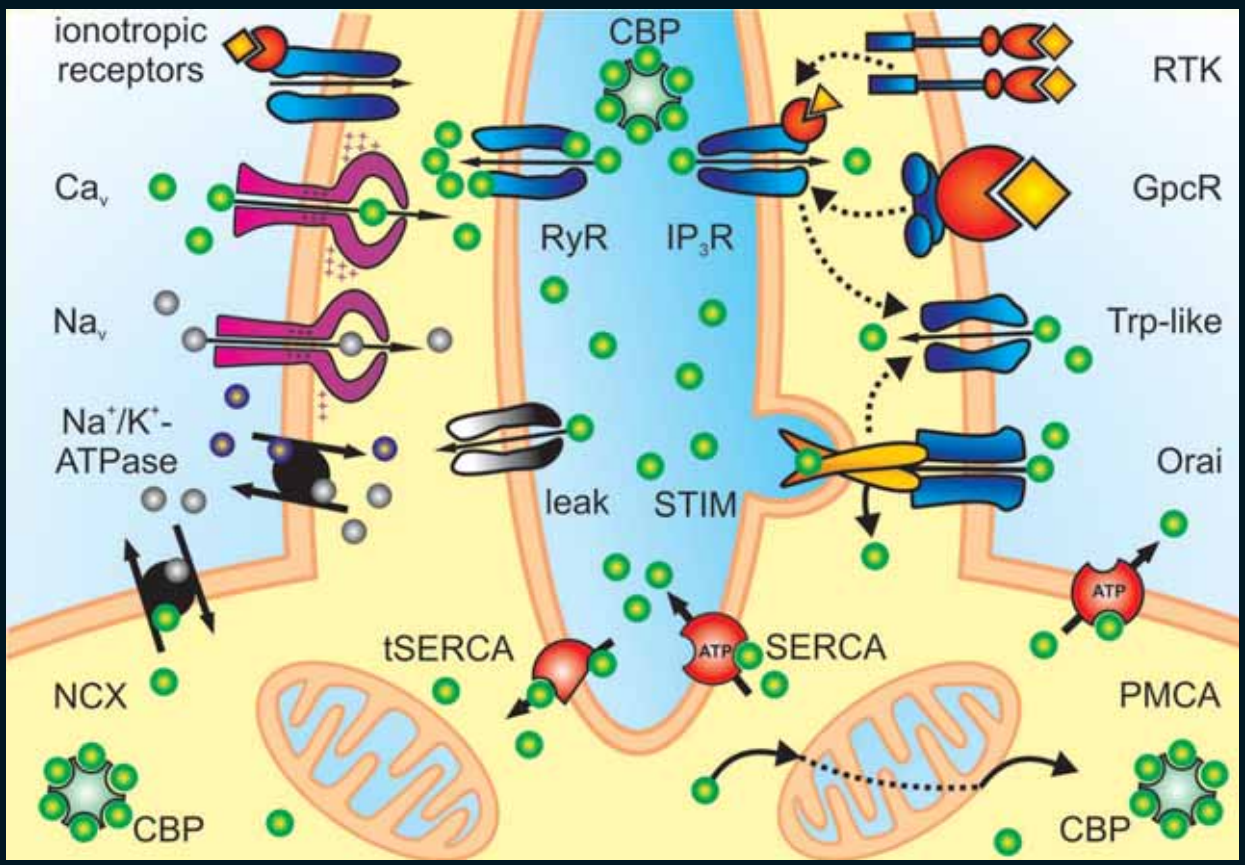
Cell News

Newsletter of the German Society for Cell Biology Volume 36, 4/2010



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CONTENT

DEUTSCHE GESELLSCHAFT FÜR ZELLBIOLOGIE

Cover image:
Cartoon showing the diverse membrane proteins of the plasma membrane, the endoplasmic reticulum and of mitochondria that are involved in Ca^{2+} homeostasis and signaling in neuronal cells.

Going Quarterly

Here we present the fourth issue of *Cell News* (formerly *Zellbiologie aktuell*) in 2010. Its major incentive is to highlight the upcoming "Annual Meeting" of the society in Bonn in March 2011. On pages 3 and 4, you will find the tentative program with 5 plenary sessions and 15 symposia. This program highlights again that our society is able to gather leading scientists from various disciplines – including many prominent colleagues from abroad – and to organize an absolutely "top notch" congress, as in previous years. Those who prefer to go to special meetings should however realize that we will have, together with the poster presenters, several "special meetings" side by side within this congress. By and large, with approximately 600 participants over the four days, it is still a comfortable meeting with many opportunities for discussion and socializing among "the flock". Please, note the abstract deadline (January 15th) and the very low registration fee for student members!

In addition, the next "International Meeting of the DGZ" in June 2011 in Potsdam is now introduced in more detail. It is a pleasure to see that the organizers, Manfred Schliwa and Ralph Gräf, wrote an accompanying perspective, *"The HiStory of Microtubules"*, that will inform you on various aspects of this highly versatile filament system.

Meeting Report

The organizers of the "Actin Dynamics" meeting of this September, held at the *Carl Zeiss* facilities, give as announced in *Cell News* 3/2010, a detailed report "All in the name of actin" on page 41. As you will learn, all participants found this meeting so important and timely that they decided to have it from now on every two years, here in Germany: A big opportunity for young scientists to hear about the latest developments and to connect to leaders in the field. The board of the DGZ is happy to confirm the continuous support of our society for this endeavour. We also welcome very much that the organizers of this meeting are going to organize again a *Summer School* on this topic, half of the students will come from a *Priority Program* funded by the German Research Foundation (DFG) on "Actin Nucleator Complexes", the other half will be selected from applying students and will be supported by the DGZ for those who are members.

Young Scientist Meeting

The next *"Young Scientist Meeting"* will be organized by Sven Diederichs (DKFZ & University of Heidelberg) and Dirk Grimm (Bioquant, Heidelberg). It will be held in Jena, September 2011. The topic will be "RNA and Disease", and the meeting will cover long non-coding RNA, microRNA, snRNA, snoRNA and maybe piRNA. We are confident that these eminent, young researchers will establish a fantastic program. Please, draw the attention of students in the field, who are not DGZ members yet, to this event. As in previous years, we will have space for up to 50 students.

Soft Matter – Tough Cells

In an attempt to further enhance the collaboration of scientists from biophysics and cell biology, Josef Käs (University of Leipzig) will organize, together with the DGZ, a Special Interest Meeting focussing on cell biomechanics. The meeting will take place on two days next autumn in Leipzig. A flavour of this subject can be obtained from the article "Probing the physics of tumor cells from mechanical perspectives" by Mareike Zink and colleagues in this issue (p. 17-21). Leipzig excites with a breathtaking atmosphere and a natural charm. It offers very attractive architecture (Jugendstil), interesting art exhibitions and a special cuisine. In summary, a reason to stay a bit longer as it offers definitively more than what you can grasp from the background scenes in "SoKo Leipzig".

Editorial Issues

In this issue you will find a *"Highlights"* contribution by Pavle Krsmanovic, a PhD student from my group, who was amazed by a series of publications on nuclear organization and dynamics. Working on the function of telomeres in his master thesis, he realized the tremendous progress that is taking place in the field of nuclear architecture and function, and therefore he decided to briefly review these papers for a wider, non-specialist audience. I enjoyed reading his article and would strongly encourage other PhD students and young post-doctoral fellows to follow his example. What is going on with cancer stem cells; any news about motors; how diverse is the knowledge about the mammalian *Target of Rapamycin* (mTor) by now; which new microscopic techniques are about to revolutionize our way to analyze cells? – and many other topics that most of us are not able to follow any more in sufficient detail. Engaged contributions, kind of "in a nutshell"-presentations of the latest developments in once' own field are highly welcome. Of course, the principal investigators should/could mentor such a contribution.

With this expectation, I wish that you will use the last two weeks of the year to get at least some of the things done that you intended to finish in 2010, and last but not least

Frohe Weihnachten und ein gesundes Neues Jahr.

Yours
Harald Herrmann

34th DGZ Annual Meeting – German Society for Cell Biology

30 March – 2 April 2011, Bonn

CARL ZEISS LECTRUE

Ueli Aebi, Biozentrum Basel
(M.E. Müller Institute for Structural Biology Biozentrum, University of Basel, Switzerland)

PLENARY SESSIONS

Plenary session 1: Cell polarity
Chairs: Elisabeth Knust and Julie Ahringer
Speakers: Elisabeth Knust, Julie Ahringer, Frank Bradke, Matthieu Piel

Plenary session 2: Cellular mechanics
Chairs: Jan Lammerding and Sarah Köster
Speakers: Jan Lammerding, Sarah Köster, Chris Chen, Peter Friedl Jochen Guck

Plenary session 3: Cell cycle and cancer
Chairs: Ingrid Hoffmann and Zuzanna Storchova
Speakers: Ingrid Hoffmann, Zuzanna Storchova, Jan-M. Peters, Geert Kops, Holger Bastians

Plenary session 4: Cytoskeletal dynamics
Chairs: Walter Witke and Robert Grosse
Speakers: Walter Witke, Robert Grosse, Melina Schuh, Thomas Surrey, Maria Vartiainen

Plenary session 5: Stem cells
Chairs: Bernd Fleischmann and Martin Zenke
Speakers: Bernd Fleischmann, Martin Zenke, Oliver Brüstle

SYMPOSIA

Symposium 1: Frontiers in microscopy
Chairs: Rainer Pepperkok and Werner Kühlbrandt
Speakers: Rainer Pepperkok, Werner Kühlbrandt, John Briggs, Stefan Jakobs

Symposium 2: Signalling and Rho GTPases
Chairs: Alexander Pfeifer and Gudula Schmidt
Speakers: Alexander Pfeifer, Gudula Schmidt, Cord Brakebusch, Olivier Pertz

Symposium 3: Nuclear envelope and NPCs
Chairs: Angelika Noegel and Birthe Fahrenkrog
Speakers: Angelika Noegel, Birthe Fahrenkrog, Roland Foisner Bas van Steensel, Martin Hetzer

Symposium 4: Inflammation
Chairs: Manolis Pasparakis and Jürg Tschopp
Speakers: Manolis Pasparakis, Jürg Tschopp, Frauke Zipp, Florian R. Greten

Symposium 5: Host pathogen interactions
Chairs: Theresia Stradal and Albert Haas
Speakers: Theresia Stradal, Albert Haas, Steffen Backert, Hubert Hilbi

Symposium 6: Muscle cell organisation
Chairs: Dieter O. Fürst and Frank Schnorrer
Speakers: Dieter O. Fürst, Frank Schnorrer, Christophe Jagla, Mathias Gautel

Symposium 7: Cellular neurobiology
Chairs: Christian Steinhäuser and Ampero Acker-Palmer
Speakers: Christian Steinhäuser, Ampero Acker-Palmer, Erin Schuman, Etienne Audinat

Symposium 8: Vesicle trafficking
Chairs: Volkmar Gieselmann and Paul Saftig
Speakers: Reinhard Jahn, Paul Saftig, Wim Aennaert, Stefan Höning

Symposium 9: Cellular immunity
Chairs: Waldemar Kolanus and Klaus Rajewsky
Speakers: Waldemar Kolanus, Klaus Rajewsky, Michael Reth

Symposium 10: Cell biology of addiction
Chairs: Andreas Zimmer and Brigitte Kieffer
Speakers: Andreas Zimmer, Brigitte Kieffer, Ian Kitchen, Rafael Maldonado López

Symposium 11: Proteostasis
Chairs: Jörg Höhfeld and Frauke Melchior
Speakers: Jörg Höhfeld, Zevi Elazar, Elke Krüger

Symposium 12: Cell adhesion
Chairs: Reinhard Fässler and Johanna Ivaska
Speakers: Reinhard Fässler, Johanna Ivaska, Guido Serini, Carsten Grasshof

Symposium 13: Molecular mechanisms of ageing
Chairs: Michael Hoch and Christoph Englert
Speakers: Michael Hoch, Christoph Englert, Dirk Bohmann, Joachim Lingner

Symposium 14: Non-coding RNAs
Chairs: Gerhard Schratt and Elisa Itzauralde
Speakers: Gerhard Schratt, Elisa Itzaurralde, Kenneth S. Kosik, Gunter Meister

Symposium 15: Intermediate filaments: novel functions
Chairs: Thomas Magin and Rudolf Leube
Speakers: Thomas Magin, Rudolf Leube, Colin Stewart, Michael Labouesse

Programme overview

Wednesday, 30 March 2011

13:00 Opening of congress

13:15 *Plenary session 1: Cell polarity I*

14:45 Coffee break

15:10 *Plenary session 1: Cell polarity II*

17:30 DGZ Awards
- Walther-Flemming-Medaille
- Binder Innovationspreis
- Werner Risau Prize

18:30 Carl-Zeiss lecture: Ueli Aebi
(M.E. Müller Institute for Structural Biology Biozentrum, University of Basel, Switzerland)

19:30 Poster session I and Welcome reception

Thursday, 31 March 2011

09:00 Symposium 1-4
Symposium 1: Frontiers in microscopy I
Symposium 2: Signalling and Rho GTPases I
Symposium 3: Nuclear envelope and NPCs I
Symposium 4: Inflammation I

10:20 Coffee break

10:50 Symposium 1-4
Symposium 1: Frontiers in microscopy II
Symposium 2: Signalling and Rho GTPases II
Symposium 3: Nuclear envelope and NPCs II
Symposium 4: Inflammation II

12:10 Lunch break

13:00 Poster session II

15:30 Plenary session 2-3
Plenary session 2: Cellular mechanics I
Plenary session 3: Cell cycle and cancer I

16:50 Coffee break

17:20 Plenary session 2-3
Plenary session 2: Cellular mechanics II
Plenary session 3: Cell cycle and cancer II

19:30 DGZ Member Meeting

Friday, 1 April 2011

09:00 Symposium 5-8
Symposium 5: Host pathogen interactions I
Symposium 6: Muscle cell organization I
Symposium 7: Cellular neurobiology I
Symposium 8: Vesicle trafficking I

10:20 Coffee break

10:50 Symposium 5-8
Symposium 5: Host pathogen interactions II
Symposium 6: Muscle cell organization II
Symposium 7: Cellular neurobiology II
Symposium 8: Vesicle trafficking II

12:10 Lunch break

13:00 Poster session III

15:30 Plenary session 4-5
Plenary session 4: Cytoskeletal dynamics I
Plenary session 5: Stem cells I

16:50 Coffee break

17:20 Plenary session 4-5
Plenary session 4: Cytoskeletal dynamics II
Plenary session 5: Stem cells II

20:00 Get together

Saturday, 2 April 2011

08:30 Symposium 9-12
Symposium 9: Cellular immunity I
Symposium 10: Cell biology of addiction I
Symposium 11: Proteostasis I
Symposium 12: Cell adhesion I

09:50 Coffee break

10:10 Symposium 9-12
Symposium 9: Cellular immunity II
Symposium 10: Cell biology of addiction II
Symposium 11: Proteostasis II
Symposium 12: Cell adhesion II

11:30 Poster awards

11:45 Lunch break

12:30 Symposium 13-15
Symposium 13: Molecular mechanisms of ageing I
Symposium 14: Non-coding RNAs I
Symposium 15: Intermediate filaments: novel functions I

13:50 Coffee break

14:10 Symposium 13-15
Symposium 13: Molecular mechanisms of ageing II
Symposium 14: Non-coding RNAs II
Symposium 15: Intermediate filaments: novel functions II

15:30 End of the DGZ Meeting

General Information

Local organizers	Prof. Dr. Dieter O. Fürst, Prof. Dr. Walter Witke	
Conference presidents	Prof. Dr. Dieter O. Fürst Institute for Cell Biology University of Bonn Ulrich-Haberland-Straße 61 a 53121 Bonn E-Mail: dfuerst@uni-bonn.de	Prof. Dr. Walter Witke Institute for Genetics University of Bonn Römerstraße 164 53117 Bonn E-Mail: w.witke@uni-bonn.de
Conference organisation	Conventus Congressmanagement & Marketing GmbH Nadia Al-Hamadi Markt 8 • 07743 Jena Tel.: +49 (0)3641 35 33 22 36 • Fax: +49 (0)3641 3 53 32 71 E-Mail: nadia.al-hamadi@conventus.de	
Language	English	

Information, registration, abstract submission: **www.dgz2011.de**

Registration

Please register online or via registration form. You may pre-register until 28 March 2011. From this day on it is still possible to register on site. Once your online or fax registration has been finished you will receive your confirmation of registration in form of the invoice via mail.

REGISTRATION fees (includes coffee breaks)	until 15 February 2011	from 16 February 2011
DGZ member	€ 145,00	€ 185,00
DGZ non-member	€ 205,00	€ 245,00
Student* member	€ 60,00	€ 90,00
Student*	€ 115,00	€ 155,00
Invited speaker	free of charge	

DAY TICKETS (includes coffee breaks)	until 15 February 2011	from 16 February 2011
Member or non-member	€ 85,00	
Student* (member or non-member)	€ 55,00	

Social (includes food and beverages)	until 15 February 2011	from 16 February 2011
Welcome reception, 30 March 2011	included in registration fees	
Get together, 1 April 2011	€ 20,00	

* Please provide confirmation

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

Abstract Submission

Submit your abstract until 15 January 2011 (24:00 hrs) on **www.dgz2011.de** to the following topics:

- Cell adhesion
- Cell biology of addiction
- Cell polarity
- Cellular immunity
- Cellular mechanisms
- Cellular neurobiology
- Cell cycle and cancer
- Cytoskeletal dynamics
- Frontiers in microscopy
- Host pathogen interactions
- Inflammation
- Intermediate filaments: novel functions
- Molecular mechanisms of ageing
- Muscle cell organisation
- Non-coding RNAs
- Nuclear envelope and NPCs
- Proteostasis
- Signalling and Rho GTPases
- Stem cells
- Vesicle trafficking

Abstracts are to be submitted online as poster or oral or poster and in English only.
The review team will decide whether the abstract is accepted as poster or as poster and oral presentation. (Every accepted abstract will result in a poster.) There will be rewards for the best posters which will be awarded in the award session on Saturday, 2 April 2011.
Please note that the abstract registration does not include the conference registration. You have to register separately as participant of the conference via www.dgz2011.de.
ATTENTION: If your abstract is not accepted, a cancelation of your registration is possible at no charge.

Obligatory guidelines for the procedure of abstract submission are indicated on the conference website.
The abstract and its submission are free of charge!

For any question regarding the abstract submission please contact:

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We are looking forward to your submissions for our **CELL OF THE MONTH** picture that serves as the eyecatching opener of our new homepage. Use the opportunity to advertise yourself and your own work with your beautiful images. Please submit your images in their original size and resolution in JPEG format to dgz@dkfz.de

Travel grants for young DGZ members

Young researchers and students with no or only half-time positions are eligible to apply for a DGZ travel grant for participation at the DGZ annual meeting. Prerequisites are active participation at the meeting with a poster or oral presentation and membership in the DGZ.

Grants will be giro transferred to the account given by the applicant.

Please do not send joint applications, only personal applications will be considered.

Please send your application per mail or e-mail until **31 January 2011** to

Deutsche Gesellschaft für Zellbiologie (DGZ)
Sekretariat, Frau Reichel-Klingmann
c/o Deutsches Krebsforschungszentrum
Im Neuenheimer Feld 280 • D-69120 Heidelberg
E-mail: dgz@dkfz.de

Applications received after the deadline cannot be considered anymore.

Please refer to the following points in your application:

1. Personal data (name, title, address, date of birth)
2. Grade of education (subject of study, subject of theses, supervisors)
3. Title and co-authors of your presentation at the meeting
4. Information about your income
5. Travel expenses for a second class train return ticket to Bonn.
6. Your bank account data for reimbursement

DGZ Member Meeting 2011

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

Thursday, 31 March 2011, 19:30 in Bonn.

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"

We are looking forward to seeing you in Bonn.

The DGZ executive board

Walther-Flemming-Medaille 2011

The German Society for Cell Biology offers a research award named after Walther Flemming, one of the pioneers of cell biological research. In 1875 he provided us with a detailed description of processes during cell division, which he named mitosis.

The Walther Flemming Medal is awarded annually for outstanding scientific merits from all fields of cell biological research. Eligible are researchers up to an age of 38 years. The award consists of the medal itself and a prize money of 2.000 € sponsored by the *European Journal of Cell Biology*.

Both individual applications and nominations are accepted. Applications will be reviewed by an independent commission. The award ceremony takes place at the annual meeting of the DGZ, which will be held in Bonn from March 30th - April 2nd, 2011.

Please send your application in parallel by mail (only one copy) and email to:

Deutsche Gesellschaft für Zellbiologie e.V. (DGZ)
Sekretariat
Frau Reichel-Klingmann
Deutsches Krebsforschungszentrum
Im Neuenheimer Feld 280
D-69120 Heidelberg

Deadline: 15 January 2011

Binder Innovationspreis 2011

The Binder Innovation Prize is founded by the Binder GmbH in Tuttlingen and awarded by the DGZ. It is endowed with 4.000 € and was awarded the first time in 1998. The award is given for outstanding cell biological research with a focus on cell culture or the use of cell cultures.

Candidates may apply for the prize themselves. A DGZ membership is desired but not required for the application.

Applications have to consist of a cover letter, a research profile and a CV and have to be submitted to the DGZ office.

Applications will be reviewed by an independent commission of the DGZ. The award ceremony takes place at the annual meeting of the DGZ, which will be held in Bonn from March 30th - April 2nd, 2011.

