# Cell News

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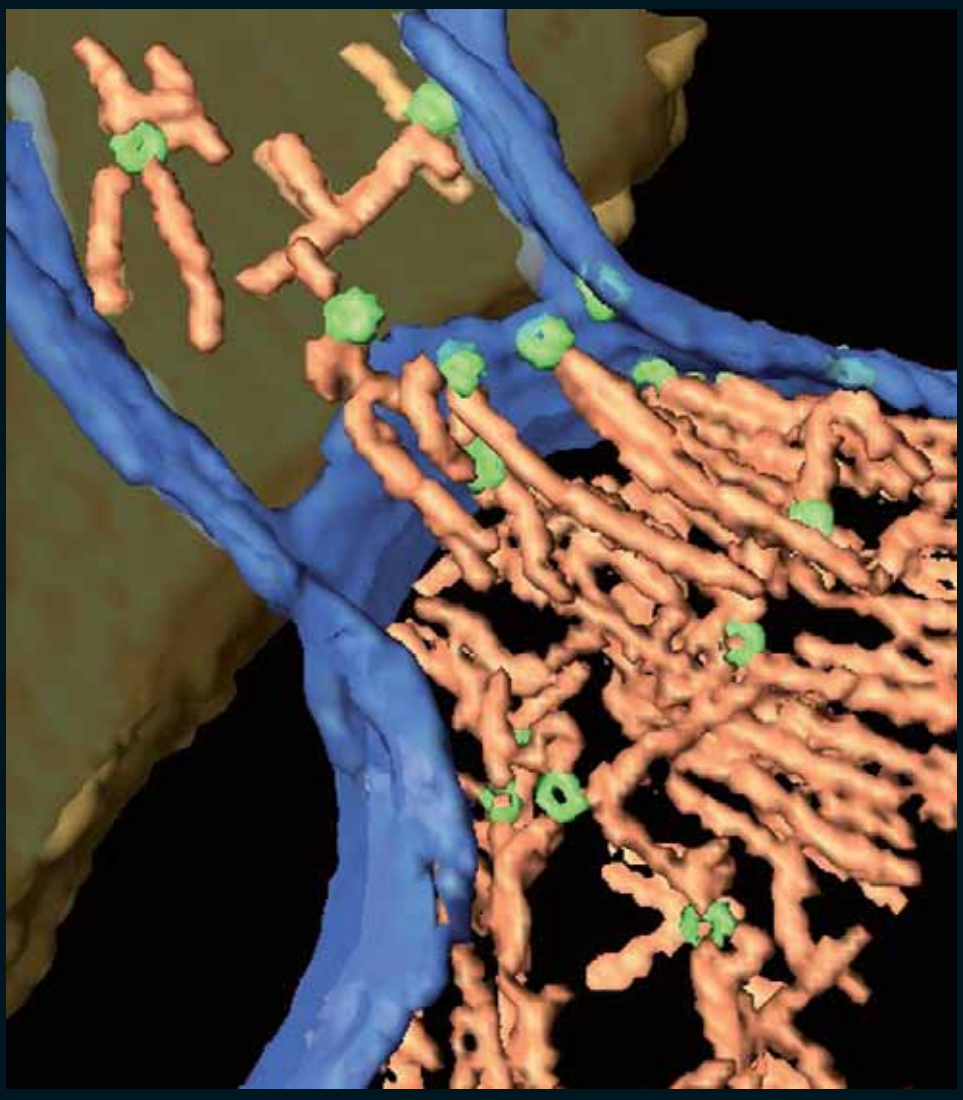

DGZ

# IHRE WELT VON MORGEN SOLL NOCH BESSER WERDEN. DARAN ARBEITEN WIR.

Kinder sehen die Welt mit anderen Augen: Gegen Krankheiten müsste man einfach eine Medizin erfinden, die alles heilen kann – und Autos sollten nur noch mit Luft fahren. Um diese Technologien von übermorgen zu entwickeln, brauchen wir heute schon große Fortschritte. BINDER Konstantklima-Schränke tragen einen wichtigen Teil dazu bei: sie schaffen einzigartig konstante Umgebungsbedingungen, um darin sensible Gegenstände aufzubewahren oder Produkte auf Herz und Nieren zu testen. Alles mit dem Ziel, die Welt noch sicherer und gesünder zu machen. Wie das genau funktioniert, erfahren Sie unter www.binder-world.com.com

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**We encourage our members to submit discussions of articles that provide fundamental progress in cell biology.**

**Cover image:**
Surface rendering view of a 480 nm x 480 nm area of the cytoplasmic aspect of the plasma membrane of REF52 fibroblasts, depicting adhesion-related particles (light green) connect the plasma membrane (blue) with cytoskeletal filaments (reddish brown), most likely representing actin filaments (I. Patla, T. Volberg, N. Elad, V. Hirschfeld-Warneken, C. Grasshoff, R. Fässler, J. P. Spatz, B. Geiger and O. Medalia (2010) Dissecting the molecular architecture of integrin adhesion sites by cryo-electron tomography. Nat. Cell Biol. 12, 909-915).

# A Great Loss for our Society

During the preparations for the first "International Meeting of the German Society for Cell Biology (DGZ)", we came to know that our member, colleague and friend Jürgen Wehland had died on August 16th. Jürgen was a long-term member of the DGZ and served our society as vice president from 2006 to 2010. It was actually at the council meeting of the society in November 2009, organized by Jürgen, that we decided to try out a new type of meeting. Jürgen very strongly supported the idea of giving members of the society the opportunity to organize meetings with a distinct focus on one special topic and with an international scope. Having organized the Annual Meeting in 2006 (Braunschweig) himself, Jürgen knew that the traditional meeting format with broad topic coverage is to some extent a compromise in order to give scientists of all fields of cell biology a chance to regularly present their data. "Actin Dynamics", a first suggestion made by Reinhard Fässler, the president at that time, was agreed on and subsequently organized by Theresia Stradal, Klemens Rottner and Eugen Kerkhoff. During the meeting, several of the invited speakers savoured and honoured the many essential contributions to the field that Jürgen has made.

In the name of the society, I wish to express the sympathy of all our members to the family of Jürgen Wehland. Moreover, two long-term collaborators and friends of Jürgen, Volker Gerke and Klaus Weber, have written an obituary to remember him as an outstanding member of the scientific community.

## First Impressions from the International Meeting

From September 15th to 18th, 250 participants met at the Carl-Zeiss facilities in Jena, to discuss the latest results in the actin field. In the next issue of "Cell News", the organizers will give a detailed report. It is fair to say that the meeting was a big success. The discussions were intense such that the allocated time never sufficed – luckily enough the chairpersons were indeed relaxed and discussions went on. The proportion of young scientists present was very high so that the impact made by the top-notch international speakers on their future research cannot be overestimated. Vice versa – the speakers were all enthusiastic about the outcome of the meeting and many stated they would be happy to have it regularly happen in Jena. From this experience, we expect that the next one on the "The Spider's Web: How microtubules organize cellular space", organized by Ralph Gräf and Manfred Schliwa, to be held in Potsdam in June 2011, will be a success as well. For 2012, Thomas Magin (University of Leipzig) suggested to organize a meeting on the various functional aspects of epithelia. Nevertheless, we would be happy to receive more suggestions for future meetings. Because of the big success of the first meeting, we do not rule out the possibility to support more than one "Special Interest"-type meeting per year.

## Support for Junior Scientists

For 2011 and 2012 we are asking our members (or future members) for suggestions to organize a young scientist meeting. The format is such that two organizers invite 14 speakers and give talks themselves. These lectures will be complemented by poster presentations of young scientists, master students, PhD students and young Post-Docs. From their contributions, 12 will be selected for a "Short Talk"-session. In the past, about 35 to 50 scientists have taken part in a two and a half day meeting at the Carl-Zeiss facilities (when available). Attractive potential subjects could be on "Noncoding RNAs in development", "Cancer Stem Cells" or "mTOR's role in ageing and cancer". Sure enough, here we would appreciate receiving feedback from the members.

During the council meeting, Eugen Kerkhoff reported on the outcome of a summer school on actin dynamics that took place immediately before the Jena meeting. Hence, the organizers took advantage of the fact that several of the speakers would agree to take part in that enterprise including Margot Quinlan (UCLA, USA) and Henry Higgs (Dartmouth Medical School, USA) as teachers. The success of this summer school was exceptional, and Eugen suggested therefore that we should draw the attention of members to the possibility to organize summer schools together with the DGZ.

In order to help students to attend the annual meetings, we will reduce the registration fee for student members. Moreover, we will support a limited number of students by travel fellowships upon application. We trust that groups with solid budgets will not apply, however, this offer is meant such that no student with significant results in her/his thesis is not able to come simply because of lack of money. The financing will be done using surplus money that has been generated during the recent annual meetings.

## The Next Annual Meeting

Next spring, from March 30th to April 2nd, we will have the Annual Meeting of the society in Bonn. The organizers are Walter Witke and Dieter Fürst and they have assembled a very attractive program. This includes an impressive list of chairs, which all are leading experts in their field. An overview is given on page XX, and we expect that we will be able to present a complete program in the next issue of "Cell News" in December. To get a flavour of what is going on in the laboratories of the organizers, we present in this issue the "Research News" provided by them. Furthermore, the Chief Operating Officer of the society, Ralph Gräf, introduces the topics of his

research. Last but not least, we introduce Roland P. Piekorz from the Heinrich-Heine University of Düsseldorf with a research profile.

### Opening the Society

During the meeting in Jena we were asked several times by "foreigners", if they were allowed to become regular members of the German Society for Cell Biology. Our answer was, of course, positive. Saying yes, however, made it at the same time clear that the language of our newsletter should be English. For scientific contributions in "Zellbiologie aktuell" it was consistently English for the last years as it always was for the annual meetings. Hence, we now will "internationalize" the newsletter from the first to the last page. When we were looking for an appropriate title, translating "cell biology update(d)", Manfred Schliwa made the suggestion "Cell News". The council easily agreed easily, and here we are presenting "Cell News". With this new make up we wish our members to go actively "fishing" in their institutions for new members. Sometimes, a brief mentioning to our foreign colleagues that they can become members and that there is a journal of the society they can read and understand, will have a great impact: They will feel that they are wanted, and we will gain new colleagues that together with us will undertake the enterprise of research in modern cell biology.

*Harald Herrmann*

# Jürgen Wehland (1952 – 2010)

Jürgen Wehland died suddenly on August 16, 2010, during a sailing holiday with colleagues in Sweden. We have lost a long time friend and a scientific colleague whom we admired and respected and we write this tribute to Jürgen with great sadness.

Well known for his work on cytoskeletal dynamics Jürgen was a central figure in German Cell Biology and a driving force behind the DGZ. It is thus more than appropriate that the DGZ journal pays tribute to Jürgen's achievements.

Jürgen studied biology in Göttingen and already in April 1975 as an undergraduate he was drawn by his desire to do cutting edge science to the Max Planck Institue (MPI) for Biophysical Chemistry. His interest in cell biology, his ability to observe and understand how cells change their shape and organization in different environments, was best fulfilled in the (at that time) new Department of Biochemistry and Cell Biology that had introduced novel fluorescence based techniques and highly specific antibodies to study such events. These early years in cell biology shaped Jürgen's scientific life. He remained fascinated by the cytoskeleton, and by its different facets and dynamics. And this is where he contributed most to our current knowledge. The actin cytoskeleton dominated his PhD years at the MPI in Göttingen. Jürgen mastered the – again at that time cutting edge – technology of microinjection and introduced labelled phalloidin and actin to study actin dynamics in living cells. His Postdoc then took him to the group of Ira Pastan at the National Institutes of Health (NIH) where Jürgen contributed seminal papers to the area of membrane transport, again using microinjection approaches and ultrastructural analyses to analyze clathrin-coated pits in endocytosis and the Golgi exit and exocytosis of Vesicular Stomatitis Virus. The years at NIH and his return as a research assistant to the Max Planck Institute in Göttingen in 1983 also marked a switch in his interest to a second cytoskeletal filament system, the microtubules, and its relation to the positioning of the Golgi. Inspired by a monoclonal antibody against tyrosylated alpha tubulin, Jürgen set out to understand the dynamics and function of this special form of posttranslationally modified microtubules. In series of elegant and insightful papers Jürgen described the reversibility of this modification and its relation to microtubule stability and he identified, purified and characterized the enzyme responsible for tubulin tyrosination. Readers of the DGZ are encouraged to look at these papers of Jürgen's, mostly published in the Journal of Cell Biology and the EMBO Journal, to appreciate the clarity and beauty of the cell biology and biochemistry involved. Throughout his career Jürgen remained attached to this enzyme, the tubulin-tyrosine ligase (TTL), and by generating TTL-deficient mice he was able finally to reveal a crucial function of reversible tubulin tyrosylation in neuronal development and the recruitment of microtubule plus end proteins.

Following the Göttingen years and initiated by his move as a group leader to the German Research Center for Biotechnology (GBF) in Braunschweig, now the Helmholtz Center

for Infection Research (HZI), Jürgen again explored new ground. In an environment concerned with microbial genetics and pathogenesis, his interest in the mammalian cytoskeleton took another turn. Jürgen merged the two fields and as one of the first in a now large community of scientists he introduced cell biological approaches and thinking into the area of bacterial pathogens. In a series of key experiments he and his group showed that the ActA protein of Listeria monocytogenes plays the central role in subverting the host cell's actin cytoskeleton for the purpose of the bacteria. By recruiting actin regulators such as the Arp2/3 complex and members of the Ena/VASP family, ActA initiates actin nucleation at the bacterial surface and thereby the formation of actin comet tails that propel Listeria and allow bacterial cell-to-cell spreading. These results from Jürgen's group in Braunschweig done in part with his long term collaborator Trinad Chakraborty in Giessen not only provided a molecular explanation for the intracellular dynamics of certain pathogenic bacteria such as Listeria and Shigella, they also introduced the use of pathogens as tools to study the cell biology of the host and thus had groundbreaking character. The EU Consortium VIRLIS in which Jurgen, Trinad Chakraborty and Pascale Cossart were among the partners was focussed on Listeria and in 2007 was awarded the prestigious Descartes prize for transnational collaborative research.

