingen zoomed on the nuclear envelope and its assembly and disassembly in the course of the cell cycle. Using the elegant combination of biochemical characterising protein-protein interactions biochemically on one hand and dedicated in vitro systems on the other he focused on the striking structural rearrangements during mitosis. Particularly he emphasized the intriguing mechanisms how nuclear pore complex components are reinserted into the membrane and how their distribution between the outer and inner nuclear envelope is regulated.

Werner Franke (DKFZ Heidelberg) revisited the different types of cell junctions. He showed a plethora of examples, where junctional proteins that in textbooks appear to be limited to a defined set of junction types mix and form intermediate or novel kinds of junctional complexes. He also highlighted the use of junctional proteins as diagnostic markers in disease, particularly in certain tumours. The talk was spiced by the intermittent reflections on philosophical and ethical questions that deal with research and researchers.

Finally, *Jürgen Klingauf* from the University of Münster highlighted the various tools and methods for visualizing membrane trafficking at the presynapse. This hotspot of exo- and endocytosis is of considerable interest for neurological and physiological research, but also fundamental basic questions are addressed in this model system. Displaying and explaining a variety of imaging methods from high resolution to superresolution fluorescence microscopy techniques combined with the use of pH-dependent dyes, he elaborated how these modern techniques validate or contradict longstanding models of how the different vesicle pools act in membrane/vesicle retrieval and excitation of signals. His talk resulted in a lively discussion about the many uses of these techniques for analyzing membrane trafficking events in virus infections.

Besides the invited speakers, the participants displayed in a number of talks and posters the wide variety of cell biological features of viral infections. Topics ranged from transport of papillomavirus interactions with



One condiment of good scientific discussions at the meeting – barrels of excellent Riesling wine.

sequence-specific polysaccharides, manipulations of cellular membranes by adenoviruses and herpesviruses, involvement of histone deacetylases in the endocytosis of viruses, transport of papillomaviruses along microtubules, analysis of Influenza A virus induced and modulated signalling, to systems biology approaches to study nucleo-cytoplasmic transport of herpesviral proteins, - just to name a few. On the other hand the participants represented as well a wide expertise in techniques covering a range from specialized life cell imaging at cutting edge speed and sensitivity, small molecule techniques, RNAi screens, high resolution cryo electron microscopy, to dedicated studies in 3D tissues.

Possibly with the help of the excellent Riesling served during the compulsory wine tasting, the meeting proved again to be a platform that stimulated interdisciplinary discussions, provided valuable feedback and served as a nucleation point for a number of new collaborations. It also saw an increased recognition of the value of this interdisciplinary meeting by the European research community. While previous meetings were mostly attended by researchers from Germany, more and more scientists from Switzerland, France, and the United Kingdom joined the meeting. Currently, negotiations with the European Society of Virology are under way to establish this meeting on a European level. As for now, the meeting series will continue as in earlier years though with some change in organization. We have organized the meeting for the last three years, and it was time to pass the torch. New chairs for the meeting have been elected for the following three years period – Susanne Bailer (Munich) and Harald Wodrich (Bordeaux) invite you to this year's 10th workshop that takes place from September 21st-23rd again in Deidesheim. The new organizers would like to emphasize and explicitly extend their invitation to all researchers in fields of cell biology as well as pathogen modulated host cell behaviour to join them in autumn 2011. We are sure that this series of workshops will continue to stimulate interactions between researchers, both, with cell biological and virological interest. For us, the last three years were most rewarding in that in particular young scientists expressed their view that this meeting series would be one of the most helpful, interactive, and scientifically stimulating meeting that they had attended and the numerous successful collaborations initiated at this meeting speak for themselves.

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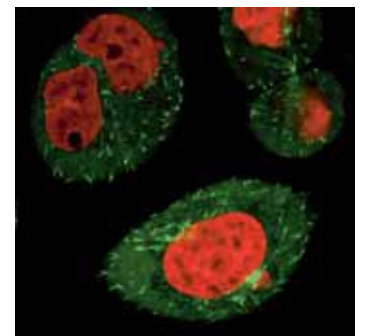
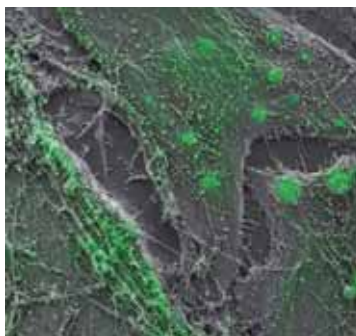
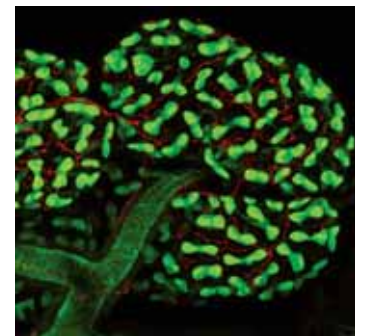
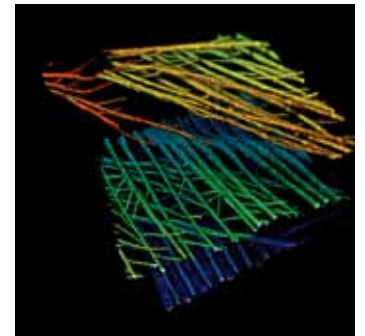
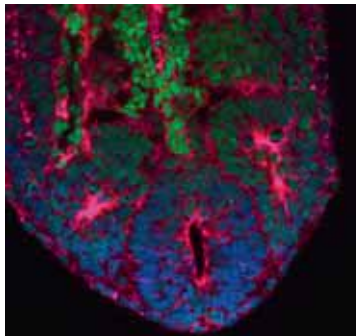
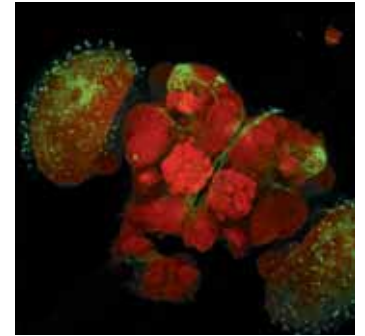
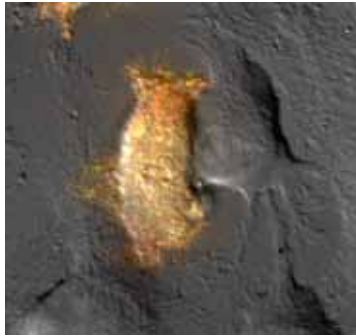
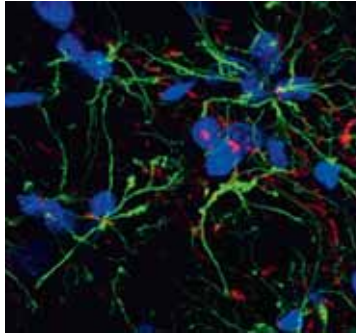
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