Please send your application in parallel by mail (only one copy) and email to:

Deutsche Gesellschaft für Zellbiologie e.V. (DGZ)
Sekretariat
Frau Reichel-Klingmann
Deutsches Krebsforschungszentrum
Im Neuenheimer Feld 280
D-69120 Heidelberg

Deadline: 15 January 2011

Werner Risau Prize 2011 for Outstanding Studies in Endothelial Cell Biology

Together with the German Society for Cell Biology (DGZ) the prize comitee will award a prize will for "outstanding studies in endothelial cell biology" to candidates within the first 5 years after obtaining their PhD or MD (except in the case of maternal leave). The Prize will be awarded for an article already published or in press, and consists of a personal diploma and a financial contribution of 4000 Euro. Applicants are requested to send a letter of motivation together with their CV and one copy of the article by e-mail (preferably in pdf format) to the

Werner Risau-Preiskomitee
c/o Prof. Dr. Britta Engelhardt
University of Bern
Theodor Kocher Institute
Freiestrasse 1, CH-3012 Bern, Switzerland
bengel@tki.unibe.ch

Deadline for applications: **15 January 2011**

The prize will be awarded during the Annual Meeting of the German Society for Cell Biology (March 30 – April 2, 2011) in Bonn, Germany.

The HiStory of Microtubules

The first 100 years

Manfred Schliwa and Ralph Gräf

It was Theodosius Dobzhansky (1964) who summed up a life's work on evolutionary processes in the timeless dictum "Nothing in biology makes sense except in the light of evolution". The breathtaking advances in molecular cell biology tempted Gerhart and Kirschner (1997) to rephrase this statement as "Nothing in evolution makes sense except in the light of cell biology". And today's cell biologists might in turn be inclined to proclaim "Nothing in the cell makes sense except in the light of spatiotemporal control". This seems justified in light of the notion that cells are dynamic systems of billions of molecules and molecular complexes whose interactions are precisely orchestrated in time and space. For example, cellular compartments such as the nucleus, endoplasmic reticulum, Golgi apparatus, mitochondria are maintained in a specific yet dynamic and pliable relationship to each other linked by highways of structural communication. This is owed, in large measure, to the existence of a fairly rigid yet highly dynamic (one is temped to say "*stabile*") array of polymers that pervade the cytoplasmic matrix like a spider's web: the microtubule apparatus. It endows the cell with an ordering network for organelle positioning, orientation and communication. In the spirit of Carl Sagan's statement "You have to know the past to understand the present", it may be worthwhile to recount briefly the developments of the field through the decades. How did we come to know what we know? What does this knowledge suggest about future developments? We will see that microtubules mutated from virtual nonexistence to one of the most-researched cell components. Spurred by technical advances, their molecular workings are now largely understood.

$CH_2(CH_2CHO)_2$

The microtubule world and, indeed, the entire field of cell biology changed radically with the advent of a small organic compound: glutaraldehyde. Actually, advent is not the right expression; the molecule was known for a long time, but in 1963 it was first introduced into cell biological research (Sabatini et al. 1963) and revolutionized electron microscopic visualization. Now tiny tubes were found to be ubiquitous; before there were sporadic and much disputed sightings. Thus the history of microtubules can be divided into two periods, BG and AG (before and after glutaraldehyde). Moreover, with the exception of the last few years BG, descriptions of microtubular assemblies, or even individual fibers, can only be recognized as such in hindsight. Thus the first clear microscopic sighting of microtubules is that by Ballowitz (1888) of flagellar fibers of macerated sperm. As we now know, axonemal microtubules are exceptionally stable and therefore could survive his rather harsh treatment (Fig. 1). Remarkably, in favorite samples Ballowitz noted up to 11 fibers, suggesting

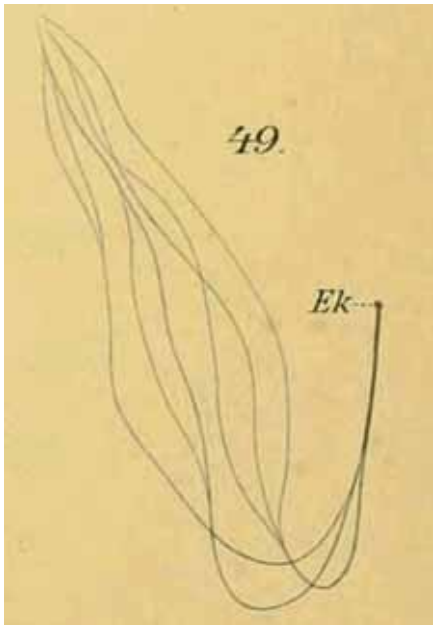

Fig. 1.: Macerated sperm tail splayed into fibers as seen by Ballowitz (1888). Ek: Endknöpfchen (basal body in today's diction)

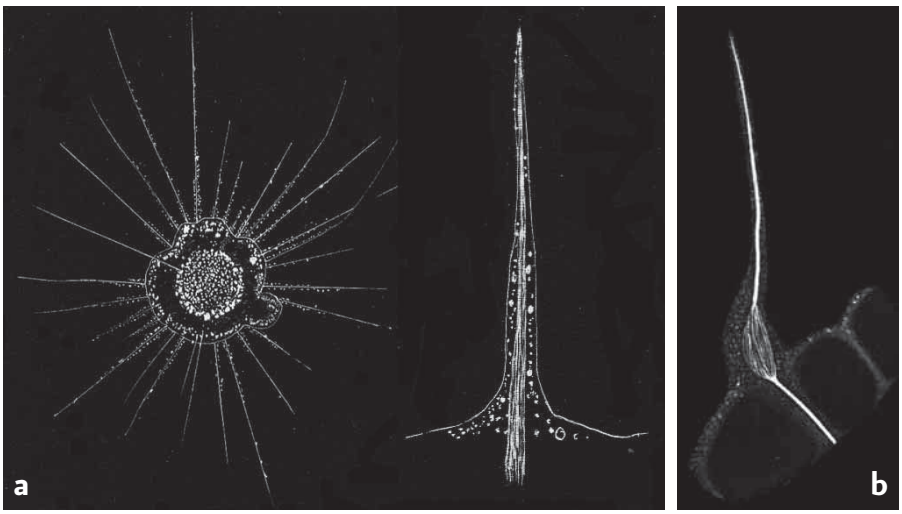

Fig. 2.: (a) Darkfield image of Actinosphaerium (left) and a single axopodium (right) clearly showing the Achsenfaden (axial rod). From Doflein (1916). (b) Axopodium in which the axial rod is splayed at the base into minute fibers. From Roskin (1925), shown here in reverse contrast.

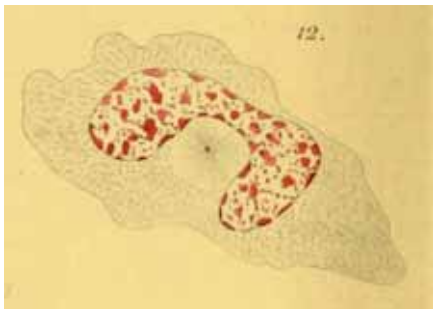

Fig. 3.: Isolated Leukocyte showing the attraction sphere, a radial array of fibers centered upon the centrosome. From Flemming (1891).

that even singlet microtubules appeared in his preparations. The nature of the involvement of these fibers in ciliary force generation was unknown, though they were believed to be contractile, as contractility was the only force known to cause biological movement at the time.

What about cytoplasmic microtubules? Some protozoa harbor astonishingly complex and stable arrays which attracted the attention of early light microscopists. Examples are the core structures of axopodial extensions of radiolaria and heliozoa, unfortunately also termed axonemes, like the ciliary ones. Initially they escaped the keen eyes of their first students (Johannes Müller and his disciple Ernst Haeckel), but the darkfield technique of Doflein (1916) brought them to light (Fig. 2a). Favourite samples (Roskin 1925) demonstrated them to be composed of a bundle of extremely fine fibers (Fig. 2b). The labile arrays of single cytoplasmic microtubules, however, remained invisible, except for the so-called attraction spheres (Fig. 3) seen in some preparations (Flemming 1891). In that respect a paper by Klemensiewicz (1902) is a remarkable find. It shows fibers in leukocytes extending from the Zentralkörperchen (today's centrosome) to the cell periphery (Fig. 4a) that closely resemble those seen much later by immunofluorescence microscopy (Fig. 4b) – in our opinion a clear demonstration of single long cytoplasmic microtubules.

Following the development of improved fixation and staining procedures in the late 1800s, another ephemeral structure immediately captured the attention of many cell biologists: the mitotic spindle (Fig. 5). Its appearance obviously had something to do with cell division. Though seen by many, its importance was not undisputed until several decades later improved polarization optics revealed a similar structure apparently composed of oriented fibers (Schmidt 1937) (Fig. 6). Initially it must have been a big disappointment when – more often than not – electron microscopy showed no corresponding oriented structures in the spindle (Rozsa and Wyckhoff 1950). But that was 13 years BG. So what (and who) is to be trusted? The dispute smoldered. Inoué (1953) then convinced the science community that spindle fibers do exist in living cells, and Mazia and Dan (1952) lend first evidence to the notion that the mitotic apparatus is a structured entity that can be isolated biochemically. Finally, a decade later, refined electron microscopy showed unequivocal oriented structures (then still called filaments) in the spindle (e.g., Roth and Daniels 1962). The plot thickened, but the culprit was neither identified nor convicted.

Assembly

The floodgates opened in year 1 AG, 1963. A flurry of papers established the ubiquity of these funny tubes in the cytoplasm, initially referred to as "microtubules" in quotation marks (Ledbetter, and Porter 1963), but the name quickly caught on. Soon a protein responsible for the formation of these structures was identified and dubbed colchicine-binding protein owing to the fact that the spindle poison colchicine avidly binds to it (Borisy and Taylor 1967). Mohri (1968) then named it "tubulin", initially also set in quotation marks. A mere 3 years AG, Keith Porter (1966) postulated the following properties of these new structures:

> *Microtubules must (a) be distributed unevenly according to some prescribed pattern, (b) be anchored at one end and free to grow at the other, (c) be oriented, i.e. given a direction in their growth from a point, (d) possess some tendency to straightness, and (e) be limited in length.*

How remarkably perceptive, considering the fact that these characterizations are based solely on static images of electron micrographs! One by one, these statements should later be substantiated by solid evidence from various experimental avenues.

One of these avenues was biochemistry. The microtubules that form the mitotic spindle appear rather solid and stable, yet at the same time they change their distribution and thus must be somewhat mutable. A protein that builds these microtubules – tubulin

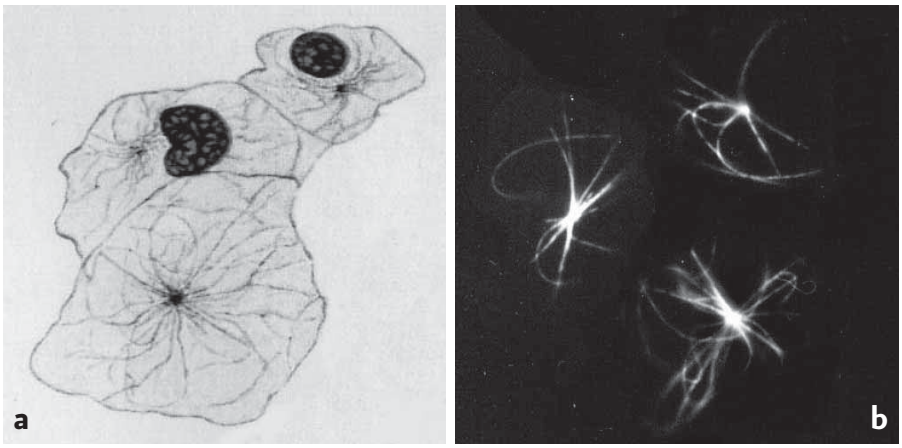

Fig. 4.: Microtubules in Leukocytes (a) as seen after staining with iron haematoxylin (Klemensiewicz 1902) or (b) antibodies against tubulin (Koonce and Schliwa 1982, unpublished).

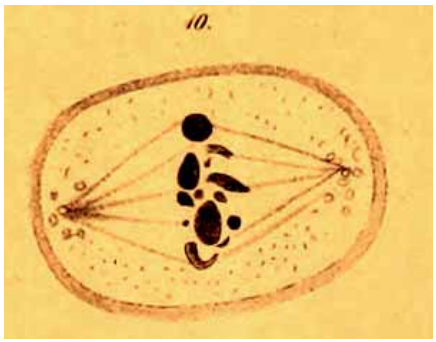

Fig. 5.: Cartilage cell of a young frog at metaphase. A spindle is clearly visible. Remarkably, this is a live-cell preparation! The author considered it to be an artifact („...ein accidentelles Product"). From Schleicher (1879).

– was identified, so the dynamic properties of that protein must be studied in vitro. In several labs, years of intense efforts to get a hand on tubulin ended in utter frustration because it could not be isolated in a state that allowed its biochemical characterization. Then in 1972 Dick Weisenberg hit the jackpot. Microtubules can be polymerized outside the cell after all. His secrets: removal of calcium and addition of GTP. This finding opened the door for a complete characterization of tubulin assembly in vitro.

Structure

Once in vitro-polymerization was established, questions of the supramolecular structure and assembly of microtubules could be attacked at all fronts. That the wall of microtubules is built from 13 strands called protofilaments had been known since year 1 AG (Ledbetter and Porter 1963) thanks to the fact that the cell type studied (juniper) is rich in tannins. Tannic acid was later rediscovered as an agent that helps visualize protofilament organization in a variety of microtubule types (Tilney et al. 1973). Now it was found that microtubules with different protofilament numbers can be generated in vitro and also exist in vivo; that neighboring protofilaments are staggered in different ways in different types of microtubules; that single protofilaments are composed of stacks of 4 nm globular entities (i.e., tubulin monomers), and that two slightly different monomers alternate along the length of a protofilament (i.e., form alpha/beta dimers) (Amos and Klug 1974); that subunits are added at two different rates at the two ends of a microtubule (Allen and Borisy 1974); and, finally, that assembly and disassembly pass through intermediate stages of rings, hoops, ribbons, and discs (Fig. 7), though the exact sequence of events could not be determined at the time (summarized in Kirschner 1978). Thus in just a couple of years a solid foundation for our understanding of the structure of microtubules was laid.

The molecular structure of tubulin, on the other hand, was lagging behind. Though as early as 1973 partial amino acid sequences of tubulin became available which revealed a high degree of homology between alpha and beta tubulin (Ludueña and Woodward 1973), their complete sequences were established only 8 years later (Ponstingl et al. 1982). Amazingly, what takes a few minutes of cDNA sequencing today required years of painstaking protein sequencing of proteolytic fragments at the time. The atomic structure of tubulin was, however, still years away.

While the biochemist fraction of microtubule aficionados uncovered one fascinating facet after another about microtubule assembly, the hard-core cell biologists were left with a nagging feeling of uneasiness. What is the relevance of all this to living cells? This spurred studies that strived to complement in vitro findings with in vivo observations. Two examples are noteworthy.

Take microtubule nucleation. In vitro, microtubules can be grown from pre-existing microtubule fragments ("seeds") or initiated from a variety of non-microtubular components. In vivo studies suggest that microtubules growth from some sort of organizing center in the cell center towards the cell periphery. At least in animal cells, the organizing center comprises a pair of centrioles consisting of a cylinrical, nine-fold symmetric arrangement of short microtubules. The centrioles are embedded in ill-defined pericentriolar material, colloquially referred to as "fuzzy stuff". Which of the two entities is responsible for nucleation? Gould and Borisy (1977) separated the fuzzy stuff from centrioles and showed that it seeds microtubules, thus stimulating a search for non-tubulin factors that nucleate microtubules. The search found its end with the discovery of a ubiquitous tubulin isoform, called γ-tubulin (Oakley and Oakley, 1989), which turned out as a key-factor of microtubule nucleation in all organisms.

Take, as a second example, microtubule polarity. In vitro, microtubules grow at different rates at the two ends, which is a consequence of their intrinsic molecular polarity. Since microtubule polarity can be envisioned to be of great importance to the cell, how can it be determined? A serendipitous observation pointed the way. Certain unphysiological buffer conditions allow tubulin to add to microtubule walls in an enantiomorphic fashion that reveals their polarity (Heidemann and McIntosh 1980), leading to the "plus ends distal" rule still valid today for all vegetative cells (Euteneuer and

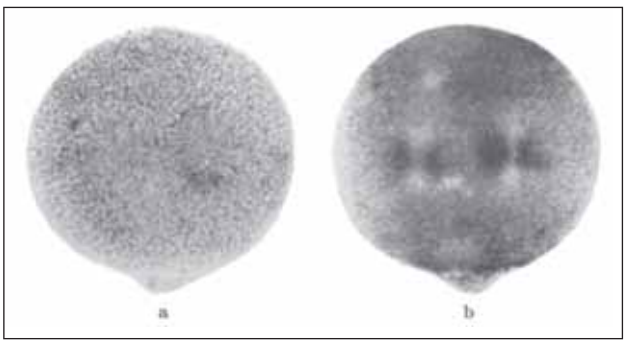

Fig. 6.: A Cerebratulus egg seen by transmission (a) and polarization light microscopy. A birefringent structure is clearly visible. From Schmidt (1937).

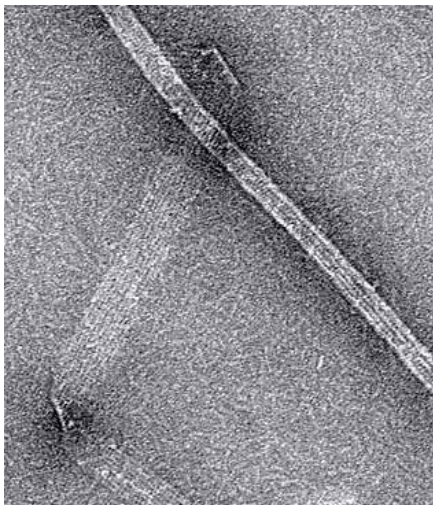

Fig. 7.: Negatively stained tubulin prep showing a closed microtubule, a ribbon, and, in the background, various forms of tubulin oligomers. Hendrik Dietz, unpublished.

McIntosh 1981). Both examples are paradigms for the successful interplay between biochemical and cell biological approaches.

More Assembly

In both these examples the cell biological side had to employ electron microscopy, a cumbersome technique that only offers limited views of selected cell areas. Wouldn't it be nice to get an overview of the entire complement of microtubules? The "big picture" was finally delivered by the resurrection of an immunological approach applied to whole cells that became known as immunofluorescence microscopy (Weber et al. 1975, Brinkley et al. 1975). Now an overview of the microtubule system could be gained at a glance. Upon seeing these first images many were doubtful: what, these curvy, bending, flaccid-looking things are supposed to be the straight, sturdy-looking tubes in my electron micrographs? The root of the discrepancy, of course, lies in the fact that in thin sections of electron micrographs, microtubules are at best a few micrometers long, whereas in fluorescence microscopy their course can be followed over several tens of micrometers. Apparently others were adamant that immunofluorescence produces artifacts and disparagingly referred to the structures seen as "these white lines". The truth was quickly revealed by correlative immunofluorescence and electron microscopy (Osborn et al. 1978) and the triumphal ascent of the technique was unstoppable.

With the new technique, hundreds of cells could now be scanned to determine patterns of microtubule organization (their "molecular anatomy" in the words of Klaus Weber), which is a big advantage over electron microscopy. What used to take at least two days to get a glimpse of part of a thin slice of a cell could now be accomplished in 2 hours and produced overviews of the entire microtubule system. Still, the patterns revealed are static snapshots of dead cells. Might it be possible to modify the technique so that living cells can be viewed, by linking the fluorophore not to an antibody but directly to the protein of interest? Of course the labeled proteins would have to be microinjected, but this would be a small price to pay considering the expected rewards, namely, live cell visualization of the molecular anatomy. In the case of microtubules, the first images so produced revealed a microtubule system very similar to that seen by immunostaining (Keith et al. 1981), but hints of dynamics were cryptic at best. At the other extreme, upon microinjection of labeled tubulin into mitotic cells in the McIntosh lab (early 1980s), the entire spindle lit up after just a few seconds. The unanimous conclusion was: something went terribly wrong, it must be an artefact! From today's vantage point we can state that everything went perfectly right because microtubules are breathtakingly dynamic in mitotic cells and therefore incorporate labeled tubulin very quickly. At the time, the nature and extent of this dynamics was not nearly expected. It took another set of breakthrough studies to establish this.

Dynamics

At sufficiently high concentrations, tubulin polymerizes into microtubules until the concentration of monomers drops to a critical value. Then what? Do microtubules just sit there in this steady state phase, rock-solid, and maintain a constant length? The suspicion grew that, though much can be learned from studies of bulk assembly, events at the level of individual microtubules may be even more significant. Albrecht Wegner (1976) had the brilliant insight that if the two ends of the polymer are chemically different (which they are) and if the critical concentrations for assembly at the two ends also differ (which they do), then there must be a combined critical concentration at which one end loses subunits at the same rate as the other end adds them. The result is treadmilling, or running in place. Wegner postulated this on the basis of studies with actin, but it turned out that treadmilling is much more dramatic in microtubules. A microtubule can actually „swim" through its environment simply by assembly/disassembly. Lots of speculations

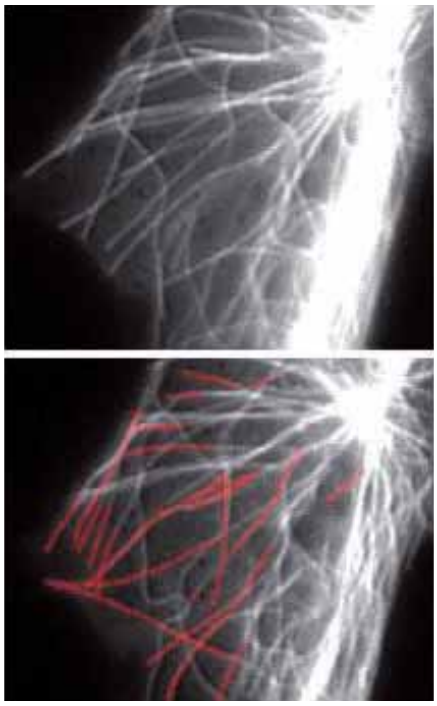

Fig. 8.: First and last frame of a movie (~10 min) showing dynamic instability of microtubules. Newly added microtubule pieces are shown in red. From the lab of G. Borisy.