Jürgen will be remembered not only for his scientific contributions but also for his many other talents. He was a visionary, inspiring and open-minded leader who assembled a group of talented and highly motivated young scientists in Braunschweig. As the Head of the Division of Cell and Immune Biology and later as the Scientific Director of the HZI Jürgen devoted much of his time to supporting young scientists. Researchers in his own group felt the freedom of research yet still the wealth of support by Jürgen backing them. Jürgen applied high standards but those fulfilling these criteria received continuous support. He also played a leading role in the selection of young scientists for the Helmholtz Young Investigator program. His support and help were experienced by young researchers and colleagues not only in Germany but all over the world. One of Jürgen's most asked questions was 'How can I help you?' and when a problem was identified Jürgen put a lot of effort and also sometimes his own resources into providing this help. In the true meaning of the word he was a selfless and generous altruist.

Many of us will also remember Jürgen for his talents in organizing and and reviewing science. He was a driving force behind the change of the former GBF into a new research center focusing on infectious diseases, the HZI, made evident in his appointment as the new scientific directer of the center in January this year. He was also a member of a number of scientific advisory boards, including those of the Max Planck Institute for Infection Biology and the Robert Koch Institute. He served on many committees concerned with evaluating science. He was a familiar face in Bonn at the DFG, where his advice was sought in the Senate Committee for 'Sonderforschungsbereiche' and in the Study Section on Biology, and he also headed the Senate Committee 'Evaluation' of the Leibniz Association. All who know him from these committees or other evaluations will remember Jürgen's deep insight and tough but always fair assessments.

Two matters which were also at Jürgen's heart should not be forgotten: his support of the cytoskeleton community – he was a member of the Governing Board of the European Cytoskeletal Forum – and his devotion to the promotion of cell biological research in Germany. For many years Jürgen was a driving force in the DGZ. He organized the 2006 DGZ Meeting in Braunschweig, and has been a vice president of the DGZ since 2006. It was Jürgen's firm belief that the DGZ is a forum to draw young researchers into this exciting field of science and that the society should provide support and and a network of communication for these young researchers. Finally he was also a strong supporter of the „Arbeitskreis für Zellbiologie und Biomedizinische Forschung e.V" a charitable foundation associated with the HZI and to which donations can be given in memory of Jürgen.

Jürgen died too young. We both have vivid memories of recent discussions we had with him. He was full of energy to start new scientific projects and to lead the HZI in new directions. Jürgen leaves behind his wife Brigitte and two children. Jürgen will be missed as a husband and father, a scientist, a mentor, a colleague and as a friend.

*Volker Gerke, Münster · Klaus Weber, Göttingen*

# 34th DGZ Annual Meeting

## March 30 – April 2, 2011, Bonn

### Organisers: Dieter O. Fürst and Walter Witke

**Wednesday, March 30, 2011**

Opening

Plenary Session 1: **Cell polarity**
Chairs: Elisabeth Knust, Julie Ahringer

DGZ Awards

**Carl Zeiss Lecture: Ueli Aebi (Basel)**

Poster session and Welcome reception

**Thursday, March 31, 2011**

Plenary Session 2: **Cellular mechanics**
Chairs: Jan Lammerding, Sarah Köster

Plenary Session 3: **Cell cycle and cancer**
Chairs: Ingrid Hoffmann, Zuzana Storchova

Symposium 1: **Frontiers in microscopy**
Chairs: Rainer Pepperkok, Werner Kühlbrandt

Symposium 2: **Signalling and Rho GTPases**
Chairs: Alexander Pfeifer, Gudula Schmidt

Symposium 3: **Nuclear envelope and NPCs**
Chairs: Angelika Noegel, Birthe Fahrenkrog

Symposium 4: **Inflammation**
Chairs: Manolis Pasparakis, Jürg Tschopp

Poster session

**Friday, April 1, 2011**

Plenary Session 4: **Cytoskeletal dynamics**
Chairs: Walter Witke, Robert Grosse

Plenary Session 5: **Stem cells**
Chairs: Bernd Fleischmann, Martin Zenke

Symposium 5: **Cellular immunity**
Chairs: Waldemar Kolanus, Klaus Rajewsky

Symposium 6: **Muscle cell organisation**
Chairs: Dieter Fürst, Frank Schnorrer

Symposium 7: **Cellular neurobiology**
Chairs: Christian Steinhäuser, Ampero Acker-Palmer

Symposium 8: **Vesicle trafficking**
Chairs: Volkmar Gieselmann, Paul Saftig

Poster session

Get together

**Saturday, April 2, 2011**

Symposium 9: **Host pathogen interactions**
Chairs: Theresia Stradal, Albert Haas

Symposium 10: **Cell biology of addiction**
Chairs: Andreas Zimmer, Brigitte Kiefer

Symposium 11: **Protein quality control**
Chairs: Jörg Höhfeld, Frauke Melchior

Symposium 12: **Cell adhesion**
Chairs: Reinhard Fässler, Johanna Ivaska

Symposium 13:
**Molecular mechanisms of ageing**
Chairs: Michael Hoch, Christoph Englert

Symposium 14: **Non-coding RNAs**
Chairs: Gerhard Schratt, Elisa Itzauralde

Symposium 15:
**Intermediate filaments: novel functions**
Chairs: Thomas Magin, Rudolf Leube

Farewell

## www.dgz2011.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 grants 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 email until
**31st of January 2011** to

**Deutsche Gesellschaft für Zellbiologie (DGZ)**
**Sekretariat, Frau Reichel-Klingmann**
**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

# 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**

# Binder Innovationspreis 2011

Der BINDER Innovationspreis Preis wird von der BINDER GmbH in 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**

# 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 Adhesome en detail – and again: *Vorsprung durch Technik*

Harald Herrmann

It was a new technique, interference reflection microscopy (IRM), applied to a cell biological problem that enabled Collin Izzard and Linda Lochner to investigate the closeness of the coverslip surface and a living fibroblast crawling on it, way back in 1976 (1). The dark areas, 0.25 to 0.5 µm wide and 2 to 10 µm long, observed at the leading lamellae were termed *focal contacts*. These structures (later also called focal adhesions or adhesion plaques) were actually seen already a year before by Michael Abercrombie and colleagues at the Strangeways Research Laboratories in Cambridge, UK, employing IRM (2). In this latter paper, the authors discussed the biomechanics of a cell moving on a substratum, on the grounds of data generated by electron microscopy and time-lapse cinemicrography.

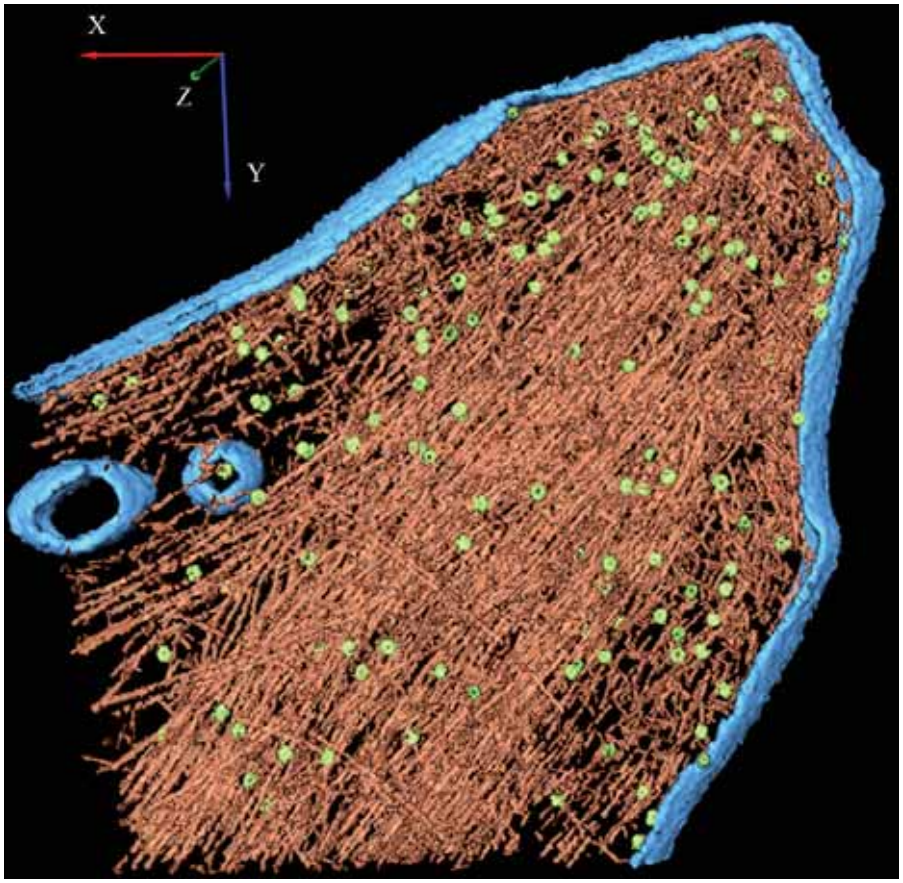


***Fig. 1.***: Cryo-electron tomography of integrin-mediated focal adhesions. This surface-rendering view of the focal adhesion site is taken from the substrate towards the cell interior (see reference 9).

Hence, in their 1971 paper, Abercrombie had summarized the EM data on fibroblast locomotion as follows: *"At certain places at the leading lamella, amounting on average to about 25% of its ventral surface, the unit membrane approaches to within 30 nm of the substratum... The cytoplasm immediately adjacent to these regions of close approach to the substratum, in a zone extending inwards an average of 60 nm from the unit membrane, was distinctly more electron-dense than the rest of the sub-surface cytoplasm... The electron-dense areas, which we term plaques, averaged 1.5 µm in length in longitudinal sections with a maximum recorded length of 14 µm... The plaques seem to be related to the oblique tracts of filaments already mentioned that run from the dorsal to the ventral surface. The ventral end of such a tract has not been clearly shown to merge with a plaque, but there is often a clear impression that it is trending towards one."* Certainly, the observation is clear and the interpretation careful, but sometimes it needs a catchy name to attract appropriate attention.

Not long after these IRM reports, Benjamin Geiger biochemically defined a principal component of focal contacts of molecular weight 130,000 that was associating with the end points of actin filaments, i.e. vinculin (3, 4). These studies started the hunt for the components of the "machinery" behind this important cell-substrate contact device. With the wide use of immunofluorescence microscopy, more and more components localizing to focal adhesion were defined by antibodies generated against isolated focal adhesion components (5). Hence, over time the interaction network described between individual proteins at focal adhesion increased tremendously, such that a few years ago Benjamin Geiger and colleagues noted in a literature survey a breathtaking number of 690 interactions occurring between 156 proteins in a cellular territory referred to as the "integrin adhesome" (6).

In a short overview on how microscopy pushed the cell biology of motility forward, Graham Dunn and Gareth Jones have recently

focussed on various light microscopic methods such as FRAP, FLIP, FLAP, FRET, TIRF and FCS (7). However, cryo-electron tomography is another rapidly developing microscopic technique that turned out to be instrumental to generate the first robust structural data of complex assemblies engaged in cell adhesion and cell motility as well as nuclear transport or protein synthesis (8). In their report - "Dissecting the molecular architecture of integrin adhesion sites by cryo-electron tomography"- Ohad Medalia and colleagues have identified now what represents the basic structural element of focal adhesions (9). The interaction between cell membrane and the actin cytoskeleton is mediated by doughnut-shaped particles with a diameter ranging from 20 nm to 30 nm. The most frequent "nearest neighbour" distances between these particles are in the range of 30 to 70 nm (Figure 1). In particular, the actin bundle shown here is about 220 nm thick, becoming 120 nm at the end of the bundle. In between the membrane and the actin bundle surfaces, the particles are situated. Seemingly identical particles were also found outside of the focal contacts, however, their density was more than 20-fold lower than within the contact zone. They are consistently found 30 to 70 nm above the carbon support coated with extracellular matrix components (the dense zone seen by Abercrombie in electron microscopy). Eventually, the authors discriminate four levels of organization at a focal adhesion site. The outermost layer consists of the plasma membrane containing the "fibronectin receptors", the integrins. The next layer inside harbours the doughnut-shaped particles; they may interact with integrins directly, however, this still needs to be shown. In a third layer, particles, studded with short filaments are found. In the fourth layer, a well organized and bundled actin filament network is covering the contact site. How these four layers are integrated at the molecular level is still elusive, however, new experiments may surely emerge from this report.

A new twist to the story emerged when the authors incubated cells with contractility inhibitors and focal contacts were looked at by correlative microscopy, combining cryo-electron tomography with fluorescence microscopy. After 3 minutes, changes were already apparent such that the diameter of the particles had decreased from 25 to 15 nm, whereas the number and the spacing of the particles remained similar, just like the overall appearance did. In summary, the data indicate that the actomyosin forces at the focal adhesions are mediated by the ***focal-adhesion-associated particles***. The authors estimate that the average tension subjected to every particle – by 2 to 3 actin filaments – is about 20 pN. This amount of force correlates with the amount that can be produced by a few myosin motors on a single actin filament. Hence, the authors describe modules that can be regulated by mechanical force. Moreover, their strategic location makes these modules ideal candidates for taking part in surface mechanosensing.