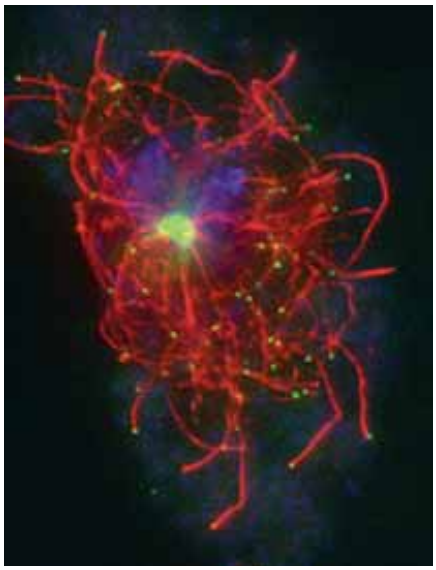

Fig. 9.: Fixed Dictyostelium cell expressing the GFP-TACCdomain as a marker for microtubule plus-ends (green). Microtubules (red) were labeled with anti-Tubulin and AlexaFluor568 (we acknowledge M. Samereier for the image).

followed whether treadmilling actually occurs in cells. However, the story is much more interesting than this. Visual inspection of small microtubule populations, either assembled free in solution or nucleated at isolated centrosomes (Mitchison and Kirschner 1984), hinted at unexpected events at the level of individual microtubules. In the first experiment, microtubule samples were checked at different time points during the steady state phase. The expectation was that the average length of microtubules would remain unchanged. It did not. Though the total polymer mass stayed constant, with time there were fewer and fewer microtubules and the remaining ones grew longer. In the second experiment, microtubule asters nucleated from centrosomes were diluted into lower tubulin concentrations. The expectation was that all microtubules shorten (lose subunits) until a new equilibrium is reached. They did not. Instead, some microtubules disappeared completely while the remaining ones remained long. Further considerations of these unexpected outcomes led to the notion of "dynamic instability". It subsumes the idea of stochastic changes between phases of elongation and shortening of microtubules under constant conditions, documented for the first time by Horio and Hotani (1986) through painstaking dark-field microscopy of individual microtubules. The principle of dynamic instability changed our view of microtubule assembly dramatically and today can be considered one of the most important concepts ever in the cytoskeleton field.

More Structure

Despite these fundamental insights the cytoskeleton field was frustrated. Structural biology was on the rise, and atomic structures of biomolecules, even obscure ones, were popping up all over. Yet two of the most abundant molecules of the cell, actin and tubulin, obstinately resisted all crystallization efforts. The reason is that at the high concentrations required for crystal formation, they rather polymerize than crystallize. This obstacle was first overcome for actin by co-crysallization with DNAse I (Kabsch et al. 1990), and later for tubulin by molecular fitting to a density map obtained by electron crystallography of zinc-induced tubulin sheets (Nogales et al. 1998). Simultaneously, the structure of the bacterial homolog of tubulin, FtsZ, was solved by conventional crystallization thanks to the use of FtsZ from a hyperthermophilic methanogen (Löwe et al. 1998). Though tubulin and FtsZ share hardly any sequence homology, their three-dimensional structures are essentially superimposable. This is not totally surprising in hindsight, since FtsZ can form tubules, sheets and minirings in vitro not unlike those formed from tubulin. It is remarkable from a physiological point of view because the two serve different functions: tubulin is involved in chromosome separation (and other tasks) while FtsZ is used for cytokinesis. The availability of the atomic structure of tubulin now allowed detailed analyses of the sites involved in binding to neighbors during polymerization and, equally importantly, the location of binding sites for associated proteins and inhibitors.

More Dynamics

Following the landmark finding of dynamic instability, the question again arose whether living cells actually make use of this behavior in their everyday life. Microinjection of labeled tubulin in theory could provide the answer but in practice proved cumbersome, though not unsuccessful (Sammak and Borisy 1988). Sensitive high-resolution visualization was required that allows long-term observations of individual microtubules in living cells. A new avenue towards this goal was opened in 1994, yet another year in which the cell biological universe changed. Marty Chalfie used green fluorescent protein to monitor gene expression and protein localization in living C. elegans organisms. It worked, and live cell imaging never was the same again. Amazingly, a large number of proteins to which GFP was linked like a molecular ball and chain still remained almost fully functional. One of them was tubulin (Carminati and Stearns (1997). When transfected into living cells, it was able to undergo multiple rounds of assembly/disassembly which, with the aid of a sufficiently sensitive camera, could be visualized for long periods of time. Thus dynamic instability at microtubule plus ends in vivo was firmly established (Fig. 8). And not only that: also treadmilling (Rodionov and Borisy, 1997); and microtubule severing (Vale, 1991); and release from the centrosome (Kitanishi-Yumura and Fukui 1987); and binding of factors to microtubule ends (Morrison et al. 1998) (Fig. 9); and and and…. Today, we can study not just the molecular anatomy, but also the molecular dynamics of these and other cell components in real time with a light microscope.

Among the most ubiquitous proteins associated with microtubules are the ones involved in microtubule nucleation and organization. γ-Tubulin was the first identified microtubule-nucleating factor but soon it turned out

that it is not able to do this job alone but requires the collaboration of up to eight more subunits. Together they form a ring-shaped complex with the diameter of a microtubule that serves as a template for its formation (Moritz et al. 2000). Such γ-tubulin ring complexes have meanwhile been found in every place where microtubules are organized. The still most prominent microtubule organizer is the centrosome. Its duplication once and only once per cell cycle and the importance of the fidelity of the duplication process for organization of a bipolar mitotic spindle was recognized already a century ago. Theodor Boveri (1914) noticed that disturbance of the duplication process leading to supernumerary centrosomes is involved in cancer. Because of its utmost importance for cell proliferation, the duplication process is the subject of intense current interest.

Yet More Dynamics…

And not only centrosome duplication. There is more to microtubules, much more. They are the substrate of numerous interaction partners: accessory proteins, linkers to other cell constituents, complexes that regulate nucleation, complexes that regulate dynamics, complexes that cap the ends, complexes that link the ends to the actin system and regulate actin dynamics, and proteins that mediate movements on their surface. They are not only biochemical but also physical entities. And they are organizers of cellular space in a very broad sense. All these are fascinating current advances that are not dealt with here and should be the subject of another (hi)story to be told in due course.

Announcement

To highlight research on microtubules, the German Society for Cell Biology, in cooperation with FEBS, will organize a conference in June 2011 – the first in several decades exclusively devoted to the subject of microtubules.

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DGZ/FEBS Workshop: The spider's web: how microtubules organize cellular space

Next year's international meeting will be held in Potsdam, Germany,
from June 29th - July 1st 2011 at the Seminaris Hotel at the nice Lake Templin.
http://www.seminaris.de/index.asp?tree_id=32

Organizers:
Ralph Gräf (Potsdam) and Manfred Schliwa (Munich)

Keynote speakers:
J. Richard McIntosh, Eckhard Mandelkow

Net Construction **Microtubule dynamics and polarity**
Sandrine Etienne-Manneville, Linda Wordeman, Gregg Gundersen

Surfing the Web **Microtubule motors and trafficking**
Vladimir Gelfand, Erika Holzbaur, Gero Steinberg

Meet the Spider **Microtubule organization and MTOCs**
Monica Dias, Alexey Khodjakov, Andrew Fry

Backbone Network **Microtubules as cellular integrators**
W. James Nelson, Orly Reiner, Frank Bradke

Location, Location **Organelle positioning**
David Stephens, Daniel Starr, Viki Allan

Net to Net **Mitosis**
Tony Hyman, Yixian Zheng, Jordan Raff

Probing the physics of tumor cells from mechanical perspectives

Mareike Zink, Anatol Fritsch, Franziska Wetzel, K. David Nnetu, Tobias Kießling and Josef A. Käs

Background

Almost 50 years ago Steinberg made the observation that two different populations of cells mixed together in a little droplet immediately separate [1]. To understand such behavior he considered cells as liquid-like and determined the surface tension of cell droplets with a self-made plate tensiometer [2]. It turned out that co-cultures of two different cells types always arrange the cells with a lower surface tension as a shell around the other cells which form a spherical core. Steinberg proposed that only cohesion forces between cells are the driving force of cell separation. Following his *differential adhesion hypothesis*, a mixture of two different cell types in a droplet culture form a spheroid in which cells with lower surface tension embed cells with higher surface tensions to decrease the surface energy of the entire system. Thus, epithelial tumor cells in droplet cultures with normal epithelial cells must surround the normal cells due to their loss in E-cadherin expression and the corresponding decrease in cohesion forces [3,4]. Nevertheless, such behavior has never been observed and just recently Schötz *et al*. together with Steinberg showed that the mechanical properties of the cells must be taken into account together with surface tension differences [5,6]. Thus, soft cells can develop more contact sides to adjacent cells compared to stiff cells even when the number of adhesion proteins on the cell surface is reduced.

How cells can squeeze along other cells and through the extracellular matrix, change their shape and contact sides to adjacent cells is a major determinant for cell demixing and the formation of compartments in the human body. Thus, the question arises: Is the formation of compartments during embryogenesis which build up the human body only driven by biochemical processes or can we describe such behavior also by considering cell mechanics and cell-cell / cell-matrix interactions? Additionally, can we understand the progression of a tumor in a similar way because a tumor is also a developing tissue? Höckel employed the term *ontogenetic anatomy* which maps the human body from its embryological origin (anlage) and characterizes the compartments representing morphogenetic units in the adult [7]. Here the compartment boundaries are primary proliferation and migration suppressors. Höckel could clearly show that women with non-metastatic carcinomas of the uterine cervix originating in the Müllerian compart-

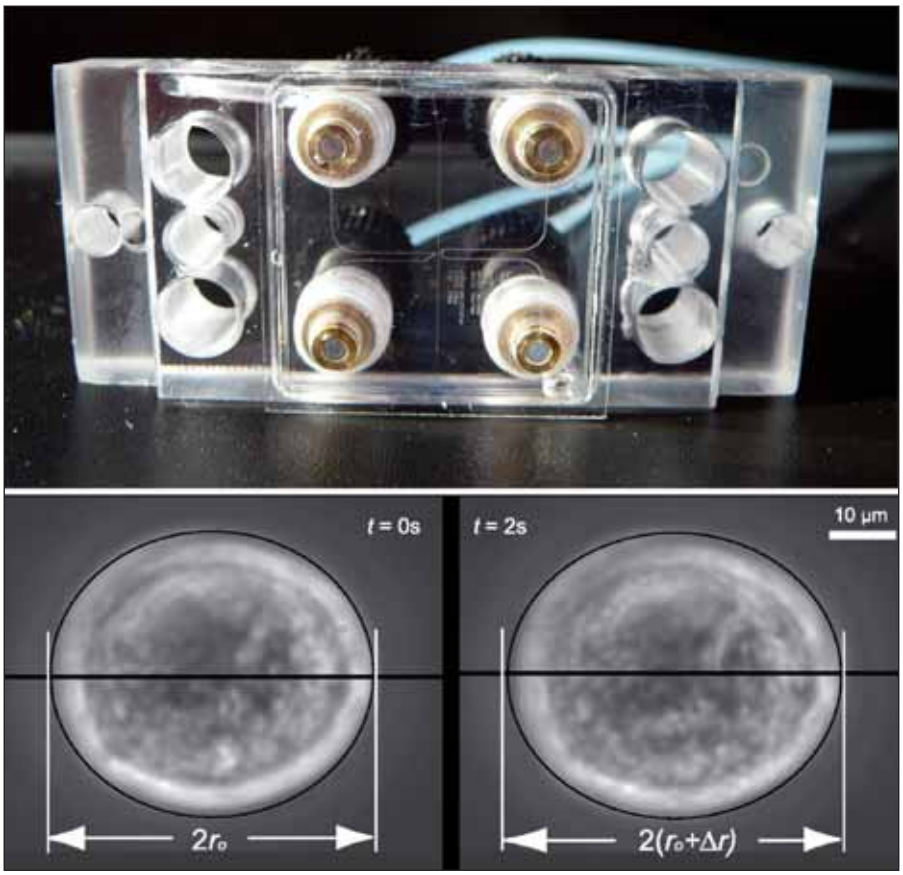

Fig. 1.: The Optical Stretcher is a tool to probe viscoelastic properties of individual cells utilizing the pressure refracted light exerts on a surface. Core piece of the Optical Stretcher is a microfluidic chip (top). The cells are in suspension in a flow channel. Individual cells are trapped between two counter-propagating divergent laser beams emitted from opposing optical fibers. By increasing the laser power, the surface stress (due to the light pressure) increases and deforms the cell along the laser axis (bottom).

ment have a reduced mortality from 20 to 4% when the total compartment is resected even without subsequent radiation or chemotherapy [8-10]. Following his compartment theory, cells of the primary tumor have the ability to spread within its initial ontogenetic compartment where the tumor originates because tumor cells and normal cells behave similarly. Major changes of the cells such as epithelial-mesenchymal transitions are a prerequisite of tumor progression beyond compartment borders and subsequently metastasis.

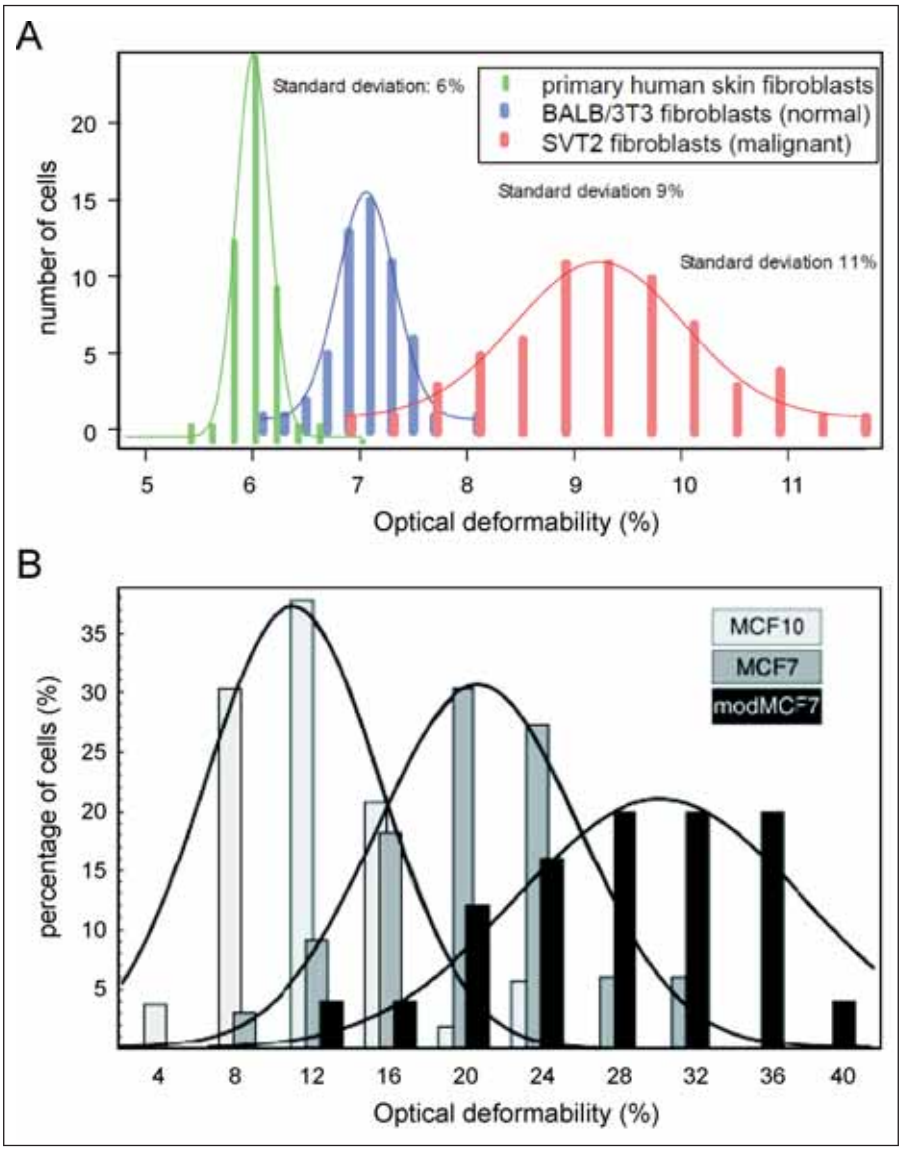

Fig. 2.: The diagrams show the optical deformability of different cells lines that were deformed in the Optical Stretcher between two laser beams of 1.5 W. The Optical Stretcher pulls on a cell with a well-defined force and determines the cell's extension with respect to its diameter, defined as the optical deformability. (A) Optical deformability of non-malignant primary human skin fibroblasts, BALB/3T3 fibroblasts and malignantly transformed SV-T2 fibroblasts. The malignant cells have the highest optical deformability. A higher standard deviation is found with higher proliferation rates due to the variations of the cytoskeleton during the cell cycle. (B) Deformability measurement of non-cancerous MCF-10, cancerous MCF-7 and modMCF-7 (metastatic) cells. Obviously, the modMCF7 are more deformable than MCF-7 and MCF-10. With increasing cancer aggressiveness the cell's optical deformability increases.

Considering ontogenetically different tissue compartments as units with different surface tensions, the question what keeps the tumor cells inside their host compartments might similarly be answered as the question why cells separate and form compartments during embryogenesis. Additionally, can the underlying mechanism be employed to understand cell demixing *in vitro*? To investigate such mechanisms from a materials science perspective, the mechanical properties of single cells must be taken into account because stiff cells exhibit less contact sides to other cells which would increase motility. On the other hand, lamellipodial motion and therefore individual cell motility is enhanced for soft cells which can also "squeeze" through the extracellular matrix.

The Optical Stretcher technology

Various methods are available to measure the local or global mechanical properties of cells. One outstanding new technology to measure global mechanical properties of living cells is the Optical Stretcher (OS) [11-13]. This fully automated microfluidic system allows to non-invasively and marker-free mechanocharacterize cells with a much higher throughput compared to other techniques such as micropipettes or scanning force microscopy. During measurement, the suspended cells are moving in a microfluidic channel. When they reach the trap chamber, the flow is stopped and the two laser beams oriented perpendicular to the channel are switched on to trap the cell between the slightly divergent laser beams. An increasing laser power up to ~1.5 W leads to a measurable deformation of the cell along the optical axis due to momentum transfer from the laser light to the cell (Figure 1). The deformation of the cells is detected by a camera and out of the recorded frames up to 48 different material properties can be calculated and compared.

In contrast to other techniques that measure the mechanical properties of adherent cells on substrates, our device determines the viscoelastic properties of the actin network which is not superimposed by additional contributions of stress fibers to the mechanical properties. The sensitivity of the Optical Stretcher to obtain even small alterations of the cytoskeleton can be attributed to the fact that the elastic modulus G' is non-linearly correlated with cytoskeletal properties such as actin concentration c: $G' \sim c^{2\text{-}6.7}$. For small deformation, the bending rigidity of the membrane during optical deformation is negligible because it is about 1000 times lower compared to the stiffness of the cytoskeleton.

Under normal circumstances the cytoskeleton is tightly regulated. During diseases such as cancer intracellular changes occur which influence the structure and the function of the cytoskeleton. When the cell malignantly transforms the well-ordered system of actin fibers, microtubules and intermediate filaments becomes more and more irregular to enhance cell motility and proliferation [14,15]. Thus, a reduction in the cytoskeleton of tumor cells is inherently amplified in measurements of the cell's viscoelasticity, and the progression of cancer becomes clearly visible in the increased softness of individual cells. The Optical Stretcher technology has the potential to allow the direct staging of the cells from early dysplasia to metastasis of a tumor sample due to respective changes in the cytoskeleton [16].

Mechanical properties of tumor cells

One of the first proofs of principle of the Optical Stretcher to investigate the mechanical properties of tumor cells was performed for fibroblasts. Even if fibroblasts are not typical tumor cells, they can be malignantly transformed. Figure 2A shows the distribution of optical deformability of BALB/3T3 ("healthy") fibroblasts and the malignantly transformed counterpart SV-T2 fibroblasts [17,18]. During the experiment, the cells were stretched with the Optical Stretcher for 1.5 s. It turned out that both cell types exhibit a Gaussian distribution of their deformability, but two characteristics are obtained to distinguish the cells: First, the malignant cells are softer; second, the distribution of the SV-T2 cells is broader.

Both properties – softness and larger variety of viscoelasticity – can be attributed to changes in the cytoskeletal structure: Confocal laser scanning microscopy of the cells clearly shows a down-regulation of the actin cortex by about 40 % of the tumor cells compared to healthy cells (Figure 3). Furthermore, is it known that the degree of order of the cytoskeletal structure diminishes for malignant cells, which leads to a greater variety of viscoelastic responses. Additionally, malignant cells divide faster and a larger number of cells in different cell cycle stages can be obtained with numerous actin fiber arrangements and, thus, distinct viscoelastic properties.

Similar observations could be made from Optical Stretcher measurements of cell lines originating from human breast epithelia [16]. We compared the optical deformability of the benign cells MCF-10, the malignant cells MCF-7 (human breast adenocarcinoma cell line) and modified MCF-7 cells (Figure 2B). These cells were treated with 12-O-tetradecanoylphorbol-13-acetat (TPA) which leads to increased invasiveness and metastatic potential. It turned out that the benign cells are stiffer and deform less compared to

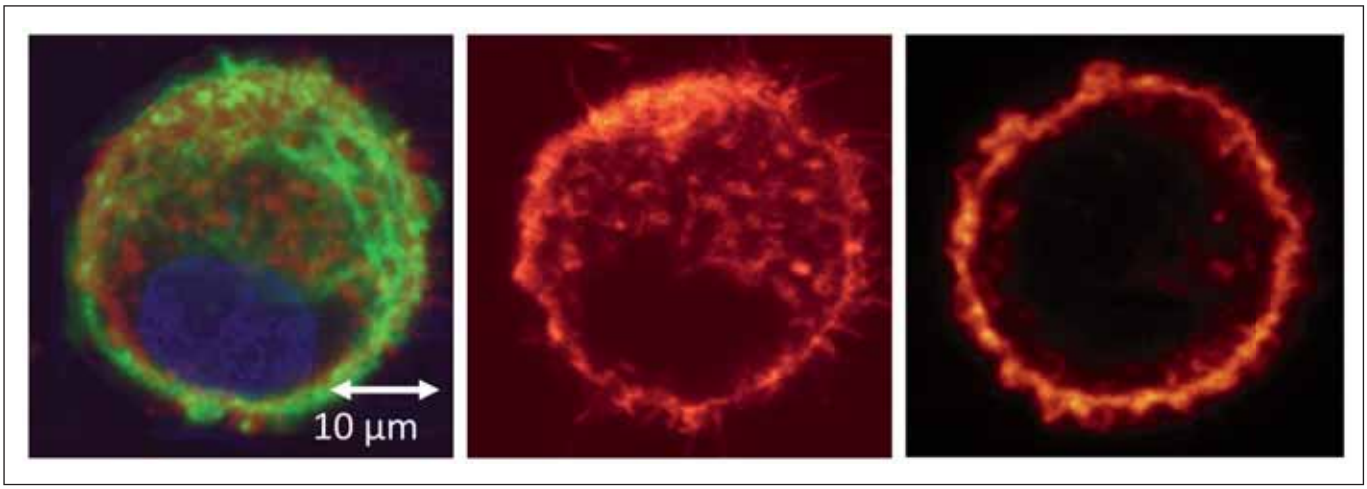

Fig. 3.: The cytoskeleton of normal and malignant cells visualized by confocal laser scanning microscopy. Left: A healthy BALB/3T3 fibroblast is shown with the nucleus stained in blue, the actin cortex lying under the cells membrane is stained in red and the microtubules distributed through the interior are shown in green. Middle: The cytoskeletal structure of the healthy BALB/3T3 fibroblast in contrast to the down-regulated actin cortex of a dedifferentiated, malignantly transformed SV-T2 fibroblast on the right. The complexity of the cytoskeleton as developed in the cause of cell differentiation gets lost during a cell's transformation to a malignant cell.

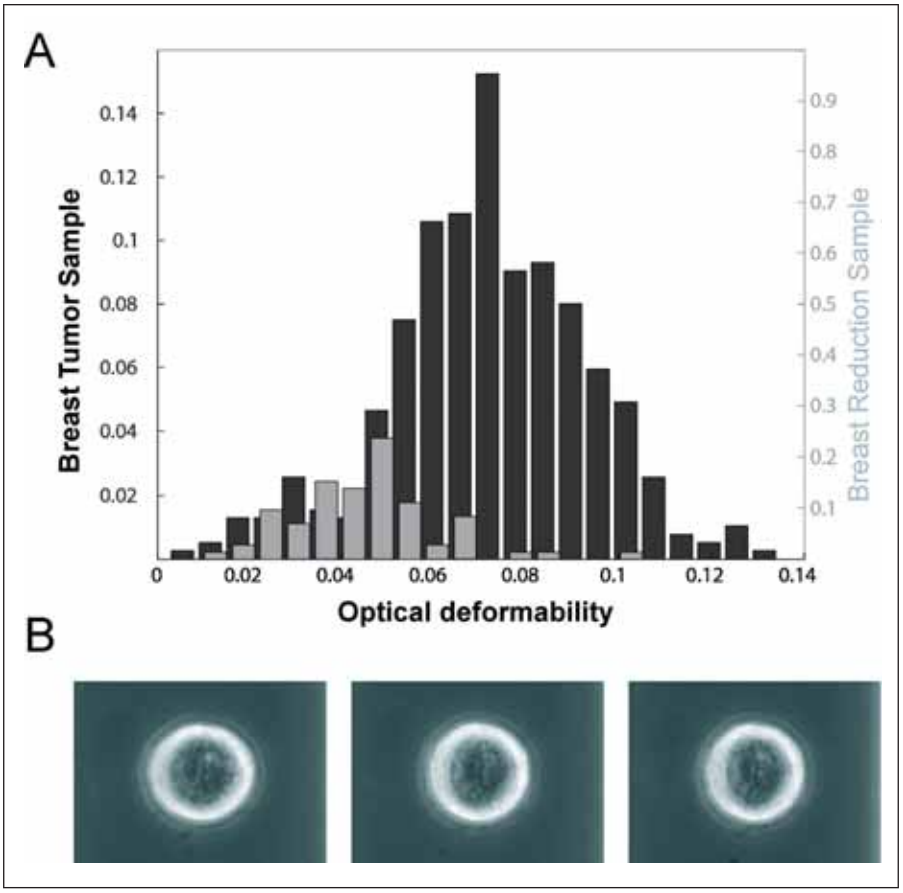

Fig. 4.: (A) Optical deformability distribution of parenchymal cells from a malignant human breast tumor (dark grey) and normal breast tissue (bright grey), measured with an Optical Stretcher and a laser power of 1.2 W. For small deformations, where a linear response is observed, the tumor shows a significantly higher fraction of softer cells than the normal cells from breast tissue. (B) When breast tumor cells are weakly stretched with the Optical Stretcher (here: 0.8 W laser power), a small fraction of tumor cells actively resists the pulling force and contracts. This can be seen by the change in cell diameter in the stretching direction.

MCF-7 cells that also exhibit a broader distribution of deformability, similarly to SV-T2 cells. In contrast, modMCF-7 cells were softest and their mechanical properties show the broadest Gaussian distribution of all cell lines. Such increased softening of tumor cells with respect to benign or healthy cells could also be observed from Optical Stretcher experiments of other cell lines such as lung and lung tumor cells.

Notably, when cells isolated from normal and cancerous tissues were compared, a very interesting difference in the deformability of both cell types was noted: Figure 4 shows the distribution of optical deformability of primary human breast cells obtained from breast reduction surgery (HUMEC) and primary breast cancer cells, both stretched with a laser power of 1.2 W. Besides the increased softness of tumor cells compared to normal cells, it is remarkable that very stiff but also very soft cells are present within the tumor sample [19]. When the laser power during stretching is reduced to 0.8 W the distributions change: Both cell types exhibit similar mean deformabilities when stretched with smaller optical forces, whereas the tumor cells have a much broader non-Gaussian distribution. Here, very soft tumor cells can be found which proliferate very fast and are not present in the healthy sample. Additionally, the tumor sample contains very few cells that actively contract when they are treated with the laser beams of smaller power. Thus, for low deformation forces these cells have the ability to withstand external mechanical excitations and instead of being stretched they actively contract. Such behavior has never been observed before for cell lines.

Tumor progression and metastasis

Why tumor cells soften in parallel with the increase in aggressiveness remains elusive. However, cell softening during malignant transformation offers several advantages for the progression of cancer: Our results indicate that soft cell exhibit enhanced proliferation because mitosis is accelerated. Furthermore, lamellipodial motion and therefore individual cell motility is improved which corresponds to the ability of the cell to invade into the surrounding tissue and metastasize [20]. However, softening alone cannot be a prerequisite for tumor cells to overcome compartment boundaries. From the materials science perspective two cellular properties are mandatory for metastatic behavior: Individual cell motion and the possibility to adapt the mechanical properties and surface tensions to the local environment. Individual cell motion is usually triggered by a loss of E-cadherin expression – a reduction of cell-cell interaction – a feature characteristic for epithelial-mesenthymal transition of the cell. Such mutations additionally result in a reduction of surface tensions which enhances individual migration through the ECM. The observed active contraction of primary tumor cells due to external forces can be considered as the ability of cells to pre-strain and stiffen the cytoskeleton to (a) reduce the number of contacts to adjacent cells and the ECM and (b) reduce the surface tension even further to overcome compartment boundaries and enter the blood stream.

Thus, tumors that already contain contractile cells have the ability to metastasize and must be considered as extremely aggressive even when no secondary tumors have been detected yet [19].

A further property of tumor cells due to their softness and the reduced actin cytoskeleton is the formation of microtentacles [21]. These are spikes of microtubules that penetrate the thin actin cortex and the cell membrane. Microtentacles are a marker for cancer aggressiveness since only cells with heavily down-regulated actin cytoskeleton and metastatic potential form such spikes, which seem to improve the success rate of cells to invade the blood stream.

All these observations discussed above can already show the clinical relevance of the mechanical properties of tumor cells. Only small alterations of cells from early dysplasia to metastatic cells can be distinguished solely from their optical deformability in the Optical Stretcher. A first clinical study exhibited the mechanical properties of primary oral squamous cell carcinoma cells from patients with early dysplasia and malignant tumors which were compared with the deformability of primary oral cells from healthy donors [22]. All cells were extracted from the patient's mouth with a cytobrush and subsequently deformed in the Optical Stretcher. The results are in line with the experiments discussed above: Tumor cells exhibit a larger softness and the distributions of optical deformability of normal and tumor cells clearly differ. Similar to the cervical Pap smear test visual inspection alone does not suffice for oral cell probes. Thus, cytobrushes of suspicious lesion, which are obtained from every patient during the annual dental exam, can be employed for cancer screening.

Summary

The Optical Stretcher is a powerful tool to investigate the mechanical properties of single cells. Even without the determination of molecular markers it is possible to distinguish normal and malignantly transformed cells by their optical deformability. Our results show that tumor cells are softer than normal cells, which results in increased proliferation and lamellipodial motion of the cell and thus enhances tumor progression.

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Microfluidic tools for quantitative studies of eukaryotic chemotaxis

Carsten Beta, Eberhard Bodenschatz

Abstract

Over the past decade, microfluidic techniques have been established as a versatile platform to perform life cell experiments under well-controlled conditions. To investigate directional responses of cells, stable concentration profiles of chemotactic factors can be generated in microfluidic gradient mixers that provide a high degree of spatial control. However, times for built-up and switching of gradient profiles are in gerneral too slow to resolve the intracellular protein translocation events of directional sensing in eukaryotes. Here, we review an example of a conventional microfluidic gradient mixer as well as the novel flow photolysis technique that achieves an increased temporal resolution by combining the photo-activation of caged compounds with the advantages of microfluidic chambers.

Introduction

Cell motility and chemotaxis are ubiquitous throughout the living world. They play an essential role for many biomedical processes ranging from embryonic development to immune response and tumor spreading [1]. Substantial progress has been made in the study of bacterial chemotaxis, where detailed knowledge of the underlying pathways and quantitative models are available [2]. Signaling in eukaryotic cells, on the other hand, is more complex and, to date, much less well understood. Our knowledge of the chemotactic pathways that link the membrane receptor to the dynamics of the cytoskeletal machinery in eukaryotic cells is based on a number of selected model organisms like neutrophils or Dictyostelium discoideum [3]. In recent years our knowledge has advanced to the point, where quantitative tests need to be performed to better understand the chemotactic signaling pathways [4]. Microfluidic flow can provide an exceptionally well controlled environment for such investigations.

To date, mathematical modeling focuses predominantly on phenomenological, low-dimensional descriptions as many molecular details of the chemotactic pathways still remain unknown. For example, a variety of models have been proposed to explain the initial stages of chemotactic signaling, generally referred to as directional sensing. They are based on different paradigms like the combination of a local activator with a rapidly diffusing inhibitory component (local excitation/global inhibition – LEGI –

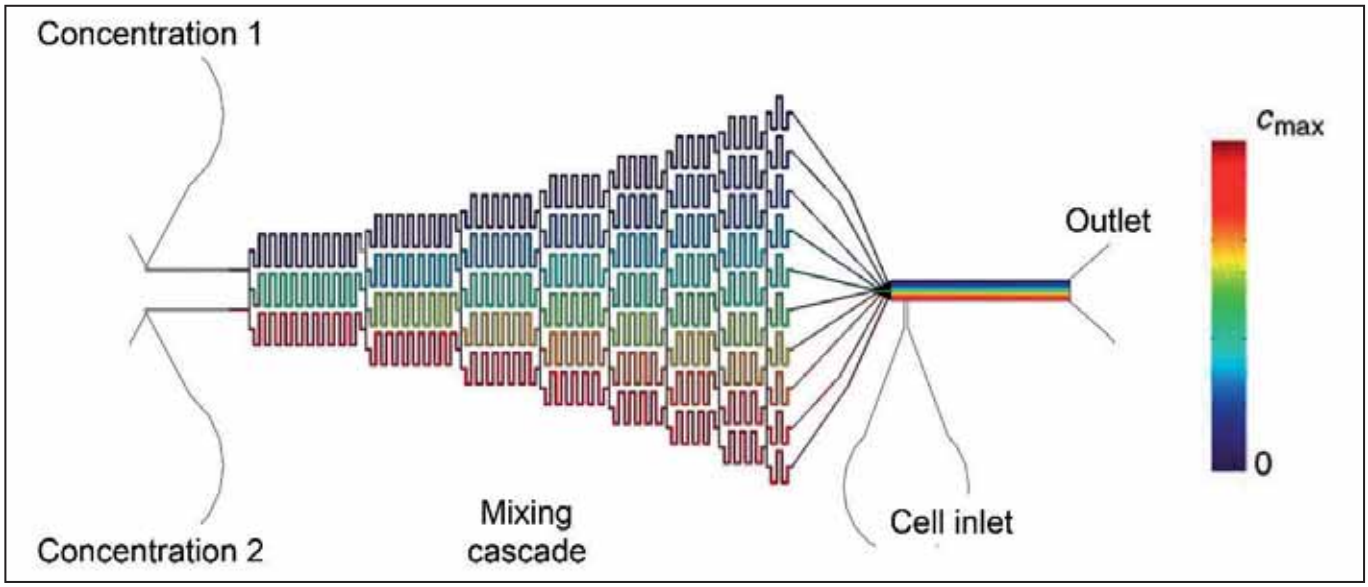

Fig. 1.: Layout of the microfluidic gradient mixer used to generate a linear concentration gradient. The color-coding displays the concentration as obtained from a two-dimensional numerical simulation of the Navier–Stokes and convection-diffusion equation in the shown geometry using FEMlab 3.1. Black lines mark the in- and outlets that were not part of the numerical simulation. Parameters: density $\rho = 10^{-12}$ g/µm³, kinematic viscosity $\upsilon = 10^{6}$ µm²/s, inflow velocity v = 3250 µm/s, inflow concentrations: zero and c_{max} = 2 nM, cAMP diffusivity D = 400 µm²/s, no-slip and isolating boundary conditions except for inlet and outlet. Reproduced from Ref. [22].

models) [5,6,7], on Turing-type instabilities [8], or on bistable dynamics [9,10]. While all of these models show an initial symmetry breaking in a chemotactic cell, they predict different dose-response characteristics and dynamics for the internal asymmetry as a function of the external gradient signal. Thus, quantitative experimental tools are needed to test the modeling predictions in single cell studies with well-controlled external spatio-temporal gradient stimuli.

The classical setup to expose cells to a spatially non-uniform concentration field is the micropipette assay, a convenient qualitative test for directional responses. However, the chemoattractant gradient is poorly defined and changes in the course of time as more substance is diffusing out of the micropipette. Early efforts to generate spatially linear and temporally stable chemical gradients led to the development of diffusion based gradient chambers. A gradient is established by diffusion inside a porous medium or a small gap between two large reservoirs containing solutions of different concentration. The most widely known versions of diffusion chambers are the Boyden [11], Zigmond [12], and Dunn chambers [13]. For a review of gradient techniques see Ref. [14].

With the advent of microfluidic techniques, an entire new range of devices became available to perform highly controlled live cell stimulation experiments with high spatial and temporal resolution [15]. In particular, with the invention of soft lithography [16], such devices became readily accessible to a wide community of users. Soft lithography is based on a two-step procedure: First, a master wafer is designed and generated based on standard photolithography. Second, molding of polydimethylsiloxane (PDMS) against the master wafer yields a microstructured polymer block that is sealed with a glass cover slip. Detailed, well-tested, and standardized protocols are available for this fabrication procedure [17]. There are a number of outstanding advantages that make these devices an excellent tool for cell biological applications. They can be fabricated with a spatial resolution on the micron scale, so that the physicochemical environment of an individual cell can be controlled with great precision. Only a moderate level of equipment is necessary to fabricate such devices. The fabrication itself is straightforward, inexpensive, and reproducible. The devices are biocompatible and, due to the integrated cover slip, well-suited for optical microscopy.

Microfluidic chambers can be fabricated in a great variety of geometries and dimensions. They can be tailored according to the requirements of a specific experiment. On the one hand, many chemotaxis studies require long time migration experiments to gather sufficient statistics of the various motion parameters, but also to allow the study of temporal variability of the chemotactic parameters of individual cells. The latter is even more important as gene expression levels and consequently the chemotactic response may vary from cell to cell. Here, larger chambers are needed that exhibit sufficient long-term stability of a chemoattractant gradient. Such devices typically come with a relatively low time resolution (i.e. gradients change in minutes) but allow for the simultaneous observation of large cell populations (i.e. hundreds of cells). On the other hand, in order to investigate intracellular signaling in chemotactic cells, the experimental setup has to meet very different requirements. The first directional signaling events occur within only a few seconds after the cell is exposed to the external gradient stimulus. Such events are typically observed on the level of individual cells with the help of fluorescence techniques. Thus, single cell stimulation experiments with a high time resolution are necessary to investigate such processes.

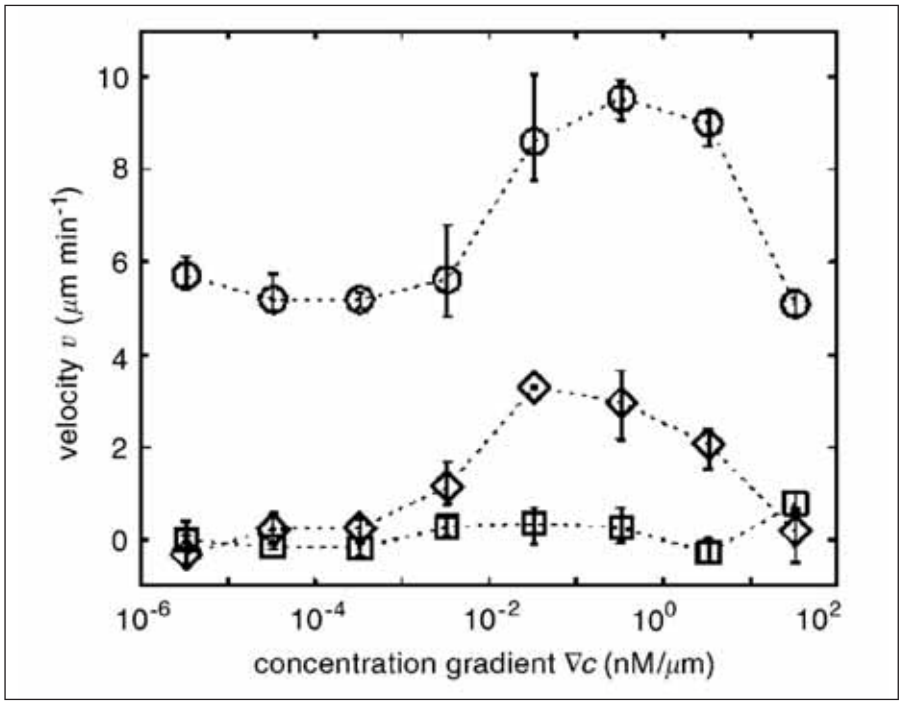

Fig. 2.: Average velocity components v_x (squares) and v_y (chemotactic velocity, diamonds) as well as motility (circles) measured for different cAMP gradients. The bars indicate the spread in velocities. Reproduced from Ref. [22].

In the following, we will exemplarily review two devices that meet these opposing demands, a classical microfluidic gradient mixer for long-term migration studies and the flow photolysis technique for rapid stimulation of individual cells.

The microfluidic gradient mixer

In 2000, the first microfluidic gradient mixer was introduced by Jeon et al. [18]. It is based on a pyramidal network of microfluidic channels that acts as a mixing cascade to generate a linear gradient between two arbitrarily chosen concentration levels. Solutions of the minimal and maximal concentration are introduced into the inlets and continuously pumped through the device. At each bifurcation in the pyramidal network, the flow is divided into an upper and a lower branch and diffusively mixed with the fluid from the neighboring channels. Consequently, a number of equidistant concentration levels are generated between the two input concentrations before all branches are finally merged in a single channel to produce a linear gradient that is oriented perpendicular to the direction of fluid flow, see Fig. 1 for an example of the layout. Since its invention ten years ago, this type of device has been used in various gradient studies [19,20,21]. It has been modified and altered in various ways and became the paradigm of a microfluidic chemotaxis assay. One of the advantage of this flow device is that the gradient is stable over downstream distances of approx. 1 cm, which allows the investigation of large cell populations. In addition, the flow washes away all cell-cell signaling agents and thus provides an ideal environment for the study of solitary cell migration.

We have used a modified version of this gradient mixer to study the chemotacitic behavior of *Dictyostelium discoideum* in stationary, linear gradients of cyclic adenosin 3', 5'- monophosphate (cAMP) [22]. The layout of the microfluidic network that was used in this study can be seen in Fig. 1. We systematically investigated gradients ranging over eight orders of magnitude in steepness. Over more than three orders of magnitude, chemotactic responses were observed. In shallow gradients of less than 10^{-3} nM/µm, the cells showed no directional response and exhibited a constant basal motility. In steeper gradients, cells moved up the gradient on average. The chemotactic speed and the motility increased with increasing steepness up to a plateau at around 10^{-1} nM/µm. In even steeper gradients, above 10 nM/µm, the cells lost directionality and the motility returned to the sub-threshold level. The results are summarized in Fig. 2. Approximating the receptor binding by simple on/off kinetics, this data can be used to estimate the limiting receptor occupancies for *Dictyostelium* chemotaxis. Based on the K_d value of the *Dictyostelium* cAR1 receptor, it was found that in the regime of optimal response, the difference in receptor occupancy between the front and the back of the cell is on the order of 100 molecules only.