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# Functional Analysis of Dictyostelium Centrosome-Associated Proteins

Ralph Gräf and Irene Meyer

## Structure and function of centrosomes

The centrosome is a nucleus-associated non-membranous organelle, which constitutes the largest protein complex in a eukaryotic cell. Its enormous size ranging from 0.5 - 1 µm already suggests a differentiated structural composition that becomes evident in electron microscopic and light microscopic analyses. Thus, it is not surprising that isolated centrosomes have been shown to be composed of more than hundred different proteins. This multitude of different proteins appears astonishing in the first glance, since most of the known centrosomal functions are directly or indirectly related to its role as the major microtubule-organizing center (MTOC) in animals, fungi and lower eukaryotes. Accordingly, it plays a key role in all microtubule-dependent processes such as organelle positioning, maintenance of cellular architecture, cell polarization or mitotic spindle organization. The centrosome is also deeply involved in orchestration of mitosis and cytokinesis. Furthermore, its integrity is required for passage through a still largely uncharacterized checkpoint in G1 [1]. A key feature of the centrosome, its duplication once and only once per cell cycle, fascinated early cell biologists such as Walther Flemming and Theodor Boveri, who realized that centrosome duplication is a prerequisite for formation of a bipolar spindle during mitosis [reviewed in 2]. Boveri realized that defects in centrosome duplication leading to supernumerary centrosomes can result in mis-segregation of chromosomes. Yet, recent research has led to a more differentiated view on spindle organization when elegant experiments with Xenopus frog egg extracts revealed that bipolar spindles can also be build in the absence of a centrosome [3]. Here spindle microtubules are organized and ordered in a bipolar fashion by chromatin-associated spindle assembly factors, kinesins and dynein motor proteins. However, in most cell types both centrosome-based and chromatin-based mechanisms for bipolar spindle formation appear to cooperate. There is no doubt that the presence of supernumerary centrosomes due to defective centrosome duplication or cell division promotes formation of multipolar spindles and, thus, unequal chromosome segregation. Supernumerary centrosomes are not only characteristic for tumor cells, they can also be the cause for transformation into the tumorous state [4]. Thus, research on the composition, regulation and biogenesis of centrosomes is of outstanding interest in biomedical research since decades.

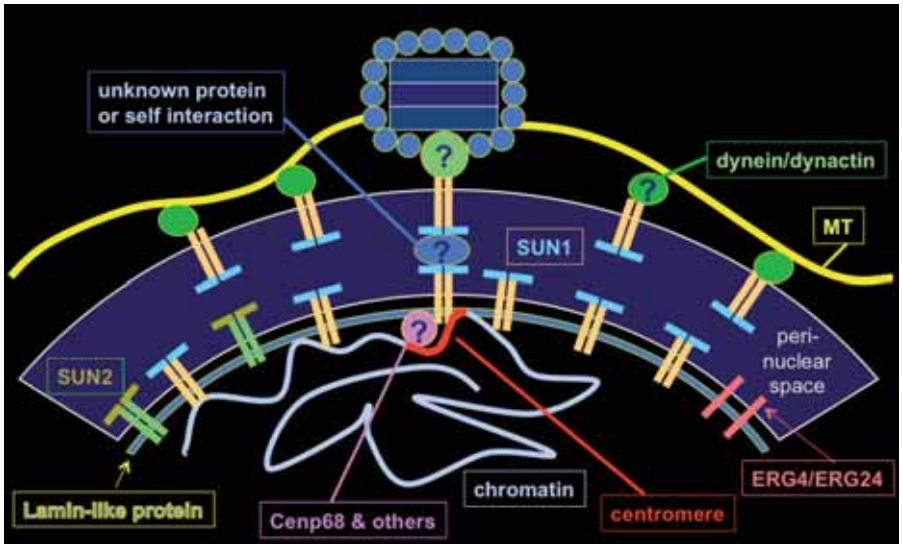


*Fig. 1.*: Hypothetical model of the organization of centrosome/nucleus attachment in *Dictyostelium*.

Despite of the universal function of centrosomes their morphology differs fundamentally in different groups of organisms. Centrosomes in animals and all other organisms bearing cilia or flagellae are characterized by a pair of centrioles. The latter are hollow cylinders composed of microtubules, which are embedded in a so-called pericentriolar matrix (PCM). The PCM is rich in γ-tubulin containing microtubule nucleation complexes. This centrosome type is opposed by the acentriolar centrosomes of fungi and lower eukaryotes like for instance the yeast spindle pole body (SPB, also called yeast centrosome) or the Dictyostelium centrosome (also called nucleus-associated body; NAB). Since the majority of all centrosomal proteins shows no catalytic activity, comparative cell biology of centrosomes in model organisms harboring different centrosome types with an at least partially conserved protein inventory is a useful approach to elucidate centrosomal functions.

Our model organism, Dictyostelium discoideum amoebae, is a lower eukaryote. However, with regard to their cell biology these cells are very reminiscent of amoeboid mammalian cells such as neutrophils or macrophages. Dictyostelium amoebae can easily be cultivated in preparative amounts and are

well suited for microscopy, biochemistry and molecular biology, especially since their genome is completely sequenced and annotated (www.dictybase.org). The Dictyostelium centrosome is composed of a box-shaped core structure with three major, dense layers and an amorphous protein sheath called corona, which surrounds the layered core and organizes the microtubules [5].

## Attachment of centrosomes to nuclei

As in all vegetative cells, the Dictyostelium centrosome is tightly associated with the nucleus and is located at its cytosolic face. This association is based on a physical linkage of the centrosome through both nuclear membranes with the clustered centromeres at the opposing inner side of the nuclear envelope. This linkage is maintained throughout the cell cycle and it is mediated by the Sun1 protein and other proteins most of which remain to be characterized [6,7]. SUN proteins are conserved from yeast to humans and are characterized by a domain (SUN-domain) with similarity to *S. pombe* **S**ad1 and C. elegans **UN**C-84. They are type II transmembrane proteins of the nuclear envelope, with their C-terminal SUN-domain oriented towards the perinuclear space. At the nuclear side, Sun1 interacts directly with chromatin and with the lamin nucleoskeleton. Within the perinuclear space it interacts with the so-called KASH-domain of transmembrane proteins of the outer nuclear envelope. The KASH domain (named after **K**larsicht, **A**NC-1, **S**YNE1 **h**omology) is found at the C-terminal end of otherwise unrelated cytoplasmic linker proteins, such as nesprins and ANC-1, which link the nucleus directly or indirectly to the cytosolic actin and intermediate filament network [8]. Dynein, which is associated with the KASH-domain protein at least in some organisms helps to reel in the centrosome close to the nuclear envelope through its microtubule minus end-directed motor activity. This situation, which was most thoroughly studied in C. elegans, appears to be clearly different in Dictyostelium, where Sun1 is located in both nuclear membranes. Furthermore, the only protein with a weak similarity to a KASH domain characterized so far is the actin-binding nuclear envelope protein interaptin [6]. However, interaptin does not interact with Sun1. Yet, dynein appears to play an important role in Dictyostelium as well, since a hypomorphic mutant of the dynein regulator LIS1 was defective in nucleus/centrosome attachment [9]. Furthermore, we have observed partial co-localization of microtubules and Sun1 in the pericentrosomal area of the nuclear envelope at least in telophase, which could be mediated by nuclear envelope-bound dynein. We have proposed a model in which Sun1 in the outer nuclear membrane interacts with the centrosome and directly or indirectly with itself in the inner nuclear membrane to tether the centrosome to the centromere cluster, which can be labeled with the marker protein Cenp68 [7] (Fig. 1). For the first time in a non-metazoan organism we recently have also identified a lamin-like protein in Dictyostelium (A. Krüger, I. Meyer, R. Gräf, unpublished results) and, thus, it could well be that a putative nucleoskeleton participates in centrosome/nucleus attachment as in higher organisms. It is unlikely that the centrosome is just tethered to the nuclear envelope through nucleoskele-

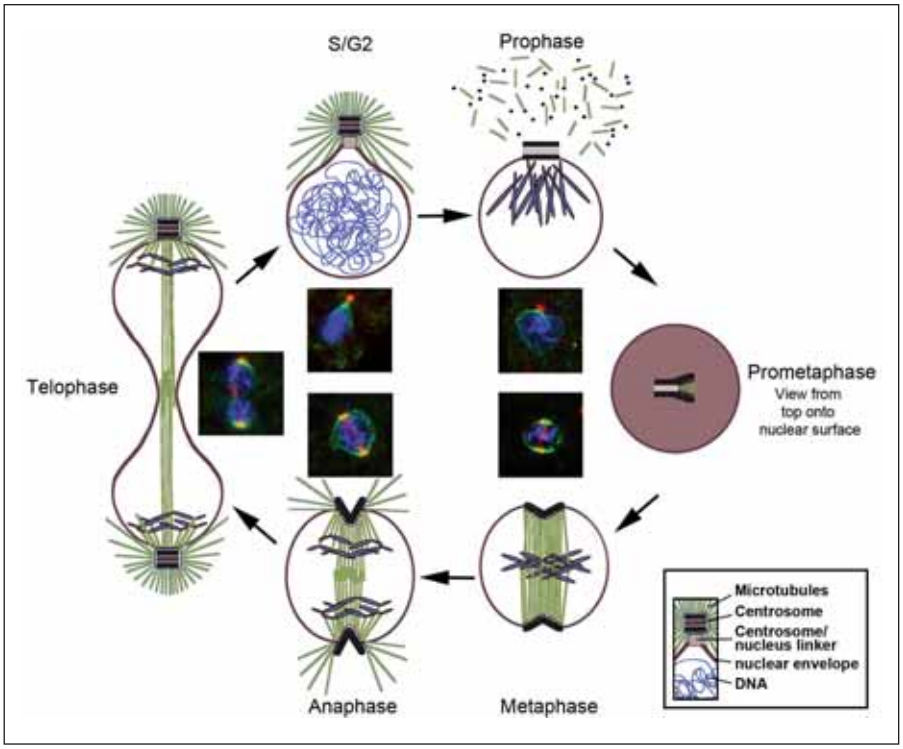


***Fig. 2.***: The *Dictyostelium* centrosome cycle. Nuclei, chromosomes and centrosomes with attached mictrotubules are shown in schematic cross sections of different cell cycle stages, except for prometaphase where a view to the nuclear surface is depicted. In the schematic drawings, the centrosome is depicted with its main structural parts. These are the layered core structure with three major layers, whereby the two outer layers are identical and different from the central one, and the surrounding corona characterized by the microtubule-organizing nodules. The corresponding immunofluorescence microscopy images show staining of the centrosomes with anti-DdCP224 (red), the nuclear envelope and the centrosome/nucleus linkage with anti-Sun1 (green) and DNA with DAPI (blue). Taken from Gräf R. (2009) Microtubule Organization in Dictyostelium. Encyclopedia of Life Sciences (ELS), DOI: 10.1002/9780470015902.a9780470021852. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission

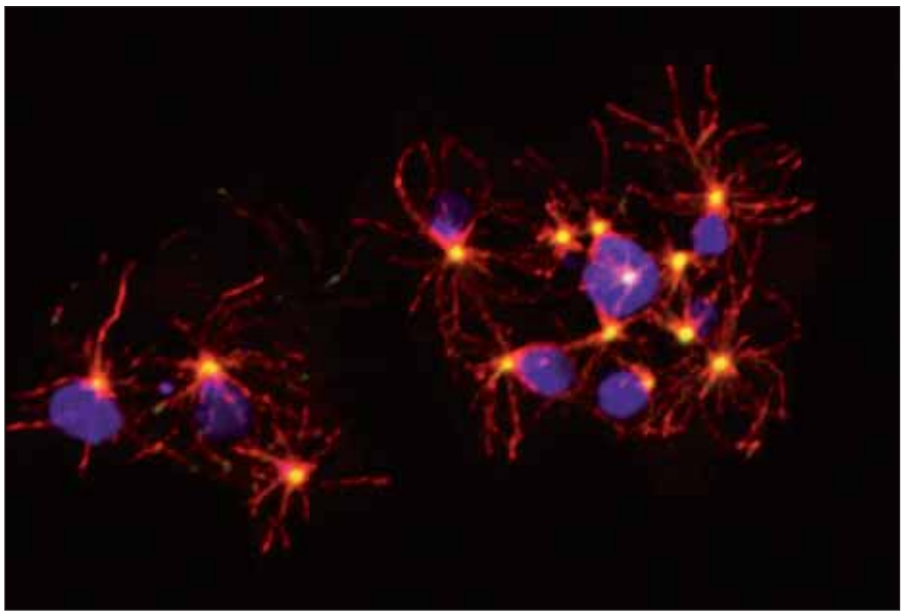

*Fig. 3.*: Overexpression of CP224 elicits supernumerary centrosomes. Microtubules are shown in red, centrosomes in green (appear yellow in this merged image) and nuclei in blue. Note that these cells possess more than one centrosome per nucleus. Furthermore, they are slightly defective in cytokinesis, which becomes apparent by their multinuclearity.

ton-anchored Sun1, dynein and microtubules, because microtubule depolymerizing agents do not cause centrosome detachment, even in mutants that respond hypersensitive to such agents and show complete microtubule disassembly in the presence of the drug [10]. Thus, further elucidation of centrosome/nucleus attachment according to our model obviously requires characterization of the so far unknown centrosomal interactors of Sun1 as well. These proteins could be part of the centrosomal core structure or of the corona.

## Composition and molecular characterization of Dictyostelium centrosomes

The centrosomal corona harbors electron-dense nodules at the base of around 30 microtubules emanating from the centrosome in a radial fashion (Fig. 2). Since the nodules are associated with γ-tubulin, the key-protein of microtubule nucleation, they are considered as the microtubule nucleation complexes. Besides γ-tubulin the corona contains further proteins involved in microtubule dynamics, such as EB1, CP224 (an orthologue of XMAP215) or TACC (= transforming acidic coiled coil protein). Similarly to fission yeast, but different from centriolar centrosomes and the budding yeast SPB duplication of the Dictyostelium centrosome is not synchronized with S-phase, but is initiated at the G2/M transition, where the core grows and corona dissociates together with all interphase microtubules. The replicating structure now is the layered core, which resembles the centrioles and the central layer of the budding yeast SPB in this respect [11]. First the central layer disappears and the enlarged outer layers split apart from each other and start to nucleate microtubules from their former inner side and organize a central spindle. Elongation of the central spindle separates the two layers that now constitute the mitotic centrosomes and are inserted in a fenestra of the nuclear envelope. At the transition from metaphase anaphase the two layers start to fold in a way that their microtubule nucleating surface becomes exposed to the cytosol. At that time astral microtubule appear as well. The folding process continues until both layers have folded back onto themselves and form the new outer layers of the two new centrosomes. Their outer, microtubule-nucleating face constitutes the new corona. The central layer is re-formed by unknown means. It is also not clear, how tubulin dimers become access to the nuclear matrix in this form of closed mitosis. We suppose that this occurs either at the two fenestrae harboring the mitotic centrosomes or through open nuclear pore complexes. We hypothesize that similar to Aspergillus nidulans some FG-repeat proteins of the central channel of the nuclear pore complex may dissociate after phosphorylation rendering the channel open for larger molecules. In Aspergillus these nuclear pore complex subunits are phosphorylated by the NIMA kinase [12]. Dictyostelium indeed contains a NIMA-related kinase, Nek2, which could fulfill this task.