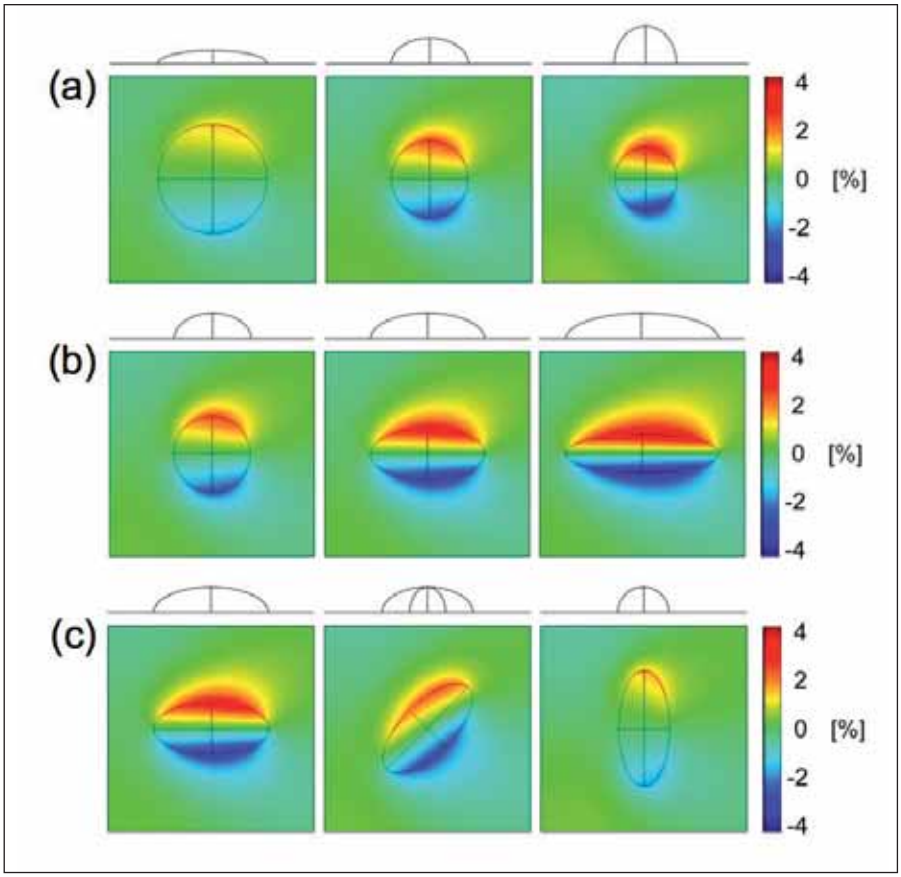

Fig. 3.: Concentration deviations from a linear gradient profile across a cell as a function of different geometric parameters. The channel height is 25 µm, the fluid flow runs from left to right with a velocity of 50 µm/sec. (a) Changing cell height from 2.5 µm (left), to 5 µm (middle), and 7.5 µm (right). (b) Changing elongation of a cell of height 5 µm. The ratio of the two half-axes is changed from 1 (left), to 2.25 (middle), and 4 (right). (c) Changing orientation of an elongated cell of height 5 µm. The angle to the direction of fluid flow is changed from 0° (left), to 45° (middle), and 90° (right). Adapted from Ref. [23].

Microfluidic gradient generators operate under continuous flow conditions. They rely on the interplay of diffusive mixing and fluid flow to produce a well-defined, temporally

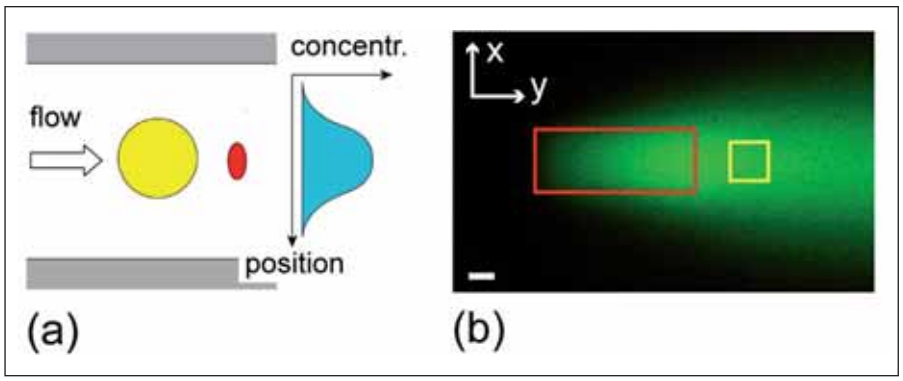

Fig. 4.: Flow photolysis experiment. (a) Schematic top view; the caged chemical is released inside an uncaging region of arbitrary shape (yellow) to produce the desired concentration profile (blue), which is carried across the cell (red) by the flow. (b) Release of 10 µM DMNB-caged fluorescein inside a rectangular uncaging region (red); scale bar, 10 µm. Adapted from Ref. [24].

stable concentration profile. In most cases, microfluidic devices are characterized for a fluid flow that runs through smooth channels and one may be tempted to assume this to be the environemt of the cell. However, when applied in live cell investigations, these conditions are rarely fulfilled. Biological cells are three-dimensional, impermeable objects in the fluid flow. When attached to the sidewalls, they extend into the channel and perturb the flow field in their vicinity, *i.e.*, the fluid is flowing around the cells. On the one hand, cells are exposed to shear forces from the flow and care must be taken that the cell's migration is not influenced by that. For mechanotactic cells the flow needs to be reduced to levels where the cell's motion along the flow remains random. On the other hand, if a non-uniform distribution of chemicals, *e.g.*, a gradient, is carried along with the fluid, these distortions by the cells can have a profound influence on the actual concentration across the surface of the cell. Depending on the flow speed, the diffusion constants of chemicals in the flow, and the characteristic length scales of the flow problem, the concentration on the cell surface can deviate considerably from the gradient profile imposed at the inflow of the chamber. In particular, for increased flow speeds, that are often used in micro-devices to avoid diffusive smoothing of steep gradients or blurring of concentration steps, these effects can become important. We have performed numerical finite element simulations of the full three-dimensional convection-diffusion problem to systematically study the influence of flow and geometry on the distribution of chemicals across the surface of a cell in a microfluidic channel. In these simulations, the cell is approximated as a half-sphere or, for elongated cases, as a half-ellipsoid. For further numerical details the reader is referred to Ref. [23].

A concentration gradient is accompanied by a continuous material flux in the direction of decreasing concentration. Close to the surface of a cell in the gradient, the diffusive fluxes in and out of a fluid element are no longer balanced, so that accumulation and depletion of chemoattractant occur at the sides of the cell pointing toward higher and lower concentrations, respectively. Thus, it is immediately obvious that the diffusivity of the chemoattractant as well as the velocity of the flow that transports the chemoattractant through the micro-channel will have an important impact on the concentration distribution across the surface of a cell. Our numerical investigations showed that the actual gradient a cell sees becomes shallower for increasing flow velocity, an effect that is commonly termed the shielding effect [23]. Also, the geometry of the cell may strongly influence the actual gradient signal at its surface. A summary of the results can be seen in Fig. 3. At the channel inflow, a linear gradient profile perpendicular to the direction of fluid flow is imposed. The deviations from this linear profile at the cell surface and the surrounding channel wall are displayed. The deviations from the imposed gradient increase with increasing cell height. They furthermore increase with increasing elongation of the cell in the direction of fluid flow and decrease with rotation of the cell from a parallel to a perpendicular orientation with respect to the flow direction.

The flow photolysis technique

In the microfluidic gradient mixer that was introduced in the previous section, concentration profiles can be defined with micrometer resolution. This is sufficient for most chemotaxis assays. The temporal resolution in this device is determined by the pumping rate and the extension of the microfluidic network. To establish a gradient signal in this device, solutions with the respective concentrations have to be pumped through the entire mixing cascade that is shown in Fig. 1, until a stable concentration profile is reached in the main channel. The time scale of this process is on the order of a minute and determines the on/off switching times as well as the times for gradient reversal in such a device. While this is sufficient for most migration studies, investigations of intracellular signaling require stimuli with a much higher temporal resolution that can be applied at the level of individual cells.

Recently, we have developed the flow photolysis technique, an approach to meet these demands [24]. It allows us to expose single living cells to well-defined chemical stimuli with a spatial resolution on the micrometer scale and a temporal resolution of less than a

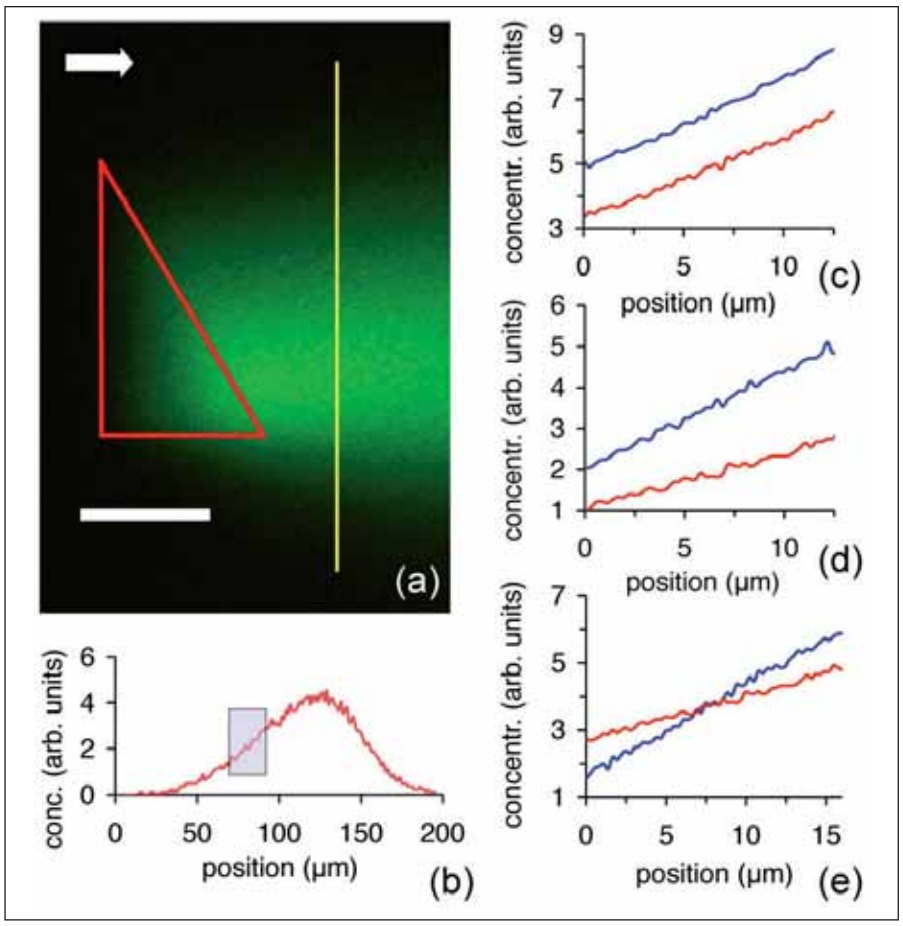

Fig. 5.: Generation of a local gradient by flow photolysis. (a) Photorelease of 5 µM DMNB-caged fluorescein in a triangular uncaging region (red); scale bar, 50 µm; arrow, direction of flow. (b) Fluorescence intensity along the yellow line in (a) for a mean flow speed of 67 µm/s. (c) to (e) Zoom of shaded region in (b) for different gradients, (c) constant slope and different midpoint, (d) constant relative gradient, and (e) different slope and constant midpoint. Adapted from Ref. [24].

second. Flow photolysis combines the advantages of microfluidic tools with the photochemical release of caged signaling molecules. Cells are placed in a microfluidic channel under a gentle, constantly running fluid flow that contains a biologically inert, caged signaling agent. To stimulate individual cells, the caging group is split off by short wavelength irradiation inside a confined region immediately upstream of the cell. The released substance is then transported by the flow downstream across the cell, see Fig. 4.

Due to the short distance between the uncaging region and the position of the cell, sub-second switching times of chemical stimuli can be achieved with this method. Furthermore, the spatial distribution of the released substance can be precisely controlled. The wider the illuminated region extends in the direction of the flow, the longer is the caged substance exposed to the light source and the more signaling substance will be released. Based on this simple interplay of fluid flow and light source geometry, a wide variety of concentration profiles can be tailored. For example, localized gradient profiles can be generated as shown in Fig. 5.

We thus achieve quantitative control of chemical stimuli on the length scales of individual cells with sub-second temporal resolution, for further details see Ref. [24]. Note that this method is limited to signaling substances that are available in a photoactivatable (caged) form. However, much progress has been made in recent years in the preparation and handling of caged compounds. A large variety of photoactivatable chemicals is available commercially, and detailed protocols are at hand for the synthesis of less commonly used caged substances [25]. Furthermore, we point out that also the flow photolysis technique will be influenced by the flow as discussed above [23].

The time resolution of chemical signals that we can reach with such techniques in a microfluidic device is limited by dispersion. We have analyzed these limits based on the theory of dispersion by Taylor and Aris [26]. Taylor-Aris theory can be applied in cases, where diffusive transport across the channel height is much faster than convective transport over the distance between the chemical source and the target, e.g., a cell. For this situation, steady state as well as time dependent solutions of the governing convection-diffusion-reaction equation can be found analytically. Moreover, even in cases where the Taylor-Aris condition is not fulfilled, i.e., the predicted switching times are not particularly accurate, they still provide a useful lower limit to the accessible temporal resolution. For typical values of the cell and channel dimensions, the flow velocity, and the diffusivity of a signaling substance, the theoretical limit of concentration switching by flow photolysis lies at 0.4 sec, a value that is not reached by other switching techniques for comparable setup parameters.

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Protein complexes and their function in cell division pathways in health and disease

Bodo M.H. Lange

Background

After the completion of sequencing of the human genome and obtaining genome sequence information for a series of important model organism, the next step is now to gain detailed information of the proteome of these organisms. Quantitative data are required that detail the expression levels and posttranslational modifications of proteins in different developmental stages and in specialised cell types. Such molecular data are essential for a comprehensive understanding of complex developmental programmes and for the analysis of the deregulation of signalling pathways in diseases. While great progress has been made to analyse fractions of the proteome in the order of several thousand proteins [1], obtaining information on the whole proteome on a routine basis is currently not yet feasible. In addition, to really understand complex developmental or disease driven traits we require an extra level of information on the protein-protein interactions and protein complexes formed. This is critical as most of the proteins in the cell are likely to be assembled as protein complexes [2] and are likely to fulfil different functions when associated with different sets of binding partners in multiple cellular locations. In the field of cell division much has already been achieved regarding these big challenges, for example through the molecular characterisation of the spindle proteome [3, 4], the midbody [5], the basal body [6], the centrosome [7, 8] and spindle pole proteome [9, 10], [reviewed by [11-13].

Subsequently proteomics studies were followed up by systematic RNAi screens or proteomic work was combined with RNAi experiments that provided information on the function of proteins for spindle or centrosome organisation identifying basic mechanisms of centrosome or spindle assembly [14-17]. A comprehensive study on protein-complexes related to cell division was recently published [18]. In addition there is already a large body of work concerning the functional and biochemical characterisation of centrosome related proteins, mainly focussing on the single gene or protein level. These important studies are too numerous to be discussed here but were already previously reviewed by [12, 19-21]. All in all we already have a very comprehensive overview of the centrosome's proteome with the current count of centrosome components having reached about 300 proteins listed in specialised databases [22, 23].

The centrosome is integrated in multiple cellular signalling pathways

We have recently analyzed the protein complement of the syncytial Drosophila embryo centrosome and identified 250 proteins of which 48% showed a centrosome related and/or cell cycle related function. Interestingly, the highest level of functional conservation between human and Drosophila was found in the group of proteins that function in centrosome duplication and segregation [7]. The centrosome proteome characterisation provides us both with information on possible structural components of this organelle but also provides interesting molecular links to other cellular signalling pathways such as protein translation, cell cycle regulation and to actin and RNA binding proteins [7, 24]. This work is currently expanded on the phosphoproteome level of the Drosophila centrosome to identify substrates of regulatory mitotic kinases (Habermann & Lange unpublished). "Another" goal was to determine the protein-protein interactions within the centrosome via tandem affinity purification in human cells (Fogeron et al. submitted). This protein-protein interaction network provides

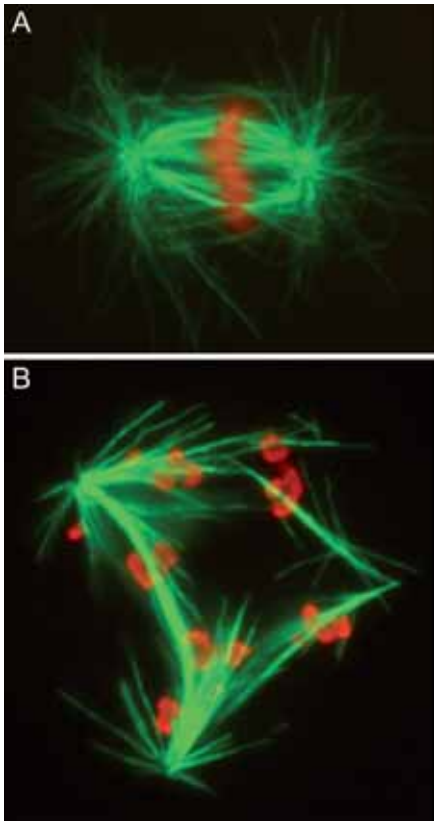

Fig. 1.: (A) In somatic cells, a normal mitotic spindle displays a bipolar organisation with aster microtubules radiating from the spindle poles. (B) In abnormal mitosis, here caused by depletion of a putative tumor suppressor, bipolar organisation is lost leading to highly abnormal chromosome segregation defects. Microtubules are labelled in green, mitotic chromosomes (marked by phosphorylated histone 3) in red.

us with a series of new centrosome candidate proteins and links centrosome and centriolar components to regulatory proteins, including also centrosome interacting partners that are deregulated in cancer.

Disease relevant protein-protein interaction networks

We are currently investigating protein-protein interaction networks that are relevant in cancer and neurodegenerative diseases to pinpoint biochemical and functional differences that might contribute to disease progression. We are applying tandem affinity purification (TAP) of bait proteins that are expressed from a single chromosomal integration site in human cells with the same (isogenic) genetic background. The mass spectrometry analysis of the TAP samples is employing iTRAQ isotope labelling [25] that quantitatively discriminates differential protein-protein interactions between wild type and several mutant variant proteins.

This research is carried out as part of the IG Mutanom consortium (www.mutanom.org), which analyses the consequences of tumor relevant mutations on a biochemical and functional level. The proteomics data are complemented by mutational profiling, mRNA profiling and functional assays that investigate cancer relevant traits (cell migration, cell division & proliferation, cytoskeleton organisation and cell cell contacts) (Figure 1). The aim is to develop a systems biology model from the obtained data to predict disease progression and to improve diagnosis and treatment. In the future, combining the quantitative information obtained, the underlying genetic profile of a patient might facilitate an opportunity to optimize treatment regimes, reduce drug side effects and improve on the prediction of disease outcome.

Cancer triggered by infection, environmental circumstances and or by genetic predisposition is still a major cause of death in developed countries. For most of the 3.2 mio cancer patients and 1.7 mio people dying of cancer in Europe (2006) every year mutations are playing the key role for the development and progression of the disease. These mutations are accumulating over the lifespan of a person with most of the mutations possibly being harmless while a particular set of mutations (so called driver mutations) finally result in the advance of cancer [26]. The mutations frequently occur either in components of signalling pathways that are required for physiological functions in short-term cellular signalling or in components that interfere with the long term developmental genetic program of an individual. This complexity has made the deployment of new generic cancer drugs - in spite of highly increased development costs - very difficult [27]. The efficiency of cancer medicamentation is to a large extent diminished by drug resistance and lack of specificity, which can result in severe side effects for the patient. Hence for the prevention, diagnosis und therapy a detailed knowledge of these processes (both in normal and in cancer cells) is required. Because cancer is such a complex multifactorial disease, for the future development and application of new and efficient drugs both the genetic profile of the patient and of the tumor will have to be taken into account. In addition, combining multiple drugs that target several cancer pathways at the same time might offer another opportunity to achieve tumor regression when treatment with single compounds failed. Hence we urgently require new methodologies that exploit the data from functional genomic studies for the individual patient. The Systems Biology modelling of the disease process that aims at predicting the outcome of a particular genetic profile is likely to be a highly promising tactic. The rapid progress in sequencing technologies (second generation sequencing methods) increasingly permits more efficient detection of cancer relevant

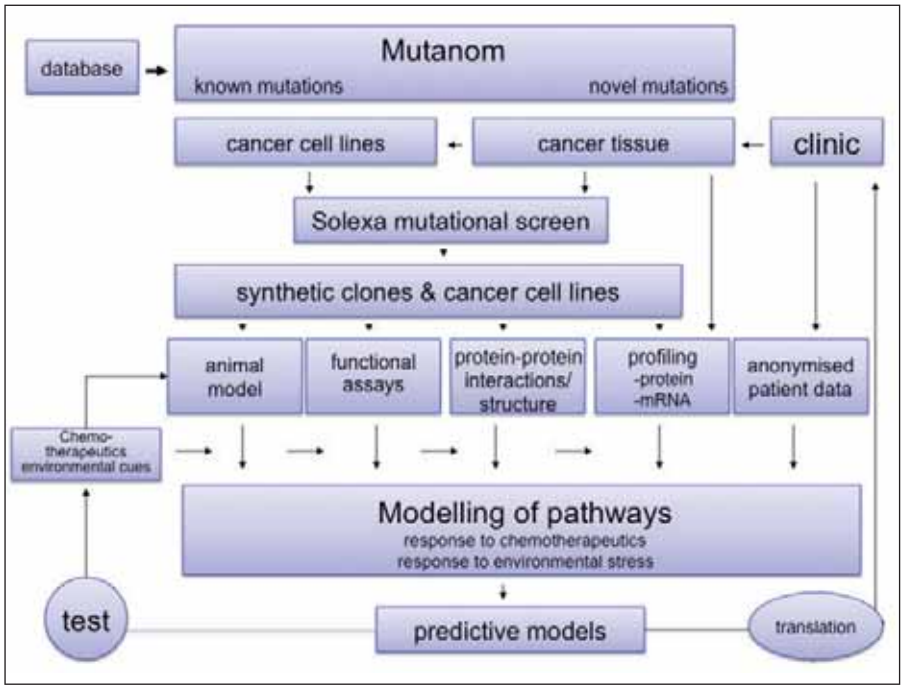

Fig. 2.: The diagram shows the interactions and workflow of the Mutanom project that uses quantitative experimental data from cell and mouse models, protein-protein interaction data, mRNA and protein profiling data for a system biology modelling approach of cancer. A model is developed for the prediction of the functional consequence of frequent cancer mutations.