Nek2 also was the first known component of the centrosomal core structure [13]. Meanwhile, we were able to isolate and characterize four more components of the core structure after mass spectrometrical analysis of isolated centrosomes [14]. The new components were named CP39, CP55, CP75 and CP91 according to their calculated molecular mass [15]. Although none of these proteins could clearly be assigned to a specific layer, their dynamic properties during mitosis indicate that CP39, CP75 and CP91, which disappear in prophase and reappear in telophase, are constituents of the central layer, which is also absent in this period. In contrast, CP55 and Nek2, which are permanently localized at the centrosomes should be assigned to the outer layers. The recruitment of CP39, CP75 and CP91 could occur in a similar fashion as in centriole formation in C. elegans worms. Here SPD-2 (= Cep192 in humans) recruits the centriole biogenesis proteins ZYG-1, SAS-5, SAS-6 and SAS-4, which is the prerequisite for assembly of centriole microtubules [16]. Dictyostelium contains a

Cep192 orthologue as well that is located at the border between the corona and the core structure. Thus, Dictyostelium Cep192 could be a candidate for recruitment of the new centrosomal core proteins during biogenesis of Dictyostelium centrosomes. Based on the current data, we cannot judge whether the new Dictyostelium proteins act as functional homologues of the only weakly conserved worm proteins. Centrosome biogenesis does not only involve centrosomal core proteins. Several years ago we could show that regulated overexpression of the corona protein CP224 reversibly causes formation of supernumerary centrosomes [10] (Fig. 3). Due to closed mitosis non nucleus-associated supernumerary centrosomes do not interfere with bipolar spindle formation since plus-ends of microtubules emanating from cytosolic centrosomes have no access to kinetochore microtubules. Although the fraction of cells containing supernumerary centrosomes is rather high in CP224 overexpressors, the number of supernumerary centrosomes per cell was rarely more than 3 per nucleus, even after prolonged cell culture. This suggested the existence of mechanisms to control centrosome number. Indeed we found two mechanisms for the reduction of centrosome number, first the fusion of supernumerary centrosomes into one entity and second expulsion of supernumerary centrosomes by the formation of a cytoplast during cytokinesis. While the first mechanism was similarly found in tumor cells as one means to control centrosome number and to assure spindle bipolarity, cytoplast formation has not been shown in tumor cells so far, but could provide a further mechanism how tumor cells could control centrosome number to retain their capacity to grow and segregate their chromosomes properly into daughter cells. In addition to its role in centrosome biogenesis, CP224 is required for microtubule nucleation and promotes microtubule elongation. The latter functions are shared by its recently characterized direct binding partner TACC [17]. TACC was identified by a tandem affinity purification approach using CP224 as a bait protein [18]. Both proteins were also found at microtubule plus ends during interphase and at kinetochores during mitosis but not at plus ends of mitotic astral microtubules. We also observed a differential requirement of TACC for CP224 localization at microtubule plus ends versus centrosomal and kinetochore localization. While CP224 required TACC for its association with interphase microtubule plus ends, its presence at centrosomes and kinetochores was independent of TACC. GFP-TACC has turned out as the first useful microtubule plus end marker for live cells in Dictyostelium. In conjunction with fluorescence recovery after photobleaching (FRAP) experiments at GFP-labeled microtubules this allowed the most thorough study of the unusual microtubule dynamics in Dictyostelium. Thus, we could show that *Dictyostelium* microtubules are dynamic only in the cell periphery, while they remain stable at the centrosome, which also appears to harbor a dynamic pool of tubulin dimers.

## Conclusions

Dictyostelium amoebae provide a useful model system to study the biology of centrosomes and its relationship to the nucleus. The conservation of key proteins that have been identified as targets for various gene mutation-based diseases such as cancer, lissencephaly and laminopathies offers the possibility to study the molecular basis and cell biology of these diseases in a simple and genetically easily accessible organism.

## Acknowledgements

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# Macromolecular assembly at its best: how to organise a muscle cell

Dieter O. Fürst, Gregor Kirfel & Peter van der Ven

## Background

Ordered macromolecular complexes are the basis for most fundamental aspects of life. Two or more macromolecules assemble into a defined three-dimensional structure, in which the constituting parts cooperate to drive cellular processes. Well-known examples include the ribosome, the proteasome, the splicing machinery and protein complexes that allow for directional movement. Numerous such assemblies are subject to intensive research, with – from the cell biologist's perspective – the main emphasis on identifying relationships between structure and function and on deciphering the principles underlying their self-assembly and turnover.

Directional movement is of paramount importance for living systems, and it is therefore found at all levels of organisation, from single macromolecules to whole animals. The most striking example are cross-striated heart- and skeletal muscle cells, which are mainly composed of myofibrils, a type of macromolecular assembly that is optimised to allow for both constant and repeated movement, e.g. in the heart for sometimes more than 100 years without any interruption.

Myofibrils are remarkable in many aspects: Their components are packed in an extraordinary degree of order and in amounts unequalled in nature. This regularity is reflected by an almost crystalline arrangement of its parts, which in turn has allowed studying the principles of its structural layout and of the contraction process. Taking into consideration that cross-striated muscles make up on average 40 % of our body mass, one can conclude that almost half of our bodies are of nearly crystalline design (Fig. 1).

To make the story even more fascinating, the myofibril is anything but a rigid structure: proteins are constantly turned over, damaged proteins have to be replaced selectively, the contractile system must respond to altered mechanical needs and strains, either by incorporating subunits with different kinetic properties or by increasing the sheer amount of myofibrils, and – last but not least – various diseases have also an impact on the structure and/or performance of the contractile machinery. Naturally, the initial assembly as well as turnover and adaptation have to be controlled in very stringent ways by distinct signalling cascades. Their elucidation will be among the most exciting next chapters in muscle research.

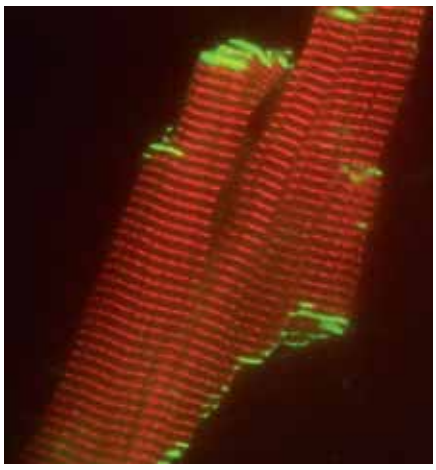

***Fig. 1.***: Immunofluorescence micrograph of an isolated mouse cardiomyocyte stained for a Z-disc epitope of titin (red) and the intercalated disc protein Xin (green). Note the striking regularity of the contractile apparatus.

## Structure and composition of the myofibril

In order to render the course of movement most efficient, the contractile machinery needs to be organised in a very regular arrangement. The building principle was uncovered by electron microscopy and X-ray scattering techniques in the 1950s and 60s (Huxley, 1969). Thus the myofibril may be seen as a succession of sarcomeres, in which actin-based thin filaments, held together at the Z-disc, interdigitate with myosin-based thick filaments, which in turn are kept together at the central M-band (Fig. 2). Most striking at this point is the uniformity of the lengths of the constituting myofilaments and up to now we are far from understanding the underlying control mechanisms.

While this basic structural layout, which was a prerequisite to explain muscular movement by a thick – thin filament sliding mechanism, has been known for more than 50 years now, the precise protein composition is still unknown. Systematic ligand screens have yielded more than 20 M-band proteins and more than 30 Z-disc components, so far. This at first glance frightening list begins, however, to reveal new concepts about the cross-talk between predominantly structural and contractile components on the one hand and regulatory signaling networks on the other (see below).

In the M-band, thick filaments are linked by the carboxy-terminal portion of titin, obscurin, obscurin-like 1 and myomesin into a hexagonal lattice. Based on a combination of biochemical data and electron microscopic epitope maps we suggested a first three-dimensional model of M-band structure (Fig. 3B and C). A further important function of the M-band is its role as a platform for organising energy metabolism by anchoring the muscle variant of creatine kinase (Hornemann et al., 2003) and glycolytic enzymes such as enolase (Foucault et al., 1999). The machinery required to produce ATP is therefore ideally positioned at the site of the highest energy demand.

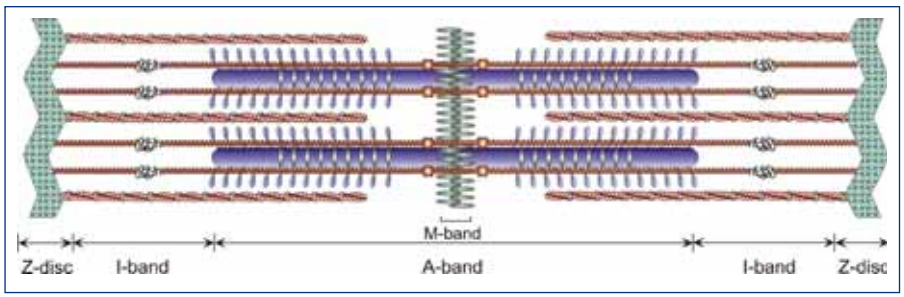


*Fig. 2.*: Sketch illustrating the structural layout of the basic contractile unit, the sarcomere. Thin and thick filaments are held in register at the Z-disc and M-band, respectively. Titin molecules, spanning half sarcomeres, are the only components linking both sub-assemblies, and provide continuity.

The carboxy-terminus of titin harbours a unique kinase domain (Mayans et al., 1998), which seems to be capable of sensing mechanical load and in combination with Nbr1, p62/SQSTM1 and MURFs it translates variations of mechanical load into either atrophic or hypertrophic signalling and thus regulates protein turnover and gene transcription (Lange et al., 2005).

Our current view of Z-disc structure is still largely based on electron microscopy studies of plastic-embedded material, in which Z-disc width varies, depending on the fiber type, between 30 and 140 nm (Luther, 2000; Luther et al., 2002). Both the major structural and regulatory components must therefore be arranged in a distinct manner. The prime task of the Z-disc is to cap the ends of thin myofilaments by capZ and to cross-link them via a-actinin. a-actinin molecules are thought to be arranged in layers with a periodicity of 19 nm, but titin and nebulin were suggested to influence the precise lattice spacing. Additional knowledge about the precise molecular layout and functional properties of the Z-disc will require elucidating detailed structures of Z-disc protein complexes, like the titin–telethonin complex (Zou et al., 2006), which was the basis for understanding its biomechanical properties (Bertz et al., 2009).

For a long time this small group of proteins were the only components specifically assigned to the Z-disc. However, our current inventory encompasses more than 30 proteins that can be part of this region, and a larger number of further proteins link the Z-disc laterally to neighbouring myofibrils or to the plasma membrane at sites of cell-cell- or cell-matrix-contact (see Fig. 4). Interestingly, several members of signal transduction pathways were either localized at the Z-disc or shown to interact with Z-disc proteins, emphasising a critical role of the Z-disc as a mechanosensor and a signaling platform that plays a critical role in the maintenance and regulation of proper myocyte function.

We have focused our work on filamin C, a member of a family of large cytoskeletal proteins that crosslink F-actin filaments either into parallel bundles or dynamic three-dimensional meshworks. Moreover, they interact directly with a plethora of cellular proteins of great functional diversity, indicating that they act as multifunctional signaling adapter proteins (Feng and Walsh, 2004; Popowicz et al., 2006). At the amino-terminus filamins possess an actin-binding domain, which is followed by a semiflexible rod comprising 24 highly homologous immunoglobulin-like domains, serving as an interface for the interaction with numerous filamin-binding proteins (see Fig. 5). The carboxy-terminal Ig-like domain 24 is responsible for the formation of dimers (Himmel et al., 2003). Most of the filamin in muscle cells is localized at the Z-disc, where it binds to different proteins including myotilin (van der Ven et al., 2000) and FATZ/calsarcin/myo-

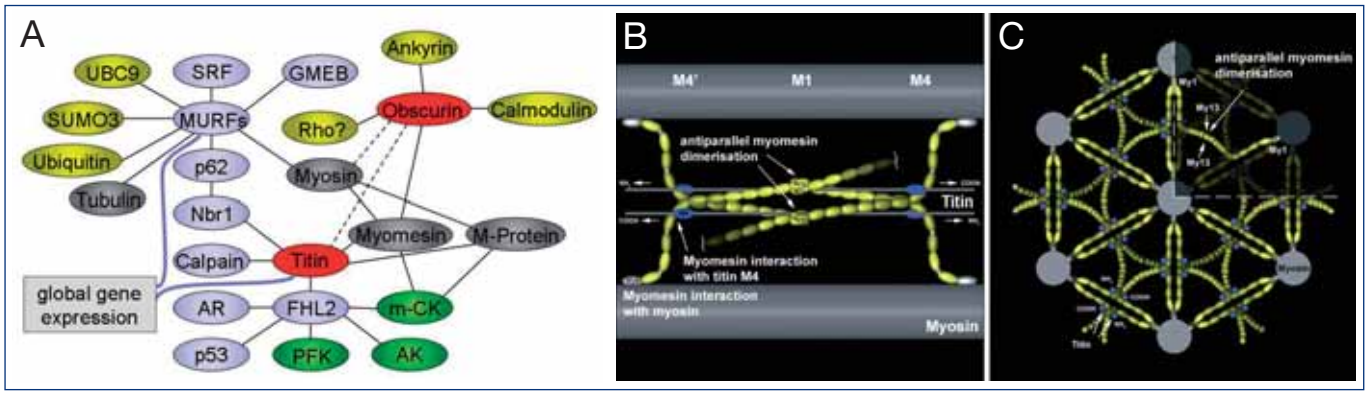


*Fig. 3.*: M-band interactions and proposed structure. A: Diagram illustrating protein-protein interactions in the M-band. Combined biochemical and microscopical data proposed the molecular layout of major M-band proteins in B (longitudinal direction) and C (cross-section). (A: modified from: Lange et al., 2006; B and C: reproduced from: Lange et al., 2005).

zenin (Faulkner et al., 2000; Takada et al., 2001). At the sarcolemma, filamin C interacts with γ- and δ-sarcoglycans (Thompson et al., 2000) and in myotendinous junctions with Xin (van der Ven et al., 2006).

In recent work we could further show that BAG-3 coordinates the formation of a multi-component chaperone complex at the Z-disc, comprising the small heat shock protein HspB8 (also known as Hsp22), Hsc70, and the CHIP ubiquitin ligase (Arndt et al., 2010). This complex recognizes filamin C damaged during contraction and mediates its disposal. Intriguingly, ubiquitynated filamin C is not sorted to the proteasome in this case, but instead, the autophagic ubiquitin adaptor p62 is recruited, which initiates the formation of an autophagosome, leading eventually to lysosomal degradation of filamin C (Arndt et al., 2010). We therefore coined the term chaperone-assisted selective autophagy, CASA, for the BAG-3-mediated degradation pathway.

## Formation and adaptation of the Z-disc

Myofibril formation is a multistep assembly process which starts on actin filament-based stress fiber-like structures shortly after onset of the myogenic differentiation program by specific transcription factors. The first sign is the appearance of α-actinin-containing Z-bodies, flanking assemblies of short non-muscle myosin filaments (Rhee et al., 1994). Concomitant with their replacement by muscle-specific myosin isoforms, their spacing increases and a series of Z-disc proteins is sequentially integrated (see e.g. for the Z-disc portion of titin in Fig. 6). We found out that initial assembly complexes include e.g. α-actinin, titin, telethonin, filamin C and myopodin, while proteins like myotilin and synaptopodin are expressed and integrated at much later stages (Ehler et al., 1999; van der Ven et al., 1999; Salmikangas et al., 2003; Linnemann et al., 2010). The precise timing of Z-disc formation and the specific function of its individual constituents, however, still remains to be resolved.

The striking stability of the contractile machinery even after detergent extraction has embossed our concept of the myofibril as a structure composed of rigid constituents that are more stable than most other complex protein assemblies. This concept has been, however, proven entirely wrong in the context of sarcomere formation, fiber type transitions, hypertrophic and atrophic remodelling, mechanosensing as well as protein turnover. The analysis of human muscle diseases has given a first indication of the highly dynamic state of the Z-disc. For instance in Duchenne muscular dystro-

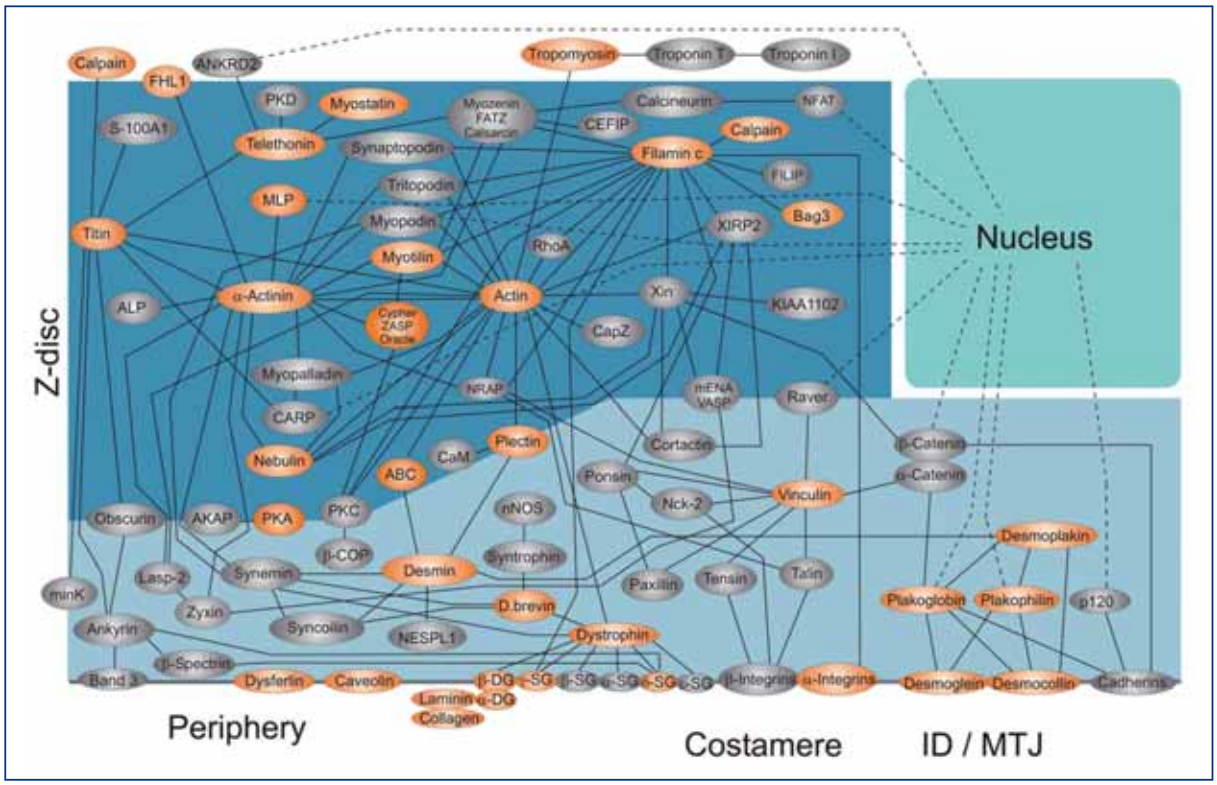


*Fig. 4.*: Diagram illustrating protein-protein interactions in the Z-disc (dark blue background) and in the structures linking Z-discs to cell-cell- and cell-matrix-junctions (light blue background). Some dual compartment proteins shuttle between a cytosolic and a nuclear localization (dashed lines). Genes/proteins associated with human neuromuscular diseases are highlighted in red.

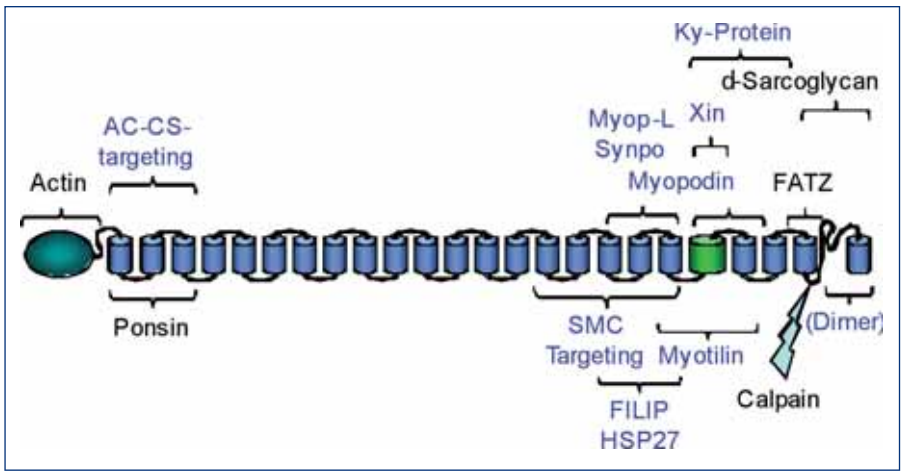


***Fig. 5.***: Schematic representation of the molecular structure of filamin C and its known binding partners. The interactions with myotilin, Xin, myopodin, synaptopodin (Synpo), Myop-L, Ky-Protein, FILIP and HSP27, highlighted by blue writing, have been revealed in systematic ligand screens performed by our group (AC-CS = actin cytoskeleton).

phy, which is caused by mutations in the membrane-cytoskeleton protein dystrophin, the normally mainly Z-disc-associated protein filamin C is dramatically relocalized to a predominantly subsarcolemmal position (Thompson et al., 2000). Subsequently, we observed pronounced relocalization for filamin C and its ligands myotilin and Xin not only in several myopathies but also in central core disease and in inflammatory myopathies (Bönnemann et al., 2003; Claeys et al., 2009). Based on these results our antibody to filamin is now widely used by neurologists and neuropathologists for diagnostic purposes.

These examples illustrate the highly dynamic nature of many Z-disc proteins and imply that in these diseases certain signalling pathways may be misregulated. The multitude of proteins involved in signalling that have meanwhile been found at the Z-disc, including protein kinase C, the phosphatase calcineurin and $Ca^{2+}$/calmodulin-dependent kinase II, have thus stimulated the novel concept of the Z-disc as a nodal point in cell signaling and mechanotransduction (Frank et al., 2006). An exciting field of future research will therefore be to precisely decipher the signalling events that control assembly and remodelling processes at the molecular level.

## Medical Relevance

The great importance of the Z-disc for the physiological function of cross-striated muscles is emphasized by the finding that mutations in many genes encoding Z-disc-associated proteins as well as failures of signalling at the Z-disc cause a wide variety of neuromuscular diseases and/or cardiomyopathies (proteins highlighted in orange in Fig. 4). Moreover, mouse knockout models for several Z-disc proteins have revealed a phenotype of cardiomyopathy and/or skeletal myopathy, including cypher/ZASP/oracle, muscle LIM protein (MLP) and calsarcin/FATZ/myozenin (reviewed e.g. in Frank et al., 2006; Pyle & Solaro, 2004).

We have identified mutations of the human filamin C gene on chromosome 7q32, which cause an autosomal dominant myopathy that affects skeletal and cardiac muscles (4; OMIM #102565). Filaminopathies usually manifest in the fifth decade of life with slowly progressive weakness and atrophy of the lower extremities and frequently lead to respiratory insufficiency (Vorgerd et al., 2005; Kley et al., 2007). Cardiac and skeletal muscles from patients suffering from filaminopathies are morphologically characterized by pathological protein aggregates in their cytosol consisting of desmin-immunoreactive material as well as by abnormalities in their myofibrillar organisation. We have subsequently studied in detail the biochemical consequences of the p.W2710X filamin C mutation, which is the most frequent one in this gene. This mutation results in the deletion of 16 amino acids at the very C-terminus and leads to a structural alteration of the C-terminal immunoglobulin-like domain 24. As a main finding, we demonstrated that this leads to a partial disturbance of the secondary structure of the dimerization domain, which consequently results in a massive aggregation of filamin C in skeletal muscle

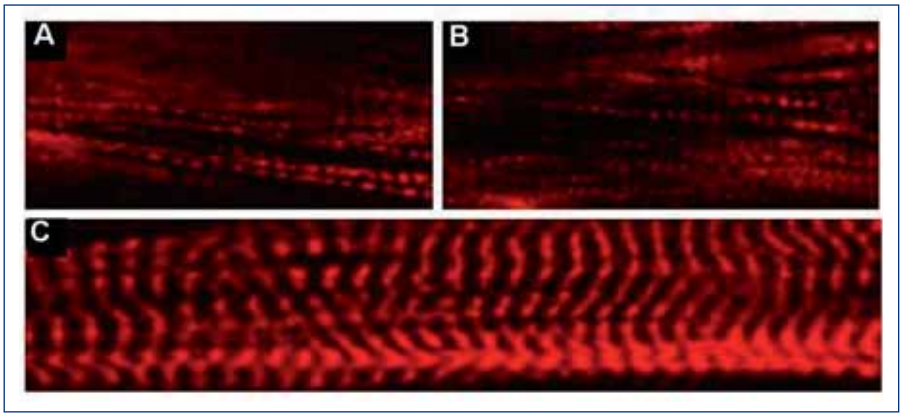


***Fig. 6.***: Differentiation of human skeletal muscle cells in culture. Cells were fixed and stained for immunofluorescence microscopy using a Z-disc titin antibody after 1 day (A), 2 days (B) and 5 days (C), respectively.

fibers of affected patients. A variety of different proteins are subsequently recruited to these aggregates, thereby destabilizing tissue homeostasis. As a result of the ectopic accumulation of γ- and δ-sarcoglycan, the connection of the myofibrillar cytoskleleton to the extracellular matrix may be weakened and the dystrophin-associated signalling affected (Löwe et al., 2007).

Though first insights into the pathological consequences of filamin C mutants have been gained, the sequential steps and exact molecular pathways leading from an *FLNC* gene defect to pathological protein aggregation and progressive muscle damage remain to be determined. In analogy to the pathophysiology of desmin-associated myopathy, the protein aggregation pathology in filamin C myopathy may impair the proteolytic function of the ubiquitin-proteasome system (reviewed in: Ferrer & Olivé, 2008). The BAG-3-directed disposal of damaged filamin C described above (Arndt et al., 2010) provides a direct mechanistic link to this pathway.

## Conclusion

Recent years have brought surprising and exciting new insight into the complexity of myofibrillar protein interactions, and it has become evident that this structure is highly dynamic. These findings challenge the existing dogma of a static architecture as it was imposed by electron microscopy snapshots and limited opportunities to investigate the biochemistry of the underlying components. An exciting goal for future research will be to unravel the molecular pathways that regulate myofibril assembly and turnover.