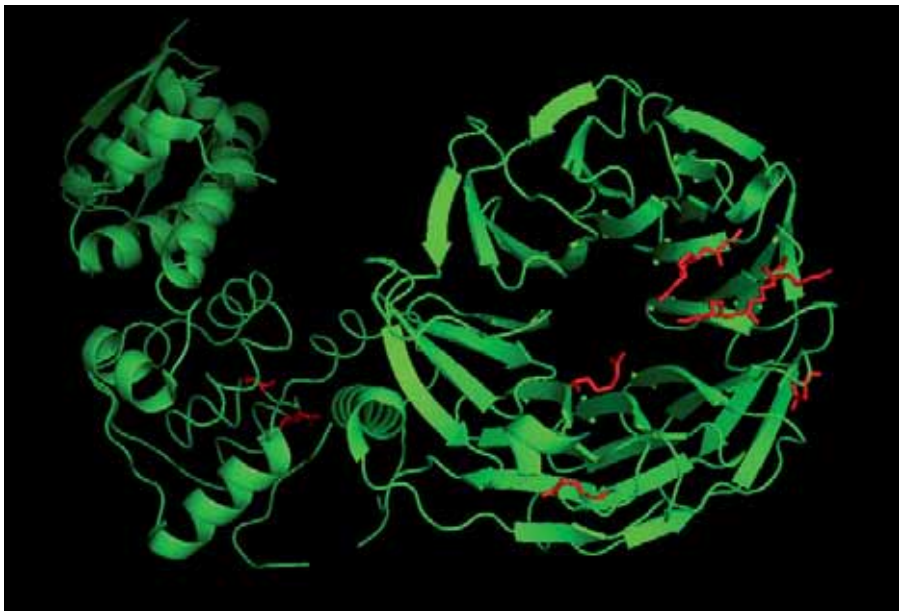

Fig. 3.: The molecular model of the tumorsupressor FBXW7. The positions of frequent mutations are labelled in red. The model aims to predict if specific cancer related mutations affect protein-protein interaction domains.

mutations as a series of studies have shown recently [28-30]. Comprehensive databases (for example COSMIC, www.sanger.ac.uk/genetics/CGP/cosmic) list currently available mutations in the genome for particular tissues or cell lines but still require urgent expansion. However, the dilemma is that while the number of identified cancer mutations is rapidly increasing, we still know very little about their consequence on physiology, survival, growth control and differentiation both on a cellular and on whole organism level. The integrated genome research network Mutanom, funded as part of the National Genome Research Network (www.ngfn.de/en/mutanom.html) is carrying out a systematic analysis of the functional consequence of some of the most frequently occurring mutations in breast, gastrointestinal and prostate cancer. The network also assembles a predictive model building on and integrating data from functional genomics, proteomics, tissue culture cell experiments, studies on model organism and also implementing clinical data (Figure 2).

Characterisation of the structural and functional consequence of cancer mutations

The characterisation of the consequence of cancer mutations in the Mutanom consortium and in our lab has revealed distinct structural, biochemical and functional effects on a cellular level. For example, the analysis and molecular modelling of tumor suppressors and oncogenes (Figure 3) that are frequently mutated in cancer displaying distinct mutational patterns on the structural level of the relevant proteins (Stehr et al. submitted). Such mutations are the likely result of a selective pressure during disease progression showing that for example in oncogenes mutations occur preferentially at positions that are critical for the protein's function. The application of isogenic cell models (described above) has been very efficient in analyzing the biochemical and functional consequence of frequent cancer mutations. We could show for example for the tumorsuppressor p53, the oncogenes PIK3C and BRAF, which are frequently mutated in a wide spectrum of tumor cells and tissues, that a subset of signalling proteins only associates with the mutant variants (Haupt, Jang, Muradyan et al. and Lange unpublished). These surprising results suggest that the characterized mutations might result in a gain of function and have an impact on cancer relevant signalling pathways. Such changes could provide the cancer cells with particular growth advantages over normal somatic cells. Hence, this work identified already a series of candidate proteins that might in the future provide opportunities to find new therapeutic or diagnostic approaches. Such results contribute to the Systems Biology Modelling approach [31] of the Mutanom project as part of an international effort to battle cancer.

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Capturing ER calcium dynamics

Juliane Jaepel & Robert Blum

Abstract

The luminal ER space is a highly dynamic intracellular Ca^{2+} store. The direct measurement of luminal Ca^{2+} release and uptake is still critical when Ca^{2+} homeostasis is analyzed in neural cells. For the analysis of Ca^{2+}-dependent signaling, synthetic Ca^{2+} indicators have become popular. The properties of these indicators allow only limited targeting to subcellular structures such as the endoplasmic reticulum (ER). Recently, we introduced a new strategy for the targeting of synthetic Ca^{2+} indicators to the lumen of the ER [1]. The method is called Targeted-Esterase-induced Dye loading (TED) [2] and is based on the targeted, recombinant expression of a high carboxylesterase (CES) activity in the lumen of the ER, which is needed to trap synthetic indicators. The method combines the selectivity of protein targeting with the biochemical advantages of low-affinity synthetic Ca^{2+} indicators. TED allows direct and non-disruptive measurement and imaging of Ca^{2+}-store dynamics.

Here, we summarize major topics in the cell biology of Ca^{2+} signaling and discuss the perspective of the TED method for the morphological and physiological analysis of temporal and spatial Ca^{2+}-dynamics in neural cells.

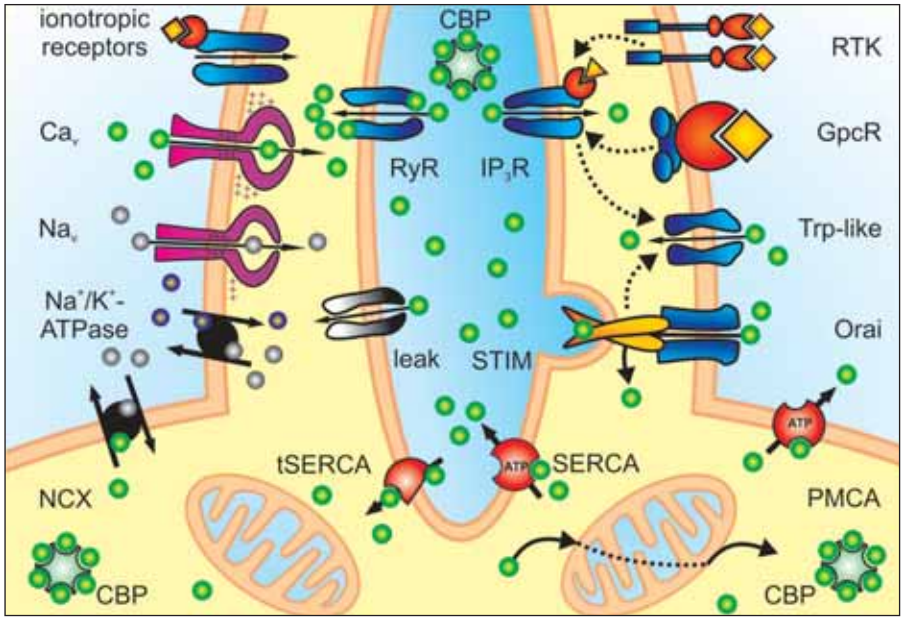

Fig. 1.: Diverse membrane proteins of the plasma membrane, ER and mitochondria are involved in Ca^{2+} homeostasis and signaling in neuronal cells. Influx of Ca^{2+} to the cytosol is mediated by (1) depolarization of cells, which leads to opening of voltaged-gated Na^+ (Na_v) and Ca^{2+} (Ca_v) channels, (2) signaling over ionotropic receptors, G-protein coupled receptors (GpcR) and receptor-tyrosine kinases (RTK) that cause either direct influx of ions or intracellular signaling over second messengers such as IP_3, (3) store-operated Ca^{2+} entry via STIM and Orai1, and/or the opening of TRP like channels, and (4) opening of ER localized Ca^{2+} channels such as the IP_3 receptor (IP_3R). The ryanodine receptor (RyR) links fast, excitatory Ca^{2+} entry to fast Ca^{2+} release. Ca^{2+} homeostasis after signaling is restored by pumping of Ca^{2+} ions in the ER via the sarco-endoplasmic reticulum ATPase (SERCA) and by transport of Ca^{2+} over the plasma membrane by the plasma membrane Ca^{2+}-ATPase (PMCA) and the Na^+/Ca^{2+}-exchanger (NCX), which is driven by the Na^+ gradient produced by the Na^+/K^+-ATPase. Mitochondria are also involved in Ca^{2+} homeostasis as they take up Ca^{2+} ions during cytosolic Ca^{2+} elevation by a uniporter, and when the intracellular Ca^{2+} concentration is lowered, they return Ca^{2+} by a Na^+/Ca^{2+} exchanger. A direct link between luminal Ca^{2+} release and mitochondria is caused via a truncated SERCA isoform. Ca^{2+}-binding proteins (CBPs) are important buffers in the cytosol (calbindin, parvalbumin) and in the ER lumen (calsequestrin, calretinin). (Green circles = Ca^{2+} ions, grey circles = Na^+ ions, blue circles = K^+ ions).

The ER is a dynamic structure

The endoplasmic reticulum (ER) is an intracellular membranous network with diverse functions in cell biology. The shape of the ER is highly variable within a cell. Dependent on the cell type, ER membranes appear to be organized in a network of cisterns, stacks or tubules [3, 4] The ER is not a solid cellular space, but a highly dynamic membrane structure. Classically, the ER is subdivided into three major distinct domains. (1) The nuclear envelope (NE), which separates the nucleus from the cytosolic compartments, (2) the smooth ER and (3) the rough EG that is characterized by membrane-bound ribosomes. ER integrity is critical for the accuracy of membrane flow between the ER, the ER-to-Golgi intermediate compartment and the Golgi apparatus, in both anterograde and retrograde directions. Its membrane integrity and spatial constitution underlie the continuous flow of intracellular membranes, proteins and milieu components that bud off from the ER and fuse with the ER as a result of intracellular trafficking pathways [5-7]. Next to biosynthesis of phospholipids, cholesterol and steroids, the ER is involved in protein synthesis, including the targeting of ribosomes, cotranslational translocation and folding and maturation of the polypeptide chain. Furthermore, it plays a role in carbohydrate metabolism and detoxification reactions [3].

Distribution of the ER in neurons

In neurons, the three-dimensional and physiological organization of the ER is extraordinary complex [8, 9]. In principal neurons, such as pyramidal neurons in the cortex, neurons in the hippocampal network, Purkinje cells in the cerebellum or motoneurons in the spinal cord, the ER extends from the somatic region to all dendrites [9-14], which are the main input region of principal neurons. The rER is predominant in somata and proximal dendrites, whereas the sER is preferentially found in dendrites and dendritic spines. Spines represent the postsynaptic compartment, where coordinated synaptic transmission is a prerequisite for neuronal plasticity [9, 10, 12, 13, 15, 16]. In hippocampal dendritic spines, only about 20% of all spines carry sER membranes [10, 12]. The differential distribution of ER contributes to local signaling and plasticity at hippocampal synapses. ER elements and functional attributes of the ER are also found in long-projection axons (cm to meter range) and their presynaptic elements [9, 17]. The importance of local protein synthesis in axonal growth cone differentiation, synapse formation and postsynaptic maturation, which involves ER-like functions, is an extensively discussed topic in motoneuron diseases [18-20].

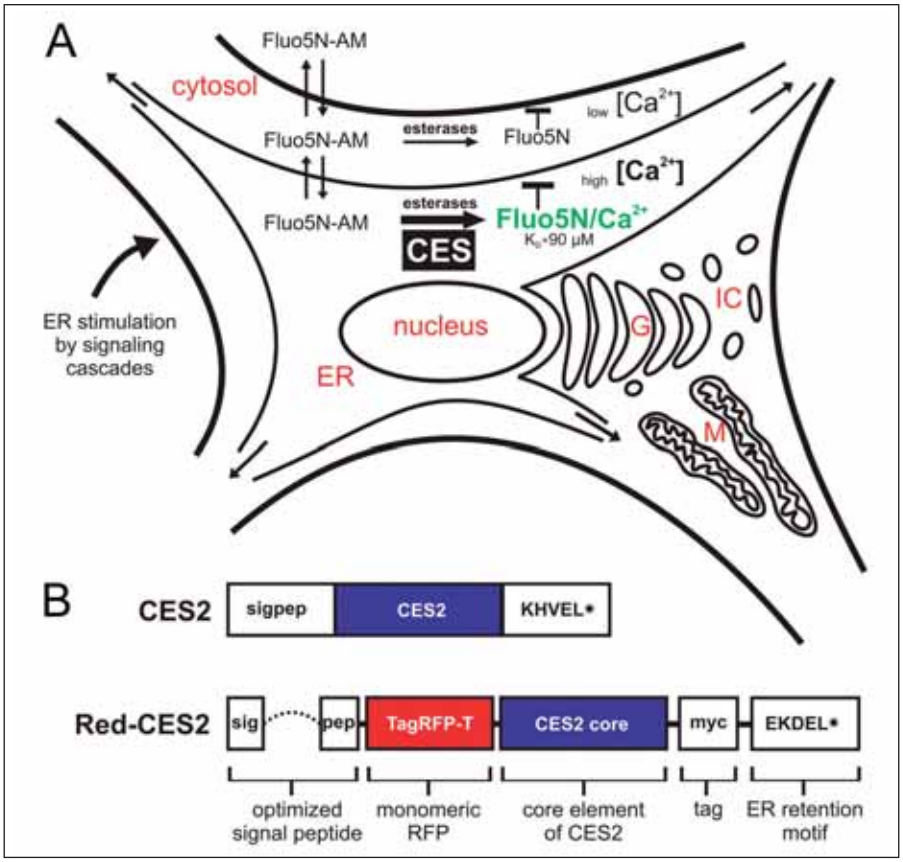

Fig. 2.: **A** Principle of targeted-esterase induced dye loading (TED). A carboxylesterase (CES) is targeted to the ER via a signal peptide and an ER retention and retrieval motif. The high activity of this carboxylesterase in the ER leads to the release of the hydrophilic dye Fluo5N, which is trapped in the ER. This low-affinity indicator dye binds to Ca^{2+} in the ER. Although a small part of Fluo5N-AM is also cleaved in the cytosol by other esterases, the concentration of the Ca^{2+} is too low to generate a Ca^{2+} signal. **B** Outline of CES2 constructs. The signal peptide and the ER retention and retrieval motif of CES2 mediate the targeting of the CES2 to the ER. The fusion protein Red-CES2 is targeted by an improved signal peptide and an ER retention and retrieval motif consisting of EKDEL. Next to the core element of CES2, it consists of the red fluorescent protein TagRFP-T and the antibody tag myc.

The luminal Ca^{2+} store

The ER lumen extends to almost all cellular regions and is the major intracellular Ca^{2+} store [21]. Available data suggest that the lumen of the ER forms a continuous, aqueous space in which Ca^{2+} and small molecules, (e.g. fluorescent dyes) can diffuse readily. The luminal Ca^{2+} concentration varies between spatial subregions of the ER [22, 23], but all subdomains of the ER are involved in its role as Ca^{2+} store, even the NE [24].

In the last years, much progress has been made in the identification of the molecules and proteins involved in Ca^{2+} homeostasis (Fig. 1). Within the ER lumen, the most Ca^{2+} is buffered by Ca^{2+}-binding proteins (CBPs) of which the proteins calsequestrin in the sarcoplasmic reticulum [25], and calreticulin in most non-muscle cells [26] carry multiple low-affinity Ca^{2+} binding sites. Functionally, calreticulin is not only a CBP, but also a multi-process Ca^{2+}-buffering chaperone [27]. The special arrangement of intra-ER Ca^{2+} buffers, characterized by low affinity for Ca^{2+} in combination with activity of the SERCA-Ca^{2+} pumps (Sarco-Endoplasmic Reticulum Ca^{2+} ATPase) keeps intraluminal Ca^{2+} at a level of ~0.1 – 0.8 mM [28], thus creating a steep electrochemical gradient directed towards the cytosol.

Ca^{2+}-permeable channels, pumps and receptors are distributed inhomogeneously over the ER [3]. A relevant consequence of this compartimentalization via the ER membrane is observed in Purkinje neurons. Purkinje cells are huge neurons in the cerebellum with more than 200.000 spine-like synapses per dendrite, which integrate sensory information and motor programs for the fine-tuning of motor coordination on a millisecond time-scale. Here, the IP_3 receptors (IP_3R) and ryanodine receptors (RyR), the two major channels involved in stimulated release of Ca^{2+} from the ER (Ca^{2+} release), coexist on

ER membranes, except for the ER in synaptic spines. In these spines, representing the input region of the Purkinje cell, only IP_3R are found [29] and are functionally important for synaptic signaling during motor coordination [30, 31].

Calcium function and signaling

The general functions of Ca^{2+} and Ca^{2+} signaling in cells are various and include cellular processes such as muscle contraction [32], gene expression (reviewed in [33, 34]) or secretion [35]. In neuronal cells, Ca^{2+} is essential for excitability, neurotransmitter release and gene expression [8, 9]. Furthermore, dendritic Ca^{2+} is important for the induction of short- and long-term changes in neuronal transmission and morphology [8, 36], representing learning and memory on a cellular level.

Astrocytes are one of the most interesting neural cell types to investigate ER function and Ca^{2+} signaling as they are currently one of the most promising endogenous cell sources for neuron repair strategies after brain injury, ischemia or stress-induced neuronal cell death. Astrocytes activate their ER Ca^{2+} store mainly by G-protein coupled receptors and subsequent IP_3 signaling and answer with long-range cell-to-cell Ca^{2+} waves and Ca^{2+} oscillations. It is under discussion whether astrocytes use Ca^{2+} waves to release gliotransmitters, which may act on neuronal networks, under physiological conditions [37].

IP_3 receptors and ryanodine receptors

Ca^{2+} homeostasis and signaling is regulated in many different ways, for example Ca^{2+} influx to the cytosol as part of a signaling process can either be mediated by an efflux of Ca^{2+} from internal stores or by an influx from the external medium. In both cases, the driving force is ensured by a high concentration gradient [8, 14, 38]. Release from Ca^{2+} internal stores, such as the ER, is controlled by channels, above all by the IP_3R and the RyR [8, 38, 39]. The activity of these ER located channels is controlled by second messengers such as inositol-1, 4, 5-triphosphate (IP_3) and cyclic ADP ribose (cADPR). IP_3 is generated by the enzyme class Phospholipase C, which cleaves phosphatidylinositol 4, 5-bisphosphate (PIP_2) into diacyl glycerol (DAG) and IP_3. There are different isoforms of PLCα/β that differ in their activation mechanism. The PLC is stimulated by G-protein coupled receptors such as the muscarinic acetylcholine receptor or metabotropic glutamate receptors (mGluRs). Receptor tyrosine kinases, such as the neurotrophin receptors of the Trk-family or other growth factor receptors like EGF and IGF receptors, activate PLCγ [40]. IP_3 then activates IP_3 receptors located in the ER membrane, which leads to the floating of free Ca^{2+} floats out of the ER, along a concentration gradient, to the cytosol (IP_3-induced calcium release, IICR). While this process is quite slow in non-neuronal cells (some seconds), it is quite fast in neurons. For example, synaptic activation of the neuronal metabotropic mGluRI causes IP_3-induced Ca^{2+} release from the luminal store within 300-500 ms in the spines of hippocampal pyramidal neurons [12]. The major activator of ryanodine receptors (RyR) is Ca^{2+} itself (Ca^{2+}-induced Ca^{2+}-release, CICR). This Ca^{2+}-gating acts as a link for fast communication between the ER and the depolarizing Ca^{2+} entry during neuronal activation [8].

Depolarization-dependent Ca^{2+} influx, followed by CICR is responsible for the increase in cytosolic Ca^{2+} needed for heart contraction. In skeletal muscle fibres, Ca^{2+} release from the sarcoplasmic reticulum is fully dependent on coordinated, non-stochastic activity of RyRs [41]. Some mutations in RyR1 cause malignant hyperthermia, a severe channelopathy, which is responsible for anesthetic-induced deaths in otherwise healthy patients. Here, defect RyR1 receptors can cause a long-lasting, irreversible Ca^{2+} efflux from the ER which results in continued muscle contraction, hypermetabolism and a critical increase in body temperature [42].