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# Crawling, pushing pulling - how actin dynamics and cell motility governs physiology

Walter Witke

## Introduction

Our body works as a perfectly fine tuned machine that can interact and communicate with the environment and at the same time protects us. If we look inside this machine, we realize that our body is anything else than a machine, but rather a conglomerate of highly dynamic cells which in a very altruistic way contribute to physiology. Some cells move in seemingly chaotic pattern, and others crawl along each other determined to find their way to an imaginary destination. Finally these cells will find a niche and contribute to organ morphogenesis or trigger a physiological response such as wound healing. In the adult brain neurons will not translocate much, but rather tickle their neighboring cells, in order to strengthen or to weaken the connections. Cell motility is what allows the brain to perform one of the biggest mysteries in biology - to remain moldable throughout life.

The genetic program of cells can determine their fate, however determination is only one aspect of morphogenesis, and cell crawling is an equally important parameter. Virtually any cell in our body has the potential to migrate or to change shape and even a resting tissue cell can be triggered to migrate autonomously when released from the tissue context.

Work over the past decades have allowed us to build up a dictionary of cell migration - lamellipodia, filopodia, focal contacts, etc., which has helped to better define and dissect the term 'cell crawling'. With a reductionist approach we identified what signals and molecules are required to make cells move. We know the proteins, which can nucleate actin polymerization, we know a plethora of actin binding proteins that control the life time of an actin filament and we have determined the structure of the three dimensional filament network (Fig. 1).

We have even learned to re-build the minimal unit of proteins, which can reproduce motility in the test tube [1]. However, the three of knowledge is getting thinner at the branches - or in other words - the exponential curve of gaining insight is asymptotically approaching a ceiling. At the end we will have a fantastically detailed catalogue of molecules, dissociation constants, structures and regulatory complexes, but we will still not fully understand the miracle of cell motility as part of our bodies physiology, unless we include studies on the ‚physiological relevance' of all our molecules and constants.

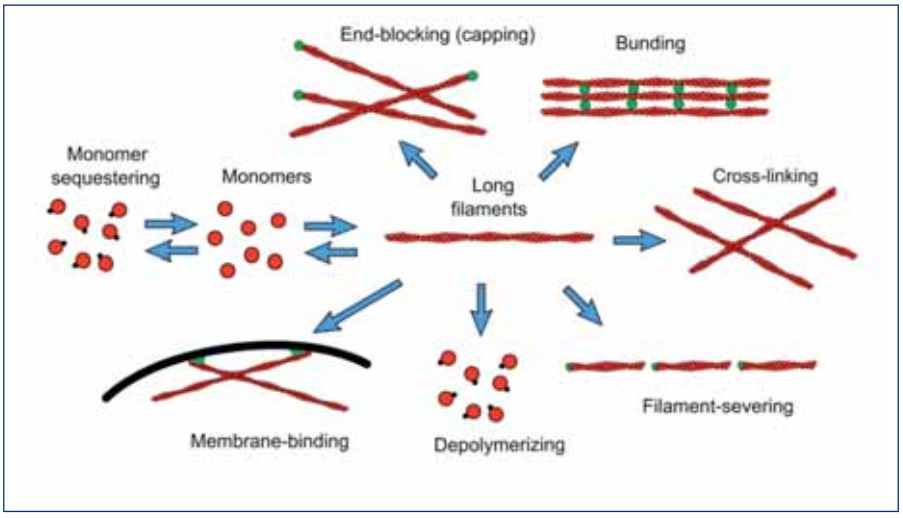


***Fig. 1.***: Cartoon of the actin cytoskeleton. Polymerization of actin filaments is controlled by monomer binding proteins and capping proteins, which block the growing end of filaments. Bundling and cross-linking proteins arrange the filaments in a 3-dimensional network. Actin severing proteins depolymerize old filaments and replenish the pool of monomeric actin.

## The Quest

The textbooks of today mostly have to rely on studies performed on single cells, in 2-dimensional cultures, on plastic or glass surfaces. However, in an organism cells move in 3-dimensions and in an environment, where the compliance of tissue is quite different from plastic. In the tissue context cells can use very different modes of migration, which in general are not as elegant as on surfaces, however if nature has for example abandoned stress fibers in migrating tissue cells, we must take this into consideration.

The quest is to extend our view and to ask whether our observations from in vitro experiments have physiological relevance and if yes in what context of physiology. Second, cell shape and cell polarity in a tissue is strictly linked to cell function, and marginal alterations, which might be difficult or impossible to spot in culture could very well impact on a physiological function. Third,

we are frequently dealing with large gene families of actin binding proteins or motor proteins, of which the precise function can only partially be revealed by in vitro experiments.

Experiments involving organisms are often considered as too complicated for studying actin dynamics and control of cell motility. My simple response to this argument would be that no in vitro assay system will ever be as sensitive as a developing organism or the morphogenesis of an organ. In the following, I will explain this view by giving some examples from our own work, showing how we can merge biochemistry and cell biology with genetic model systems in order to gain insight into mammalian physiology.

## The mouse as a model to study the actin cytoskeleton

While lower organisms such as Dictyostelium, C.elegans or Drosophila are very good models to learn about actin dynamics in development, they are quite distant to human physiology and disease. Since the early 90s of the last century, targeted mutagenesis in the mouse became more and more a routine technique in the laboratory. The establishment of conditional mutagenesis in the mouse was a second quantum leap - a technique, which became essential for analyzing essential genes and cell autonomous functions in specific cell types and tissues at any given time window in development [2]. This toolbox now allows us to model almost any kind of disease related mutation in the mouse.

## How the actin network is formed - from actin polymerization to brain function

A number of different mechanisms are used by cells to promote de novo actin polymerization, thereby increasing the amount of filamentous actin. This process is tightly regulated and occurs in a spatially controlled fashion near the cell cortex. One important player in controlling actin polymerization is a molecule called profilin - a 15kDa protein which can bind monomeric actin in a 1:1 complex and then delivers it to a growing actin filament. In mammals 4 profilin genes have been described, and while profilin1 and profilin2 have all the properties of the 'classical' profilins, the profilin3 and profilin4 are considered unconventional forms.

Over the past years our group has been interested in the role of profilin1 and profilin2 in the brain. We found that profilin2 is specifically expressed in neurons and there it localizes to the synapse. Studies in cell culture and mutant mice have suggested an additional, actin independent role of profilin2 in membrane trafficking. Profilin2 can interact with dynamin and thereby control membrane uptake as well as vesicle release [3]. We found that profilin2 can bind dynamin1 via a proline-rich domain and that this binding puts a brake on dynamins activity. Removing profilin2 increased the kinetics of membrane flow. Interestingly this regulation of dynamin1 was specific to profilin2 since profilin1 would not interact with dynamin1, despite the presence of a poly-proline binding domain in profilin1.

This work suggested that apart from actin, profilin2 can have other partners in the cell. Employing a proteomics approach, we showed that profilin2 can indeed interact with a number of novel ligands. One complex that was found to interact with profilin2 was the WAVE-complex, an actin nucleating complex that stimulates de novo actin polymerization through the activation of the Arp2/3-com-

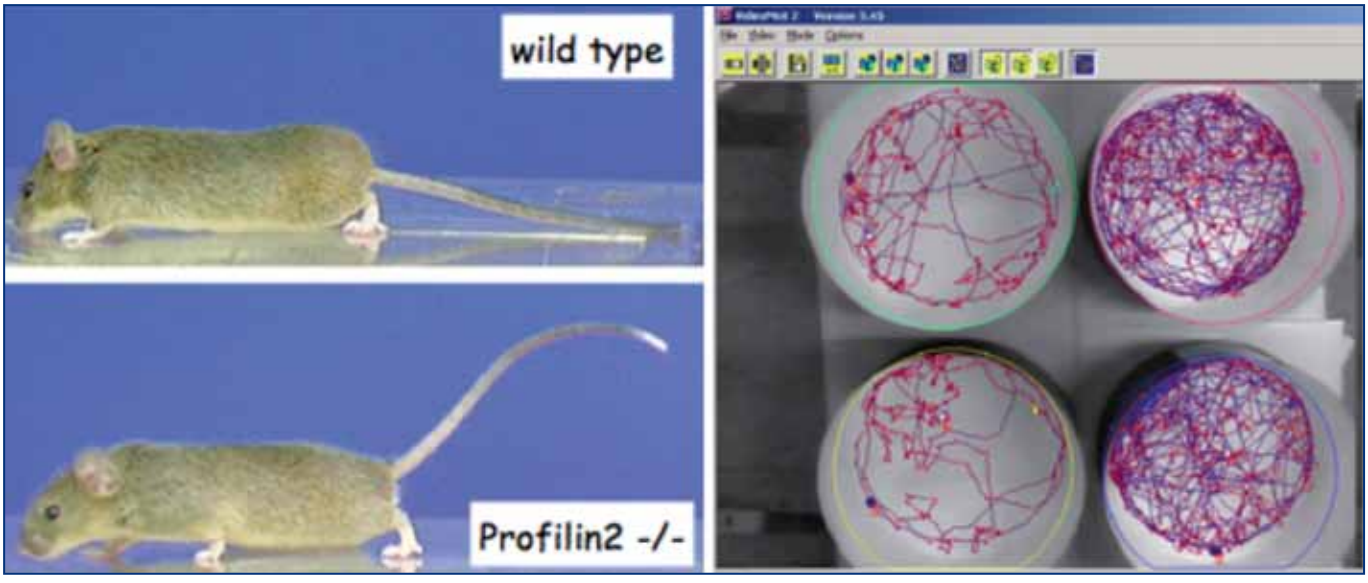


***Fig. 2.***: Hyperactivity of profilin2 mutant mice. Increased stress response of profilin2 mutant mice is illustrated by the 'Straub-Tail' position. On the right panel tracks of two control (left) and two profilin2 mutant mice (right) in an open filed arena illustrate the hyperactivity of mutants.

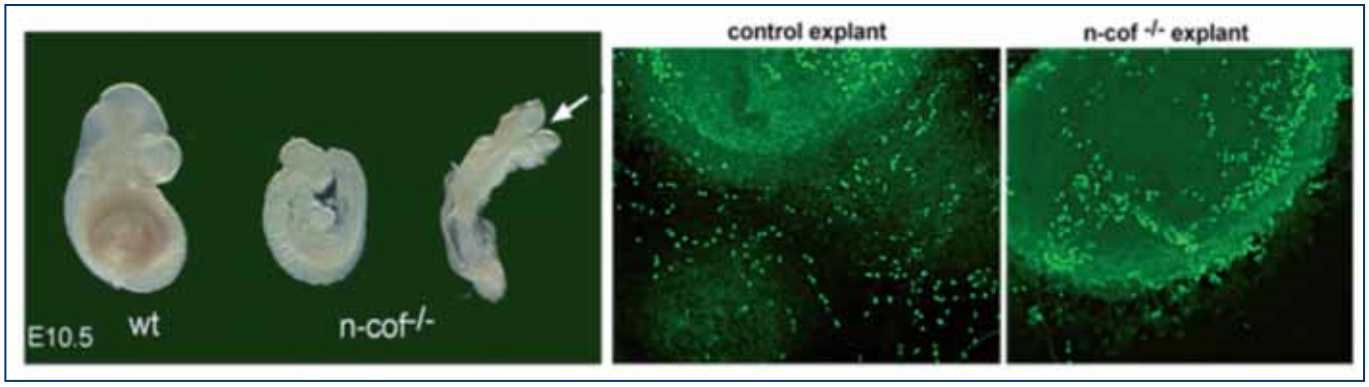


*Fig. 3.*: n-cofilin mutant embryos develop until E10.5 and show an open neural tube (arrow). Neural crest cell migration out of explanted somites is impaired in n-cofilin mutants. Neural crest cells were labeled with antibodies against Sox-2.

plex. These results suggested that profilin2 is central for controlling actin polymerization in neurons. In knockout mice for profilin2, we could then show that stimulated actin polymerization in the synapse was indeed impaired, leading to a presynaptic defect in neurotransmitter release. Loss of profilin2 facilitates vesicle release in glutamatergic neurons and results in a hyperstimulation of neuronal circuits in the striatum. These physiological alterations correlate with hyperactivity and increased novelty seeking of the animals [4] (Figure 2).

The profilin2 mouse model clearly demonstrated that actin dynamics plays an important role in the synapse and the control of complex behavior. More recent studies from our group revealed symptoms of autistic behavior in profilin2 mutant mice, and future research will focus on this aspect. Interestingly, mutations in the WAVE-complex have also been shown to contribute to autistic behavior in humans, which suggests a potential functional link to profilin2. Along these lines we would like to complement the studies by analyzing the role of profilin1 in brain physiology and determine to what extend profilin1 and profilin2 might have overlapping functions.

## How the actin network is taken apart - or why cell polarity requires actin filament severing

A large number of different F-actin severing proteins were found in men and mice, and what is common to the activity of these proteins is the rapid depolymerization of existing actin filaments. This is a crucial activity in order to replenish the pool of monomeric actin and to allow remodeling to the existing actin network.

Gelsolin has been a good example, showing that biochemical activity not necessarily correlates with physiological relevance. When purified from cell extracts, gelsolin shows the highest detectable F-actin severing activity, and the logical conclusion was, that gelsolin must be an essential activity in all cells. However, when we tested this hypothesis in the mouse this interpretation had to be reconciled with the fact that gelsolin mutant mice were viable. Work by a number of laboratories, including ours, showed a much more complex picture of gelsolin function than anticipated. It turned out that gelsolin is important for very rapid actin dependent responses such as platelet activtion, or inflammatory invasion by neutrophils, while for general cell functions gelsolin was dispensable. Interestingly, the phenotype of the gelsolin mutation was more severe after backcrossing the mutation into a pure Balb/c genetic background, suggesting that in a mixed genetic background other genes can somehow compensate the loss of gelsolin (W. Witke, unpublished observation).