In neurons, CICR is of high physiological relevance in presynaptic compartments, where voltage-gated Na^+ channels (Na_v) involved in action potentials are triggering the opening of voltage-gated Ca^{2+} channels (Ca_v), thus mediating a fast Ca^{2+} influx which subsequently activates RyRs [17, 43, 44]. This mechanism allows a gradual and fast increase in the cytosolic Ca^{2+} concentration and therefo-

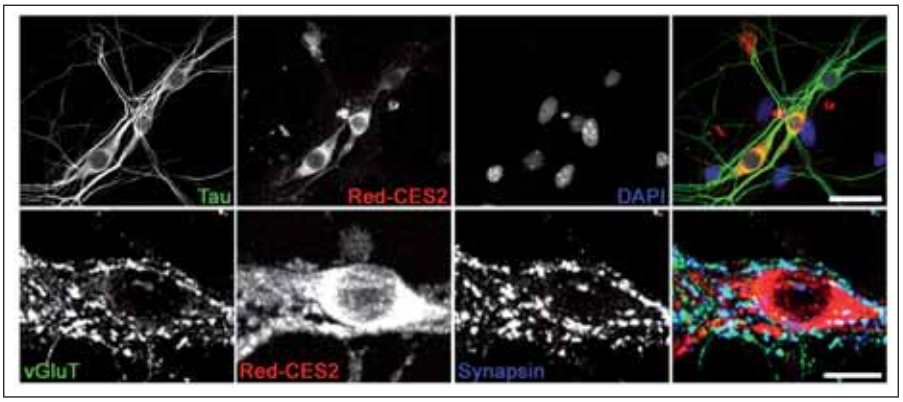

Fig. 3.: Immunostaining of cultured hippocampal neurons expressing Red-CES2. The upper panel shows its co-localization with Tau, which modulates the stability of axonal microtubules, and is therefore expressed in axons. As expected, Red-CES2 is absent from the nucleus marker DAPI. In the lower panel, a co-staining with the pre-synaptic markers synapsin and vGluT is visible, indicating the expression of Red-CES2 also in peripheral regions of the ER.(Bar, top panel: 25 µm, lower panel: 10 µm).

re presynaptic RyRs are regulators of presynaptic transmitter release [44] and contribute also to the release of neurotrophic proteins [45].

Ionotropic receptors

Ionotropic receptors, such as the nicotinic acetylcholine receptor at the neuromuscular end-plate and in the cortex, or the glutamate receptors (NMDA-type) at postsynaptic spines, are transmitter receptors and ion-permeable channels in one multimeric protein complex. Upon transmitter binding, these receptors mediate fast postsynaptic Ca^{2+} influx , which is important for the subsequent synchronization of activity-dependent signaling cascades within a cell and can cause long-term changes in cellular function such as gene expression, growth and synaptic integration [8].

Concepts of store-operated calcium entry

Stimulation of cells and subsequent release of Ca^{2+} from the ER is an initiation signal that triggers Ca^{2+} entry from the extracellular space and which is fully independent of voltage-activated Ca^{2+} currents [46]. This phenomenon has been referred to as store-operated calcium entry (SOCE) and the variety of channels involved in this process are called store-operated calcium channels (SOCCs) or calcium-release activated calcium channels (CRACs) [38, 46, 47]. While the importance of this event is still unclear in neurons, it is a major Ca^{2+} influx mechanism in non-excitable cells and replenishes the intracellular luminal Ca^{2+} stores after Ca^{2+} depletion. As the stores are acting like capacitors, this current was also termed capacitative Ca^{2+} entry (CCE) [48].

One mechanism of SOCE is mediated by stromal interacting molecule (STIM), which has diverse functions in SOCE. It senses Ca^{2+} concentration in the ER via its EF-hand and rapidly clusters and relocalizes to ER regions near the plasma membrane to interact with

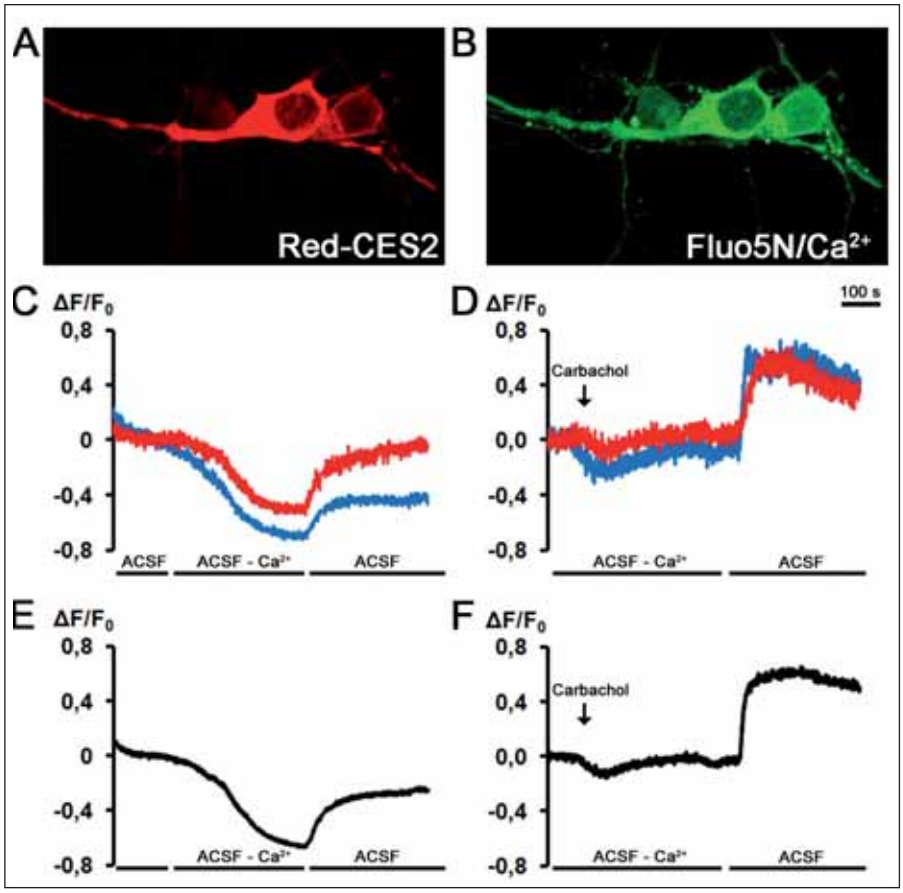

Fig. 4.: Direct imaging of ER derived Ca^{2+} signals after targeted-esterase induced dye loading. **A** Cultured hippocampal neurons transduced with a lentiviral vector for Red-CES2 expression under ubiquitin promoter and **B** with Fluo5/Ca^{2+} complexes after Fluo5N-AM loading for direct measuring of luminal Ca^{2+} signals. **C/E** Changing of the buffer from artificial cerebrospinal fluid (ACSF with 2 mM Ca^{2+}), to a Ca^{2+} free ACSF leads to a decrease in ER Ca^{2+} signals, which is reversible as a further change back to ACSF induces an increase in Ca^{2+}. **D/F** Addition of 20 µM Carbachol, an agonist of the muscarinic acetylcholine receptor, in Ca^{2+} free ACSF, causes an efflux of Ca^{2+} ions via the IP_3 receptor, and an increased Ca^{2+} influx after addition of Ca^{2+} to the extracellular media. (C, D single cell recording, E, F average of 10 cells). Experiment by Juliane Jaepel.

Orai proteins, which then form a highly Ca^{2+} selective channel pore [49, 50]. This mechanism is now widely accepted as the base of a highly specific, sustained Ca^{2+} inward current called I_{CRAC} (a current mediated by CRAC), which was first observed in mast cells [51].

Initially thought to represent store-operated channels or even I_{CRAC}, the family of the transient receptor potential channels (Trp) is one of the most interesting players in Ca^{2+} homeostasis and signaling [46, 47, 52, 53]. In mammals, the family of Trp channels is organized in six related subfamilies and ion-permeable receptors. Physiologically, they are best characterized as "sensory channels" responding to temperature, touch, taste, and many other sensory stimuli [52]. Another sensory function of Trp channels is to mediate sniff responses to chemotractants in the growth cone of long-range projection neurons during axonal growth and differentiation [54, 55].

Recently, it was found that dynamic interactions between TRPC1-STIM1-Orai1 are essential for SOCE [56]. Another study demonstrated that Orai1 mediates the communication

between STIM1 and hTRPC1. This functional interaction is important for the activation of hTRPC1 [57], thus linking CRAC components with Trp channels. Anyhow, the regulation of SOCE and the interplay with Trp channel functions remains one the most fascinating fields in cellular physiology.

Control of cytosolic Ca^{2+} concentration

Ca^{2+} signaling depends on the Ca^{2+} concentration gradient between the extracellular medium and the cytosol, and the gradient between the cytosol and the internal Ca^{2+} stores. For the ER of sensory neurons, the resting Ca^{2+} concentration reportedly varies from 60-400 µM [58, 59], while the cytosolic free concentration is of the order of 100 nm at resting levels. In Purkinje cells, some spines have a resting level of 45 nM [60]. Cytosolic increase in Ca^{2+} is roughly in the range of some hundreds of nM to the low-µM range (<5µM). Thus a high electrochemical inwards-driving force from the extracellular space (~2 mM Ca^{2+}) to the cytosol, as well as from the ER lumen to the cytosol, is driving the Ca^{2+} flux.

After Ca^{2+} has carried out its function, it is important to remove it from the cytosol by diverse pumps and exchangers. Based on the Na^+ gradient produced by the Na^+/K^+-ATPase, the Na^+/Ca^{2+}-exchanger (NCX), the plasma membrane Ca^{2+}-ATPase (PMCA), and a variety of other transporters and exchangers transport Ca^{2+} back to the extracellular space. In the heart, digitalis/ouabain class of compounds have been used for centuries to treat congestive heart failure and arrhythmias. These compounds are decreasing the driving force for the Na^+/Ca^{2+}-exchange and increase cellular content and release of Ca^{2+} during depolarization for an improved contractility of the heart. As a further Ca^{2+} export mechanism, the sarco-endoplasmic reticulum ATPase (SERCA) pumps Ca^{2+} back into the ER.

While the concentration of Ca^{2+} in the cytosol is tightly regulated, it is not clear, whether the ER calcium concentration of neurons needs to be maintained at a constant level as suggested by the concept of capacitative Ca^{2+} entry.

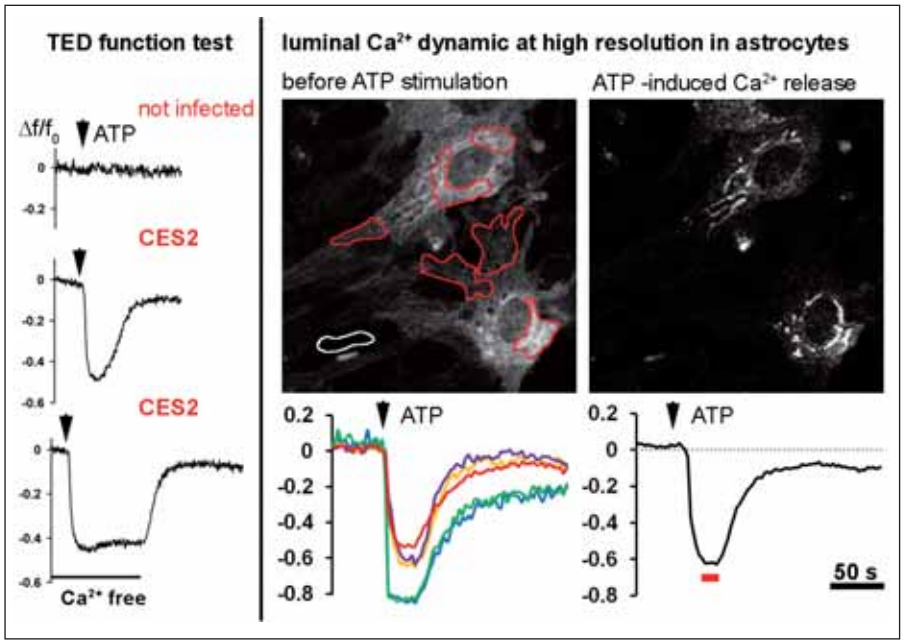

Fig. 5.: TED measurement in cultured astrocytes. (left top panel) After Fluo 5N dye loading in non-transfected astrocytes, Fluo 5N loading is ineffective. Stimulation of cortical astrocytes with 100 µM ATP, a strong agonist for the release of Ca^{2+} from intraluminal Ca^{2+} stores, does not show any increase in Fluo5N fluorescence (cytosolic signal) nor a decrease in fluorescence (direct luminal Ca^{2+} measurement). (left medial panel) In astrocytes after TED with Fluo 5N-AM and lentiviral CES2, 100 µM ATP induces the fast release of Ca^{2+} from intracellular Ca^{2+} stores. Stores are rapidly refilled, because the measurement had been performed in the presence of extracellular Ca^{2+} (2 mM). (lower, left panel) When the very same stimulation protocol is performed in the absence of extracellular Ca^{2+}, loss of intraluminal Ca^{2+} becomes visible. Re-addition of extracellular Ca^{2+} refills the Ca^{2+} store within seconds, most likely by store-operated Ca^{2+} influx. Right top panels: High-resolution imaging of luminal Ca^{2+} transients in astrocytes. Before ATP stimulation, Fluo5N/Ca^{2+}-complexes resolve the luminal membrane morphology. ATP-induced Ca^{2+} release depletes the luminal Ca^{2+} store, but not the luminal Golgi. Regions of interest are labeled and correspond to the graph in the lower panel. Lower panel, right graph: Average graph. The red bar indicates the store-depletion state. Experiment by Robert Blum [2].

Calcium-buffer mechanisms

The spread and diffusion of cytosolic Ca^{2+} is limited by Ca^{2+} binding proteins (CBPs, e.g. Calbindin, Parvalbumin) [60, 61]. In some neurons, such as cerebellar Purkinje cells, the presence or absence of mobile CBPs is a critical determinant of spine compartmentalization and dendritic integration [60, 61]. The deletion of Calbindin in mice causes ataxia thought to be caused by an imbalance in postsynaptic signal integration of Purkinje cells [62]. CBPs ensure that Ca^{2+} elevation remains a local event in spines and associated dendritic elements. High and low-affinity Ca^{2+} binding sites in CBPs distinguish fast, excitatory Ca^{2+} influx via VGCCs and slow metabotropic, IP_3-mediated Ca^{2+}-release with large changes in cytosolic Ca^{2+}. This process is thought to be important for calcium-dependent downstream signaling via Calmodulin-dependent effector molecules (e.g. Cam kinases) [60, 61].

Furthermore, mitochondria are able to buffer high Ca^{2+} concentrations [63] by up taking Ca^{2+} by uniporters with low sensitivity to Ca^{2+}. Via a Na^+/Ca^{2+} exchanger the Ca^{2+} is returned to the cytosol and is then either

pumped into the ER or removed from the cell [64]. Strong Ca^{2+} release may damage mitochondria by Ca^{2+} overload [65, 66].

Monitoring changes in Ca^{2+} in the luminal Ca^{2+} stores

For understanding the dynamics of Ca^{2+}-mediated signaling, direct measurement of Ca^{2+} in different compartments of the cell, like the ER, is an important and indispensable tool. The ideal Ca^{2+} probe for these kinds of experiments is highly specific for Ca^{2+}, covers a wide range of Ca^{2+} -affinity and a high targetability. In principle, three different kinds of indicators are used for Ca^{2+}-measurements, (i) photoproteins derived from the chemiluminescent protein aequorin, (ii) fluorescent protein indicators based on GFP and (iii) synthetic Ca^{2+} indicators. The genetically engineered protein-indicators base on aequorin or GFP-derivates and recognize free Ca^{2+} through Ca^{2+}-binding protein domains such as calmodulin or troponin [67-71]. In recent years, spectacular progress in optimizing protein-based indicators has been made, especially for the imaging of cytosolic Ca^{2+} in neurons *in vitro* as well as *in vivo* [72, 73]. Even though progress has been substantial, the development of a low-affinity indicator to analyze Ca^{2+} signaling in the ER of neurons remains a major challenge in methodological research [2].

Synthetic fluorescent dyes are still the most widely used Ca^{2+} indicators and are based on Ca^{2+} chelators (EGTA; BAPTA) [74, 75]. They are synthesized as hydrophilic versions or as lipophilic esters, which permeate the plasma membrane. Within cells, the lipophilic acetoxymethylester residues are cleaved by intracellular esterases and the Ca^{2+} indicators are trapped in a hydrophilic form. Ca^{2+} indicators are very flexible in their chemical design, thus allowing a wide range of Ca^{2+} affinities with very good kinetics and a high photon emission rate. Their targetability, however, is very poor. High affinity indicators, such as Oregon Green BAPTA1, with a dissociation constant (K_d) of 130 nM, are used to detect small changes in cytosolic Ca^{2+} while low affinity indicators are preferred for use in compartments with high Ca^{2+} content. Different protocols have been established to remove low-affinity indicators from the cytosol to allow measurement of Ca^{2+} in the ER. These techniques include permeabilization of the plasma membrane with agents as streptolysion O [39], digitonin [76] or saponin [58], and dialysis of the cell under whole-cell patch-clamp configuration [77]. Nevertheless, these techniques disturb the cytosolic environment and therefore may interfere with intracellular signaling pathways.

Capturing Ca^{2+} dynamics in the ER by TED

Recently, our group developed a new strategy for targeting synthetic Ca^{2+} indicators to the ER [1]. The method is called TED (Targeted-Esterase-induced dye loading) [2] and bases on the recombinant targeting -not of the dye- but of a recombinant esterase, which then releases the hydrophilic Ca^{2+} dye from the lipophilic acetoxymethyl ester in the targeted subcellular compartment. The esterases that are used in this method belong to the family of mammalian carboxylesterases (CES), EC 3.1.1.1. The members of this family are soluble ER-resident proteins [78], and thus they carry an ER-translocation signal peptide at their aminoterminal end and an ER-retention and retrieval motif for luminal proteins at their carboxyterminal end [79]. In the ER lumen, providing a high level of carboxylesterase activity enables rapid cleavage of AM-dyes. As the concentration of free Ca^{2+} in the ER is known to be in the range of 100 - 800 µM, the non-ratiometric Ca^{2+}-indicator dye Fluo-5N/AM is particularly useful for non-disruptive, esterase-based loading. This probe has a KD for Ca^{2+} ~90 µM. Because of its low affinity for Ca^{2+}, the small amounts of Fluo-5N that may remain in the cytosol will not obscure visualisation of the ER Ca^{2+} dynamics (Figure 2A).

The most important advantages of the TED strategy have been verified [1, 2]: (1) The method is non-disruptive and the intracellular environment remains functionally unaffected. (2) Cells that are primarily unable to activate low affinity indicators can be loaded with sufficient amounts of indicator dye. (3) Direct Ca^{2+} release events are not obscured by an increase of $[Ca^{2+}]$ in the cytosol. (4) Stable expression of the targeted esterase activity facilitates the use of indicators with different biophysical properties. (5) High amounts of Ca^{2+} indicator in the ER enable high resolution imaging at low laser intensities, thus reducing phototoxic effects. (6) Direct analysis of Ca^{2+} in the store-depletion transients in the presence of extracellular Ca^{2+} is possible, thus not modifying cell functions dependent on extracellular Ca^{2+}. (7) The direct ER-Ca^{2+} measurements do not require sophisticated experimental setups and are easy to analyze. (8) The method is not restricted to Ca^{2+} indicators, but applies to esterase-sensitive indicators in general.

Recent developments in TED

a) Simultaneous imaging of the ER structure and changes in luminal Ca^{2+}

To further improve this method, a new construct containing a fusion protein of the core element of CES2 and the red fluorescent protein TagRFP-T, was cloned (Figure 2B). The rationale was to allow co-imaging of the red CES2 fusion protein as an ER structure marker together with Fluo5N/Ca^{2+} complexes. TagRFP-T was recently described as an appropriate red fluorescent fusion partner for subcellular targeting [80]. Furthermore, the ER retention and retrieval motif of CES2 was exchanged by a traditional KDEL motif, which has been proved to lead to retention of protein in the ER [81, 82].

Immunostainings of the Red-CES2 reveal its ER targeting and the protein became visible in the perinuclear ER, in dendrites and axons

(Figure 3). To demonstrate functionality of the Red-CES construct, we compared lentivirally expressed CES2 [1, 2] and Red-CES.

As shown in Fig. 4, both Red-CES2 and Fluo5N/Ca^{2+} complexes show ER localization (Figure 4A and B) and changes in luminal Ca^{2+} can be recorded. Here, as an example, the removal of extracellular Ca^{2+} is sufficient to cause a loss of luminal Ca^{2+} via an unknown "leak mechanism" (Fig. 1) and the plasma membrane. Interestingly, the ER lumen replenishes luminal Ca^{2+}, when Ca^{2+} is re-added from outside the cell, without any stimulation. When Carbachol, an agonist of the metabotropic, muscarinic acetylcholine receptor is applied by rapid perfusion, an ER depletion signal is visible in the absence of extracellular Ca^{2+}. Surprisingly, now, the addition of extracellular Ca^{2+} induces a fast refilling of the luminal store (Figure 4C-F).

b) TED in astrocytes

Metabotropic ER Ca^{2+} signaling is of extraordinary importance for glial communication. The TED method shows an extraordinary performance in cultured astrocytes (Fig. 5), thus opening the possibility to address ER Ca^{2+} release and re-uptake in glial cells directly and to use this technique for the analysis of physiological hallmarks of neural stem cells with a glial phenotype.

Outlook: Ca^{2+} homeostasis in neurological disorders

Clinically, imbalanced Ca^{2+} homeostasis is seen in neurological disorders such as Alzheimer's [83], Huntington's disease [65] and Parkinson's disease [66]. The "Ca^{2+} hypothesis of Alzheimer's" signifies that amyloid metabolism results in an upregulation of Ca^{2+} due to increased entry of Ca^{2+} through the plasma membrane and an elevated sensitivity of channels, which release Ca^{2+} from the ER. This deregulation of Ca^{2+} might cause the learning and memory deficits that occur early during the onset of Alzheimer's disease. In Huntington's and Parkinson's diseases, mitochondrial dysfunction results in cellular damage as mitochondria are involved in diverse cellular key processes. Dysbalance in Ca^{2+} buffering (reviewed in [84]) and Ca^{2+} overload are discussed as possible causes for the mitochondrial dysfunction. However, it remains unclear, whether the additional Ca^{2+} is due to more Ca^{2+} influx through the plasma membrane or due to a deregulation of the ER Ca^{2+} release and reuptake. We are now on the way to investigate some of these questions with TED and we hope that the scientific community will profit from our functional TED biotools. It will now be a challenging endeavor to exploit the various signaling pathways involved in cellular Ca^{2+} homeostasis.