The observations made in gelsolin knockout mice led to the conclusion that other actin depolymerizing factors must provide a more general activity for cytoskeletal remodeling, and using extracts from gelsolin mutant

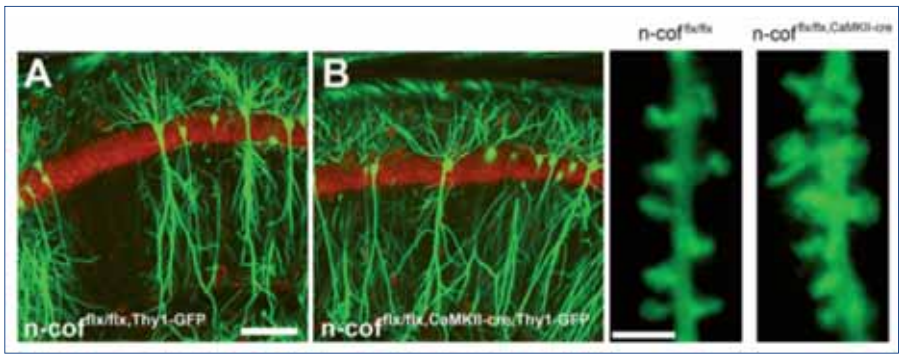


*Fig. 4.*: Neurite branching in n-cofilin mutant mice. In this experiment n-cofilin was specifically deleted in neurons of the forebrain. Hippocampal slices are shown of mice with sporadic expression of GFP in order to visualize dendritic branching. Dendritic spines in n-cofilin mutants are enlarged and show aberrant shape (right panel).

mice proteins, we identified the ADF/cofilin family as the most promising candidates.

In mouse and human three genes exist for the ADF/cofilin family - n-cofilin (non muscle cofilin), m-cofilin (muscle cofilin) and ADF (Actin Depolymerizing Factor). Gene targeting experiments in the mouse for all three members revealed common and distinct roles for these proteins and showed that this family of proteins is crucial for actin remodeling. We could show that n-cofilin is essential for neural crest cell migration and epithelial-mesenchymal transition, and null embryos did not survive beyond day 10.5 of development [5].

M-cofilin on the other hand is required for postnatal muscle function (C.Gurniak&W. Witke, unpublished observation) and ADF works in concert with n-cofilin in controlling the fate of dividing stem cells.

While in the test tube all three ADF/cofilin molecules are not much different in terms of F-actin depolymerization activity, our results in the mouse clearly show that n-cofilin, m-cofilin and ADF can have distinct roles in tissues. Of the three proteins n-cofilin is the most versatile in terms of cellular functions. In the developing brain, loss of n-cofilin leads to specific defects in radial migration of neurons in the cortex. Interestingly, these mutants phenocopy the characteristics of lissencephaly - a human disease where defects in the layer formation of the cortex lead to severe mental retardation [6].

While a role of n-cofilin in neuronal migration is intuitive, we had no information on the possible role of n-cofilin in the adult brain, at a time when all the neurons are in place and the synaptic connectivity has been established. When we removed n-cofilin at this late time point in the forebrain of adult mice, we observed an unexpected phenotype.

Basically, mice can survive without n-cofilin in the forebrain, but problems arise when mutants are asked to learn certain tasks. N-cofilin turned out to be essential for 'associative learning', but it was not required for 'explorative learning' [7]. This means that mice would for example remember where in the cage they had been walking a minute ago, but they would not be able to learn that a certain area in this cage should be avoided because an aversive situation there.

Without n-cofilin the capacity of the brain to remodel synaptic connectivity, usually summarized as 'synaptic plasticity', is impaired. Deletion of n-cofilin leads to an increase of filamentous actin in the synapse and alterations in dendritic spine size.

Importantly, n-cofilin is required for AMPA receptor mobility on the postsynaptic side of neurons, and deletion of n-cofilin significantly slows down AMPA receptor mobility [7]. AMPA receptor mobility and clustering was shown to influence synaptic strength and plasticity.

A truly surprising way by which an F-actin depolymerizing protein can control neuronal receptors and ultimately modulate learning and memory.

## Clinical relevance of actin dynamics

The actin cytoskeleton is a toolbox and its instruments have surprising leverage on many different physiological processes. Our group has extensively used mouse genetics in combination with cell biology techniques and biochemistry to obtain a better picture of the actin cytoskeleton in cell function and particularly physiology and diseases.

One interest of our work is to address the role of actin dynamics in synaptic plasticity. Alterations in synaptic plasticity not only affect learning and memory, but also seem to play an important role in the etiology of pathological syndromes such as autism and mental retardation. How the actin cytoskeleton contributes to neurological diseases is part of a long term goal together with our partners at the University Bonn.

A second important aspect of our work deals with the role of actin in the regulation of immune cells. Chemotaxis, T-cell polarization and local immune responses all depend on extensive cell shape changes and cytoskeletal dynamics, and even the uptake of viruses depends on actin dynamics. To understand the specific steps and mechanisms where and how actin remodeling contributes to immune responses might provide us with targets to interfere in pathological conditions.

In our 'actinocentric' view of the world, we believe that understanding the principals of actin dynamics will hold the answer to many questions of physiology and morphogenesis. The challenge is now to put the pieces of information provided by biochemists, cell biologists and geneticists together. Cell biologists like to take things apart and then to put them together piece by piece, geneticists try to read a phenotype and then work their way down. In our field of actin and cytoskeletal research it is timely that we are now trying to merge both approaches using the mouse as a model.

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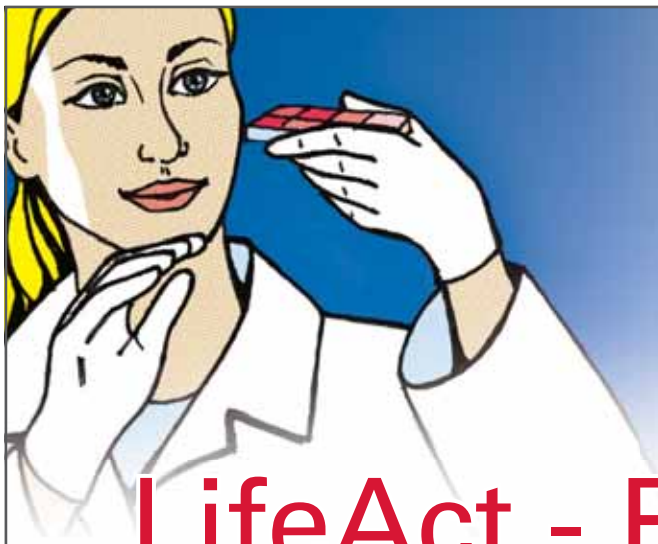

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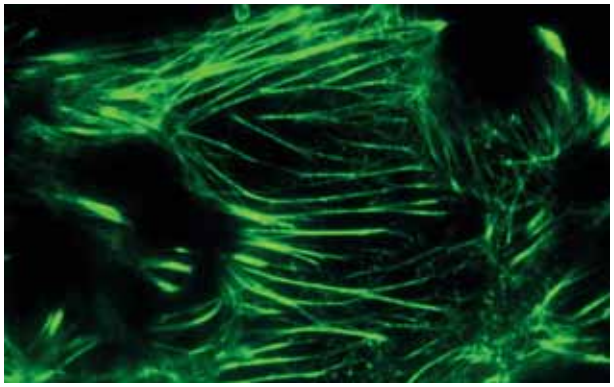

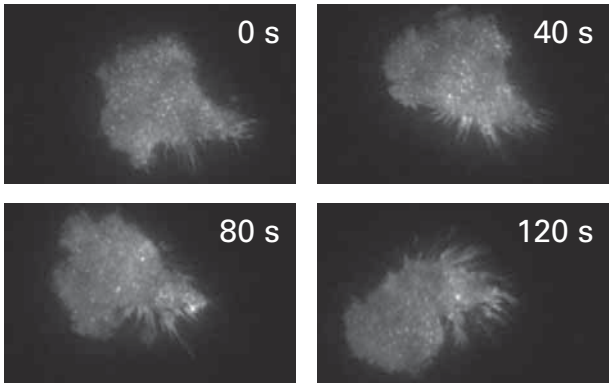


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# The centrosome and mitotic spindle apparatus in cancer and senescence

Roland P. Piekorz

## Role of the centrosome in normal and malignant proliferation

Mitotic cell division is a prerequisite for development, proliferative homeostasis and tissue regeneration. During mitosis the bipolar spindle is organized by centrosomes to ensure faithful separation of chromosomes to both daugther cells (Nigg et al., 2006). Spindle poles, kinetochores, and various microtubule-associated proteins are involved in the regulation of microtubule dynamics. The assembly of the mitotic spindle apparatus is a highly complex and dynamic process and tightly regulated by the cell cycle. On the other hand, alterations in centrosome and mitotic spindle architecture and function have profound consequences for cell cycle progression and can lead to chromosomal instability, aneuploidy/tetraploidy, and cell death (Doxsey et al., 2005; Nigg et al., 2006; and Sluder, 2005; Bastians, 2010). Cancer cells often display genetic instability and centrosomal abnormalities thus implying chromosomal missegregation and aneuploidy as driving forces in tumorigenesis. Interestingly, this concept was proposed almost 100 years ago by the pioneering work of Theodor Boveri (Boveri, 1914). However, the role of the centrosome and its amplification in generating genomic instability and as a consequence cancer is debated since amplified centrosomes can form clusters and assembly a bipolar rather than multipolar spindle resulting in normal cell division (Gergely and Basto, 2008). Moreover, analysis of mice with reduced expression of the centromere-linked motor protein CENP-E unexpectedly revealed that increased aneuploidy can act both in an oncogenic way (in the case of spontaneous tumors) and as a tumor suppressor (in the case of induced tumor formation) (Weaver et al., 2007). An alternative and intriguing concept linking centrosomal amplification to tumorigenesis and with high relevance to the cancer stem cell field was recently proposed and successfully tested in the fruit fly model. The authors demonstrated that symmetric (abnormal) rather then asymmetric (normal) cell division of neuroblasts, and not genomic instability, led to their overproliferation resulting in a transplantable cancer-like phenotype (Basto et al., 2008; Castellanos et al., 2008). Given these provocative findings it will be important to determine to which extent centrosomal aberrations occur in human cancer stem cells (Klonisch et al., 2010), and whether these defects influence an early stem cell balance between the symmetric (cancer promoting) and asymmetric (cancer suppressing) division mode (Zyss & Gergely, 2009).

## Centrosomal TACC proteins and their link to cancer

The mammalian transforming acidic coiled coil (TACC) family of centrosomal proteins consists of three members (TACC1-3) which function as important structural components of the mitotic spindle apparatus and contribute to its dynamics (Gergely, 2002; Peset and Vernos, 2008). TACC proteins are evolutionary conserved from yeast to man (Gergely, 2002; Peset and Vernos, 2008; Gomez-Baldo et al., 2010; Samereier et al., 2010) and share a 200-amino acid coiled coil motif at their C-terminus, but have only limited homology in the N-terminal part. TACCs regulate centrosome integrity, centrosome-dependent assembly of microtubules and spindle stability during mitosis (Gergely et al., 2003; Schneider et al., 2007, Yao et al., 2007). TACC3 is predominantly expressed during the $G_2$/M phase of the cell cycle where it localizes in an Aurora-A phosphorylation and clathrin heavy chain-dependent manner to centrosomes and mitotic spindles (**Figures 1 and 2A**; Kinoshita et al., 2005; LeRoy et al., 2007; Hubner et al., 2010; Lin et al., 2010). TACC3, but not the related isoform TACC2 which is rather found in postmitotic tissues, is required for efficient cell expansion and survival as indicated by its crucial and non-redundant role during embryogenesis and stem cell function (Piekorz et al., 2002; Schündeln et al., 2004; Yao et al., 2007).

TACC genes have been originally discovered in genomic regions that are amplified in cancer (Still et al., 1999a; Still et al., 1999b). Aberrations of TACC1 and TACC3 as well as their altered expression have been later linked to the etiology of breast, ovarian, bladder and non-small cell lung cancer (Cully et al., 2005; Lauffart et al., 2005; Jung et al., 2006; Kiemeney et al., 2010). Interestingly, in glioma tumors, the TACC3 gene localized at 4p16 exhibits a gain of copy number. This is accompanied by a grade-specific upregulation of TACC3 expression which is highest in grade IV gliomas (Glioblastoma multiforme, GBM) known to have a very poor therapeutic prognosis (Duncan et al., 2010). Despite of these findings, the relevance of TACC3 for the pathogenesis of GBM or other cancer types needs to be substantiated and additional functional data are required to establish TACC3 as a "driver" in tumor development and hence as a potential molecular target.