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Functional Chromosomal Dynamics: Towards Dissection of Discrete Chromatin Domains

Pavle Krsmanovic

The initial delineation of chromatin to euchromatin and heterochromatin, largely based on their apparent differences in staining properties in electron microscopy, implied that chromatin exists in two different states, as either active or inactive, respectively. Such model of organisation of nuclear DNA material during the cells' interphase implied that the bulk chromatin is predominately static. However, in a recent publication, Thomas Cremer and colleagues have challenged such a paradigm, suggesting that individual chromatin domains are not static but could rotate within the nucleus. Hence, these movements would potentially lead to the formation of novel interaction sites [1]. In a series of recent manuscripts published in Molecular Cell, Cell and Nucleus, several groups reported chromatin to be rather dynamic during different stages of the cell cycle, as well as during development. The state-of-the-art techniques employed by these groups also reflect the advance and development of new research technologies. These techniques, such as DamID [2], allowed the respective groups to analyse the nuclear proteome and chromatin structure on larger scale.

The dynamic nature of chromatin is indicative from its changes through the cell cycle alone. Some of the structural factors located at the mitotic chromosomes were also recently found to be involved in their restructuring through different stages of the cell cycle. Those factors include proteins located on chromosome kinetochores. They are also parts of the histone modification complexes [3], indicating their versatile character as

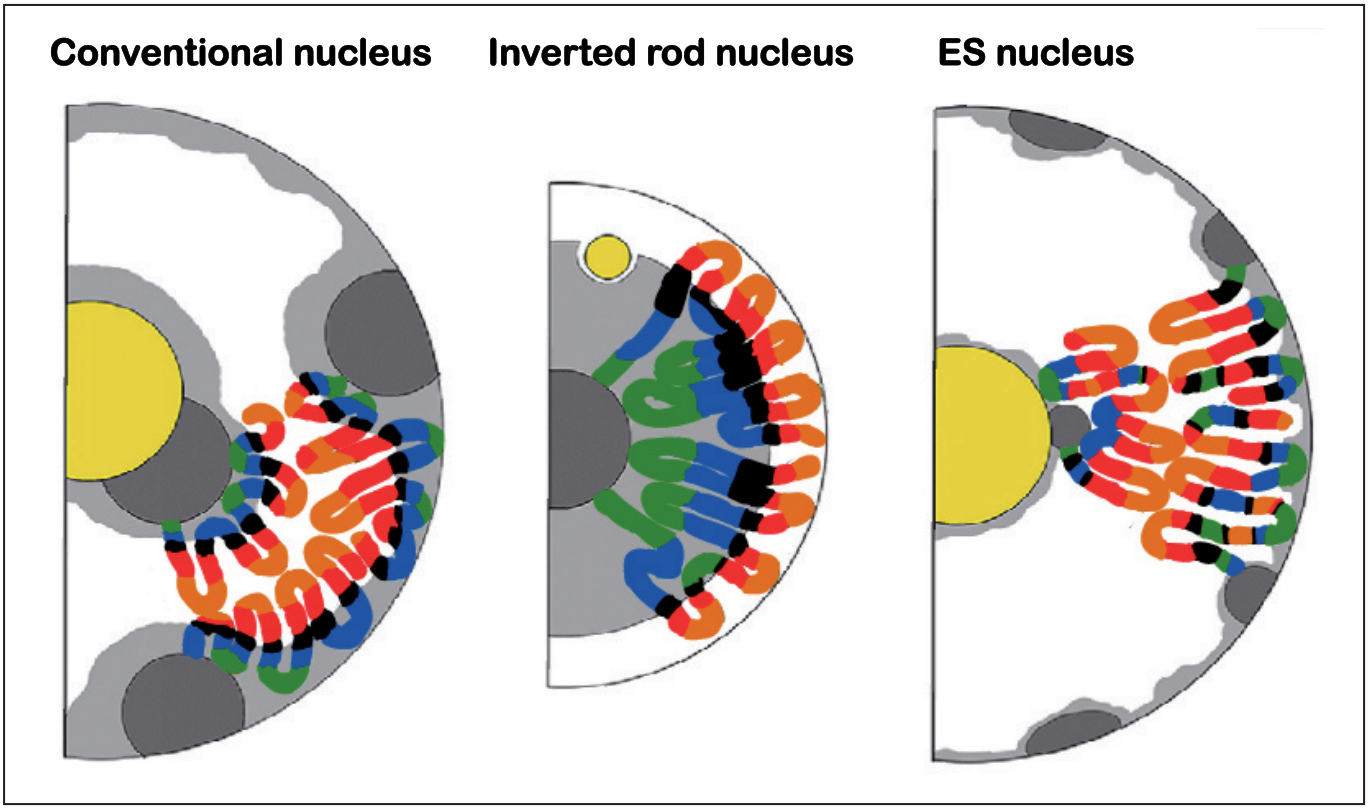

Fig. 1.: Hypothetic scheme of chromatin packing in cells with conventional and inverted nuclear chromatin organisation, as well as pluripotent (ES) cells (modified from [9]). The five different chromatin types (green, blue, black, red and orange) are included (extrapolated on from [7]). Dark grey: chromocentres; light grey: heterochromatin; white: euchromatin; yellow: nucleolus.

well as plasticity in function on chromosomes. During interphase, some factors which are not involved in condensation of chromatin were found to function in its organisation. Namely, the structural components of the nuclear pores, nucleoporins, have been demonstrated to play a role in chromatin reorganisation and coordination of gene expression [4]. Using chromatin immunoprecipitation, Martin Hetzer and colleagues have demonstrated that the DNA packed in chromatin interacts with these structural components of the nuclear envelope. In another study focusing on DNA interactions with nuclear pore components, Maarten Fornerod and colleagues have reported on interactions of nucleoporins with areas of chromatin containing developmental and cell-cycle regulator genes [5]. Using the DamID technique, they have shown that nucleoporins appear to stimulate expression of the respective genes, indicating that chromatin reorganisation is concomitantly occurring with cell maturation in development of an organism.

With respect to the structural reorganisation and packaging of chromatin, the nuclear chromatin interactions with the lamina have been in the focus of Bas van Steensel and colleagues. Applying DamID, they have determined that chromatin anchoring to the lamina actively reorganises during multiple stages of mammalian development [6]. As positioning genes relative to the nuclear lamina is an indicator of the extent of their activity, such structural changes in the chromatin organisation have clear consequences for the activity of genes in the respective areas of chromatin. Accordingly, as chromatin reorganisation is perceived as a vital process during cell maturation and differentiation, the dynamics of its remodelling are slowly getting into the main focus of developmental biology. Last but not least, looking back to the initial division of chromatin into heterochromatin and euchromatin, it is becoming increasingly apparent that such a binary classification might just reflect the initial availability of basic tools and techniques in molecular life sciences. To further dissect the chromatin states, Bas van Steensel and colleagues have once again applied the DamID method and generated DNA binding maps of over 50 different chromatin components. Using bioinformatic analysis of the data they have obtained, they have determined that chromatin consists of five different types (figure 1), based on groups of functional factors located on these particular domains [7]. The reported chromatin analysis and classification represents a giant leap toward further delineating the "classical" chromatin domains. Thereby, one could see the discrete nature of chromatin previously observed and subsequently described as crude and/or largely temporal. Such robust classification has reflected the cumulative functional status of the chromatin or merely represented a snapshot of its activity rather than depicted its dynamic and fluctuating character.

One of the exciting examples from an evolutionary perspective, of how the entire chromatin reorganisation is modified during development to the need of some organisms has been previously reported for the retina cells of nocturnal mammals [8]. These authors reported that the positioning of heterochromatin and euchromatin in retina cells is inverted with respect to the nuclear envelope. In this study, molecular biology and biophysics have merged to impressively show how heterochromatin may serve as an optical lens for nocturnal animals in order to catch even the last photon. Obviously, the position of heterochromatin changed from the periphery to the center of the nucleus. Ergo, the importance of chromatin reorganisation has been followed up not only through organisms' development, but also through their evolution.

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All in the name of actin

International Meeting on Actin Dynamics, Jena 2010

Eugen Kerkhoff, Theresia Stradal, Klemens Rottner

Inspired by the very successful Young Scientist Meeting of the *German Society for Cell Biology* (DGZ) on "Imaging Cell Migration" in Munich 2009, where outstanding international experts and as special guest Nobel laureate Roger Tsien gave highly motivating and sparkling presentations to an audience of young and motivated scientists, the former DGZ-president Reinhard Fässler suggested a new meeting format. He proposed an international meeting on a current scientific hot spot, with the leading scientists of the field as invited speakers and free access for young researchers.

The first meeting of this novel format was held this year in Jena on "Actin Dynamics". The conference was hosted at the company campus of *Carl Zeiss Jena* and its associated Fachhochschule, a venue with more than 150 years of history in manufacturing microscopes. Up till today, high end optical instruments of all kinds are manufactured on this campus, which are instrumental for driving progress especially, but certainly not only in modern cytoskeletal research. The conference was overshadowed by the sudden death of the long-term DGZ-member and former vice president Jürgen Wehland, whose contribution to the community both in Germany and worldwide cannot be overestimated. This became evident last not least through the condign expression of personal condolences by his friends, and former and present DGZ- presidents, respectively, Reinhard Fässler and Harald Herrmann (see also Cell News 3/2010).

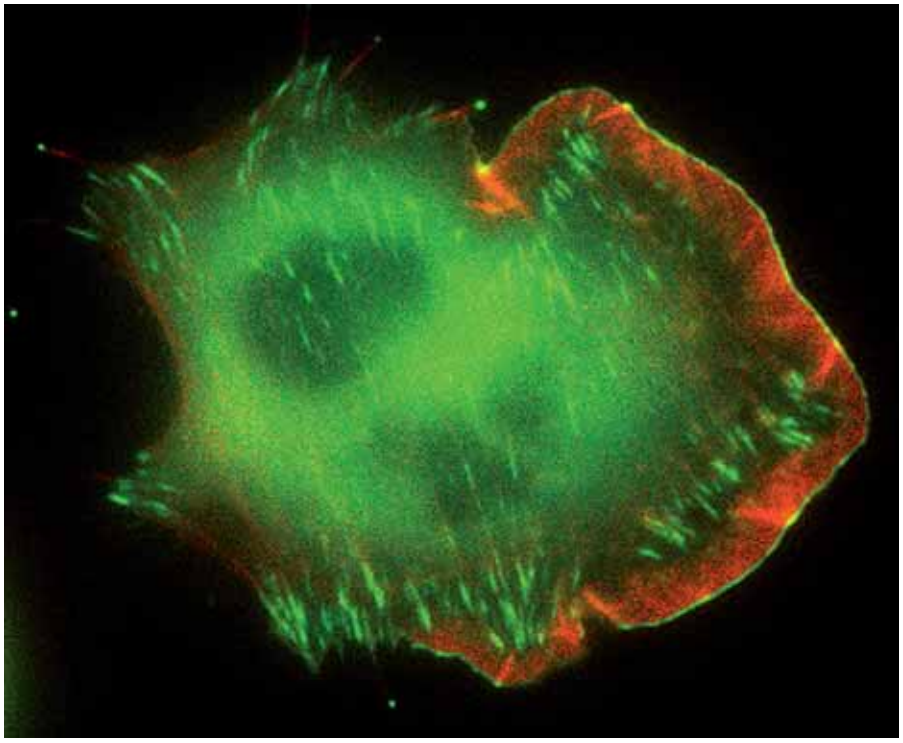

Fig. 1.: Motile melanoma cell co-expressing EGFP-VASP (green) and mCherry-actin (red). Image captured on Demo-instrument from Intelligent Imaging Innovations GmbH (3i), Göttingen, Germany.

The success of the meeting surpassed our expectations and that of the international actin community. Researchers from diverse sub-disciplines of the field came together in a completeness rarely seen before, and celebrated a scientific gala of impressing research, enthusiasm and friendship. 250 scientists from all over the world mixed up with the active German actin community, many of which are society members, and listened to as many as 38 oral presentations, and discussed 118 posters. This was outstanding in many respects. Admittedly, the programme was not only fascinating and overwhelming, but also intense and exhausting, clearly reflecting the feedback obtained from numerous participants. And we have all learned a lot!

The dynamic assembly of actin monomers into filaments and the continuous turnover of these filaments are vital requirements of nearly all cell biological processes (see e.g. articles in Cell News 3/2010 by Dieter O. Fürst and Walter Witke). The tremendous increase in information provided by the genome projects throughout the kingdoms in combination with technical breakthroughs e.g. in the visualization of protein dynamics in live cells or the characterization of individual proteins and protein complexes in cell-free systems has contributed to the emergence of multiple layers of complexity when thinking about

actin dynamics. The actin field has recently experienced the discovery of many new structures and functions, and at the same time an explosion of efforts to understand the mechanisms regulating all these functions. We are convinced that the complexity of how to build, cut, bundle or crosslink actin filaments is not only necessary for structural diversity, but also for maintaining the flexibility and effectiveness in reacting to changes in environmental conditions. Why is actin of such fundamental importance? And why are we experiencing a renaissance in interest on actin filament turnover and its associated regulators? We think there are multiple reasons for this, many of which have become apparent when following the program of our Jena conference.

First, the community has discovered different groups of novel actin binding proteins, which can catalyse the "birth", called nucleation of actin filaments (for a recent review see Campellone and Welch, 2010). Second, the more we know the less we can conceive the interrelationships and surprising connections between different actin binding proteins, structures and pathways. Third, recent developments in imaging of individual filaments or molecules both *in vitro* and *in vivo* have furthered our understanding of the molecular mechanisms of polymerisation of actin filaments and large actin filament assemblies. Fourth, physicists and mathematicians have begun to perform experiments to substantiate the biologist's simplified and naïve intuitions with quantifiable numbers and models (Fletcher and Mullins, 2010; Mogilner, 2009; Schaller et al., 2010). Fifth, the relevance of the impact of novel insights in actin dynamics on human disease has been becoming increasingly evident. Relevant human model pathogens mimic actin nucleators or abuse the actin cytoskeleton for their own needs (Haglund et al., 2010; Hamon et al., 2006; Rottner et al., 2005). And last, not least, while actin and associated motors are essential for most motile processes and drive morphological plasticity in eukaryotic life, bacteria have also evolved actin-like proteins, capable of forming filaments, and operating e.g. in cell shape maintenance or plasmid segregation upon replication (Graumann, 2009). Should we go on? In conclusion, studying and thinking about actin dynamics is more exciting than ever - and it is pleasing to the eye (Figure 1).

Although the discovery of the first, best characterised and most famous of the nucleators, the Arp2/3-complex, now already dates back more than 15 years (Machesky et al., 1994), researchers keep surprising us with the discovery of yet another of its activators (reviewed in Rottner et al., 2010), the most recent examples being WASH (Linardopoulou et al., 2007), WHAMM (Campellone et al., 2008), and JMY (Zuchero et al., 2009). However, Arp2/3-complex is only one part of the story, as eukaryotes have also evolved the formin family of proteins which can nucleate and elongate actin filaments by a totally distinct mechanism (Chesarone et al., 2010; Schonichen and Geyer, 2010). Many of us were convinced for times that actin filaments must be nucleated by either one of these two mechanisms. However, the community was amazed again only a few years ago when yet another group of proteins, Spir and friends (Ahuja et al., 2007; Chereau et al., 2008; Quinlan et al., 2005), harbouring up till 4 distinct actin binding sites were reported also to be able to catalyse actin filament assembly simply by bringing together actin monomers in proximities close enough for igniting nucleation.

Inspection of the scientific program reveals that many of the discoverers of these different molecules have honoured the conference as invited speakers: e.g. Laura Machesky (Glasgow), who discovered the Arp2/3-complex when in the lab of Tom Pol-

Fig. 2.: Coffee break discussions.

lard (Machesky et al., 1994), Margot Quinlan (Los Angeles) and Dyche Mullins (San Francisco), who discovered Spire as a nucleator in collaboration with one of the meeting organizers and authors of this article, Eugen Kerkhoff (Quinlan et al., 2005); Britta Qualmann (Jena), who discovered Cobl (Cordon-bleu) as the second prominent member of the new class of WH2 domain-containing nucleators (Ahuja et al., 2007); Roberto Dominguez (Philadelphia), who discovered Leiomodin as a nucleator in muscle (Chereau et al., 2008), together with yet another speaker, Pekka Lappalainen (Helsinki); and last, not least, Bruce Goode, who recently showed the tumor suppressor protein adenomatous polyposis coli (APC), a long-known microtubule-associated protein to also be capable of actin filament nucleation and to synergize in this process with the formin mDia1 (Okada et al., 2010).

The meeting was kicked off by two keynote lectures: Marie-France Carlier, who pioneered the reconstitution of actin-based motility with purified proteins in vitro, and is one of the most influential scientists in the field worldwide (Carlier et al., 1997; Loisel et al., 1999; Romero et al., 2004), set the stage by providing a general overview of actin-based motility followed by exciting novel insights into the intricacies of Cobl and Spir biochemistry. Dyche Mullins, yet another of the numerous, famous former Pollard collaborators among the list of speakers, changed gears and discussed his exciting works on prokaryotic actin homologs (Campbell and Mullins, 2007; Garner et al., 2007; Polka et al., 2009).

Fig. 3.: Can you lead me through your poster?

The rest of the meeting was structured into 11 sessions covering different topics or sub-disciplines in the field, ranging from biochemistry and structural biology, signal transduction, adhesion or host-pathogen interaction to modelling and physics. We refrain from providing an exhaustive list of highlights presented in each of the 38 oral presentations, which would simply be too many. However, it was more than fascinating to watch a magnetised audience even in the very last talk on Saturday evening to be glued to the lips of Daniel Fletcher (Berkeley) elaborating on how actin filament elasticity can influence Arp2/3-complex interaction. So the quality of presentations did surely not decline as the conference progressed, and besides excellent science, Saturday speakers were additionally challenged by having to throw in unrelated words into their lectures, as initiated by Michael Way (London), and the audience additionally entertained by the dry American humour of John Leong (Worcester). We also experienced a session on cellular protrusions: lamellipodia, filopodia and blebs, apparently operating in different modes of motility (Charras and Paluch, 2008; Chhabra and Higgs, 2007; Lammermann and Sixt, 2009). Jan Faix (Hannover) and Ewa Paluch (Dresden) presented electrifying presentations on functions and molecular regulation of filopodia (Breitsprecher et al., 2008) and membrane blebs (Tinevez et al., 2009), respectively. And Vic Small (Vienna) challenged the audience with his provocative (EM-)views of branchless actin filament networks in lamellipodia of different migrating cells. Based on his most recent ultrastructural work (Urban et al., 2010), he calls for reconsideration of current models of Arp2/3-complex-dependent actin filament assembly in cells (Small, 2010a), which continues to provoke a lot of opposition and thought, not only in the audience, but also in the literature (Firat-Karalar and Welch, 2010; Higgs, 2010), which is bravely fought back (Small, 2010b). The vivid debate illustrates not only that apparent gaps between the *in vitro* and *in vivo* worlds are still to be closed, but also that the 'dissenting voice' (Insall and Machesky, 2009) is indispensible for progress. If *in vivo* means tissues, organs or whole animals, the mentioned gap is even wider, as emphasized e.g. by Walter Witke (Bonn), who discussed differential functions of murine cofilins in spite of indistinguishable biochemistry (see e.g. Rust et al., 2010).

This was a large meeting, with great speakers and posters and lively discussions during coffee breaks (Figures 2 and 3). We would have loved to get one or the other additional speaker, for instance Michael Rosen (Dallas), who has just published yet another fascinating crystal structure, this time of the WAVE-complex (Chen et al., 2010); he promised to come next time! Besides the presentations by invited speakers, another specific highlight of this conference was that almost every other attendee presented a scientific poster (see above), and all of them being of highest quality. The DGZ generously awarded as many as 10 presenters with a poster prize, who were selected by an independent committee consisting of 12 invited speakers. Prize winners were Martin Bergert (Dresden), Dennis Breitsprecher (Hannover), Thomas Fischer (Würzburg), Nicole Koch (Jena), Kai Murk (Braunschweig), Margit Oelkers, (Braunschweig), Stéphane Romero (Paris), Andre Schönichen (San Francisco), Hao Ran Tang (Glasgow) and Marlene Vinzenz (Vienna). A special prize donated by the Journal *Cytoskeleton* was awarded to Gergana Gateva (Helsinki).

It is the unanimous opinion of all participants that the DGZ has struck the needs of the actin community with its new meeting

format. As a key element of the cellular cytoskeleton and a central function in cell biology, research on actin dynamics is of general interest to virtually all DGZ members. We are proud to announce that the *German Society for Cell Biology* will therefore support the international conference on "Actin Dynamics" as a bi-annual meeting over the next years, and we are thus heading for the next meeting in 2012.

In addition, the forthcoming meetings are planned to be co-organized by the DFG priority programme SPP 1464 "Principles and Evolution of Actin Nucleator Complexes", which started this year. The DGZ and the SPP 1464 will also cooperate in organizing the *Regensburg International Summer School on Actin Dynamics*, where 30 students will spend a week together with international experts of the actin field, being trained with lectures in the morning and practical courses in the afternoon. All these new activities of the German actin dynamics community will be made public and continuously updated on a new web forum starting in February 2011 under the following address: www.actindynamics.com. So please keep an eye on exact dates and venues of our future meetings, and we all hope to see you in 2012!

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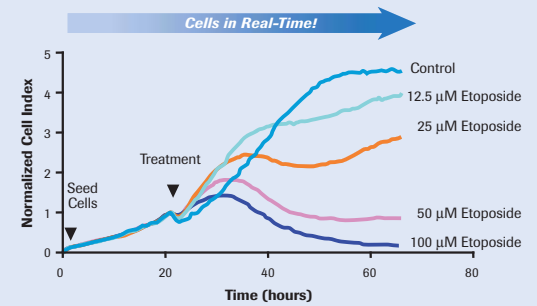

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