Irrespective of the obvious causative connection between centrosomal aberrations, overproliferation and tumorigenesis, alterations in the mitotic machinery can as well act as potent triggers for postmitotic cell cycle exit

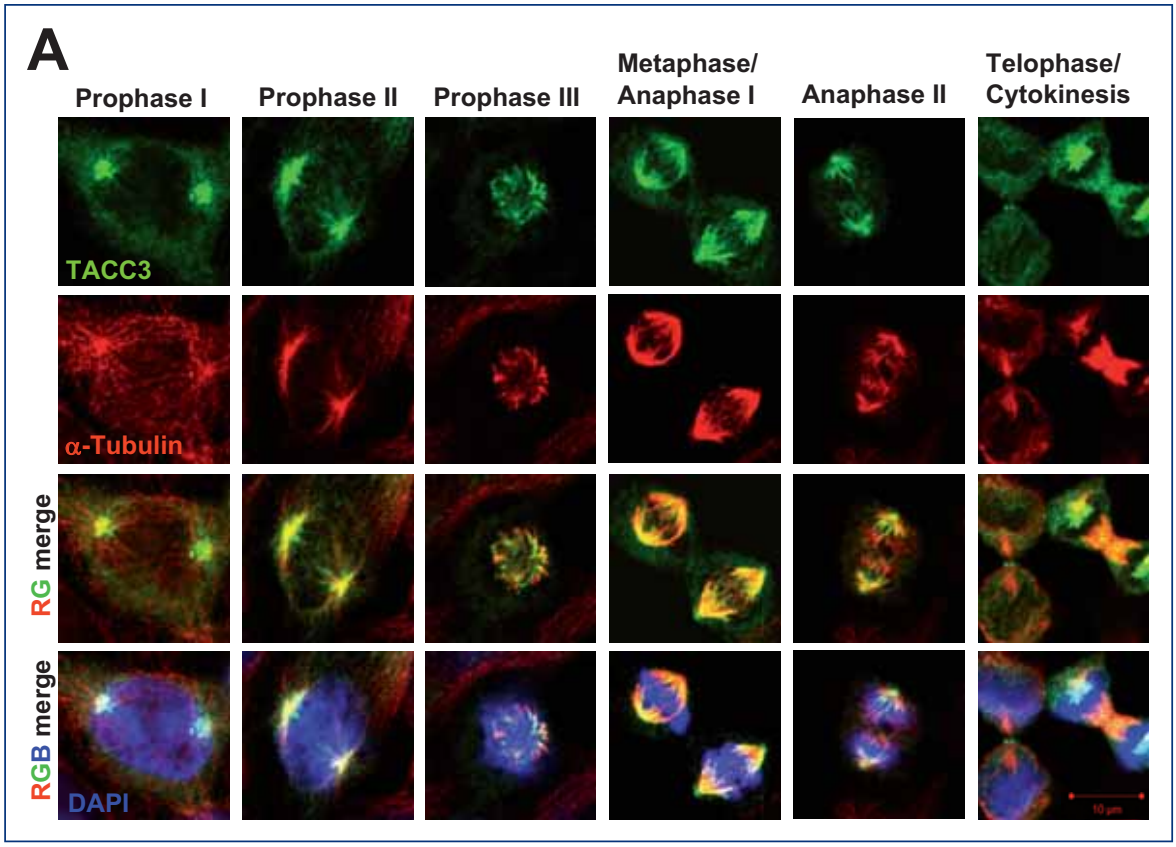


***Fig. 1.*: Subcellular localization of the centrosomal protein TACC3 during cell division.** HeLa cells were imaged by confocal microscopy during distinct mitotic phases following staining with antibodies against TACC3, the spindle marker α-Tubulin, or the centromeric region. The latter was detected by a CREST autoimmune serum recognizing CENP proteins A-C. **(A)** TACC3 shows a centrosomal localization during mitosis and decorates also the spindle apparatus up to the centromeric region **(B)**.

and permanent growth arrest. Interestingly, as discussed below and summarized in **Table 1**, various recent reports link centrosome and kinetochore dysfunction to the induction of cellular senescence.

## Cellular senescence – causes and characteristics

The phenomenon of cellular senescence was first described almost 50 years ago. Hayflick and Moorhead noted in their classical work that normal diploid fibroblasts have only a limited proliferative capacity under non-physiological conditions in culture and following a finite number of cell divisions undergo a state of cell cycle arrest, known as replicative senescence (Hayflick and Moorhead, 1961). In human cells this intrinsic type of senescence is typically elicited by progressive erosion of telomeres (Campisi and d'Adda di Fagagna, 2007). Senescence represents a major cellular stress and self-defense response with tumor suppressor function, and as such is elicited by extrinsic factors, including strong mitogenic stimuli like oncogenic Ras(G12V) (referred to as oncogene-induced senescence), DNA damage, various cytotoxic drugs, and oxidative stress in the form of endogenously and extrinsically generated reactive oxygen species (ROS) (Ben-Porath & Weinberg, 2005; Collado and Serrano, 2006). These forms of senescence represent rapid antiproliferative reponses and are collectively referred to as *stress-induced premature senescence (SIPS)*.

Senescent cells are typically characterized by growth arrest, apoptosis resistance. and altered gene expression (Collado and Serrano, 2006). The cells display several features which in part distinguish them from quiescent cells reversibly arrested in the G1 phase of the cell cycle (Campisi and d'Adda di Fagagna, 2007; Chuaire-Noack et al., 2010). In particular, senescent cells adopt (i) morphological changes, i.e., they become larger and flattened and display an increased granularity, (ii) biochemical changes including an expansion of the lysosomal compartment with increased SA-β-galactosidase expression and activity at pH 6.0, lack of BrdU incorporation and DNA synthesis, and (iii) changes in nuclear architecture characterized by the formation of senescence associated heterochromatic foci (SAHF) linked to the repression of proliferative genes. Lastly, the cell cycle regulators p53, $p21^{WAF1}$ and $p16^{INK4a}$, as well as the central mTOR (mammalian Target of Rapamycin) pathway play crucial roles in the induction and maintenance of the senescent phenotype (Campisi and d'Adda di Fagagna, 2007; Demidenko and Blagosklonny, 2008; Demidenko et al., 2009). In this regard, recent findings underline a new and key function of mTOR in determining the choice between senescence and quiescence upon p53-$p21^{WAF}$ mediated cell cycle arrest (Korotchkina et al., 2010).

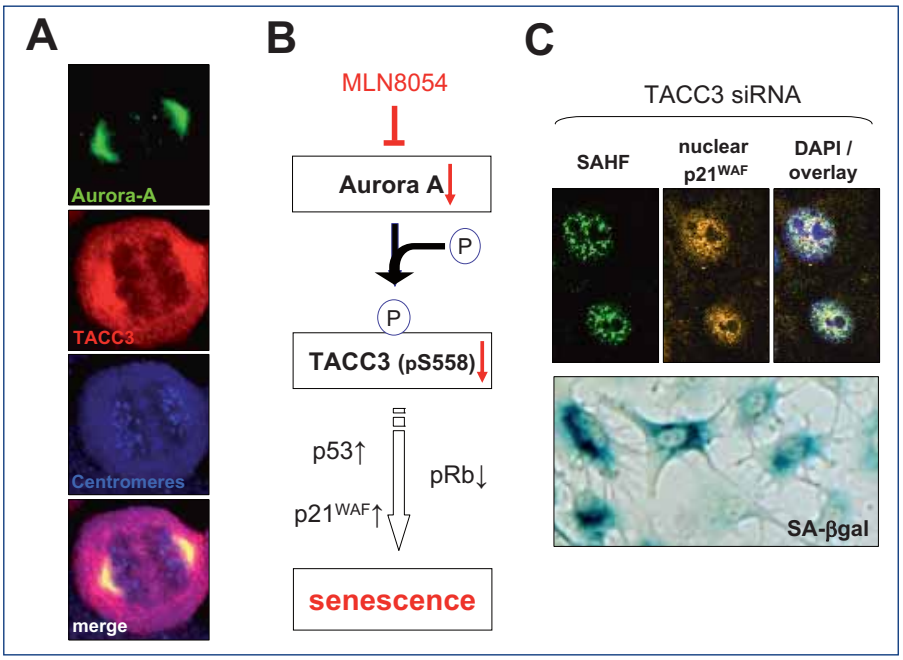


***Fig. 2.*: Inhibition of the Aurora-A - TACC3 function triggers premature senescence. (A)** Confocal microscopic analysis of Aurora-A kinase colocalized with its substrate TACC3 during cell division. Centromeric regions were visualized by the CREST autoimmune serum. **(B)** Inhibition of Aurora-A activity by the small molecule inhibitor MLN8054 (Huck et al., 2010), siRNA-mediated Aurora-A depletion, or knockdown of its downstream target TACC3 (Schmidt et al., 2010) all trigger comparable effector pathways and a premature senescence phenotype. **(C)** Senescence upon TACC3 depletion is characterized by the occurrence of HP1γ (pS83)-positive senescence associated heterochromatic foci (SAHF), nuclear accumulation of the cell cycle inhibitor $p21^{WAF}$, and increased SA-β-Gal activity (blue staining). **(B)** and **(C)** modified from Schmidt et al. (2010).

## Centrosomal and kinetochore proteins are linked via the tumor suppressor p53 to cellular senescence

Targeting TACC3 by gene inactivation or RNA interference causes centrosomal dysfunction and mitotic spindle stress which cellular outcome is decisively determined by the status of the postmitotic and p53-$p21^{WAF}$ controlled G1 checkpoint (Piekorz et al., 2002; Schneider et al. 2008; Blagosklonny, 2006). Whereas checkpoint compromised human cancer cells rapidly succumb to polyploidization and mitotic catastrophe (Schneider et al., 2007), checkpoint proficient cells primarily activate a senescence program. This response is in particular characterized by increase of p53 levels, nuclear accumulation of the p53 induced gene product and cell cycle inhibitor

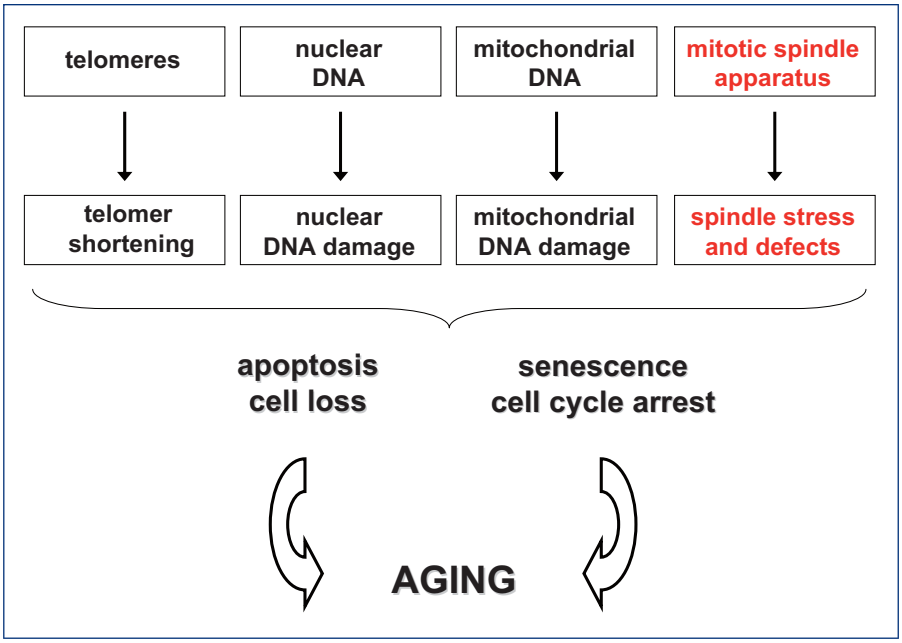


***Fig. 3.*: Cellular structures and macromolecules linked to senescence and aging.** Degeneration of telomeres and nuclear or mitochondrial DNA damage are major causes for the induction of either apoptotic cell death or cellular senescence. Recent findings indicate that a functional impairment of the centrosome and mitotic spindle apparatus is as well sufficient to provoke a a premature senescence response (**Table 1**). Thus, like telomeres, centrosomes and kinetochores may take over a crucial role in regulating commitment of mitotic cells to premature senescence.

$p21^{WAF}$, downregulation of pRb (phosphorylated Retinoblastoma protein) which promotes S phase entry, formation of SAHFs, and increased SA-β-Gal positivity (**Figure 2C**; Schmidt et al., 2010). Interestingly, a comparable premature senescence phenotype was observed in p53 proficient tumor cells when Aurora-A kinase, the upstream regulator of TACC3, was either pharmacologically inhibited or depleted (Huck et al., 2010). These findings provide evidence that the centrosomal Aurora A - TACC3 axis (**Figure 2B**) regulates commitment of mitotic cells to cellular senescence. Given that on the one hand p53 levels rise during prolonged mitosis ("mitotic clock function of p53"; Blagosklonny et al., 2006), and on the other hand Aurora-A phosphorylates p53 at Ser315 and thereby targets p53 for degradation (Katayama et al., 2004), it is conceivable that mitotic p53 levels per se link spindle stress with cellular senescence via transcriptional induction of $p21^{WAF}$ in $G_1$. Aurora-A plays in this model an inhibitory role by keeping p53 levels below a critical threshold during normal cell division, whereas Aurora-A independent mechanisms may intervene upon prolonged mitotic arrest to increase p53 concentrations necessary for G1 arrest. Possible candidates for those positive p53 regulators would be the ataxia telangiectasia mutated (ATM) kinase and the cyclin dependent kinase inhibitor p19ARF that both prevent p53 degradation by the ubiquitin ligase MDM2 (Tritarelli et al., 2004; Dominguez-Brauer et al., 2010). However, neither deficiency for ATM nor p19ARF was able to prevent the embryonic lethal phenotype caused by TACC3 deficiency (Piekorz et al., unpublished results), whereas at the same time the lethality of TACC3 deficient embryos (being likely secondary to a growth arrest phenotype) could be rescued by genetically reducing p53 expression (Piekorz et al., 2002). Moreover, Vogel et al. observed that increase of p53 levels during transient mitotic arrest occurs independent of ATM, ATR, Chk1, and Chk2 (Vogel et al., 2004). Thus, during prolonged mitosis, p53 levels may simply accumulate due to the lack of Mdm2-mediated degradation, as Mdm2 levels are dependent on transcription which is absent in mitosis and only resumes in G1 (Blagosklonny, 2006).

As summarized in **Table 1**, altered expression or suppression of various proteins required for centrosomal integrity, kinetochore organisation, or spindle assembly checkpoint function, have now been linked to p53 activation and premature senescence. For instance, silencing of the mitotic spindle checkpoint kinase Bub1 or the centromere-localized histone H3 variant CENP-A activates a stress response characterized by typical senescence markers. Consistant with these findings, downregulation of Bub1 and CENP-A has been observed under conditions of oncogenic stress and during replicative senescence (Maehara et al., 2010; Gjoerup et al., 2007). Lasty, mouse models with genetic insufficiency for BubR1 or haploinsufficiency for Bub3 and Rae1 display progressive aneuploidy, premature senescence, and pathological phenotypes typical for accelerated aging (Baker et al., 2004; Baker et al., 2006). Taken together, these data uncover a particular role of spindle assembly checkpoint proteins in the regulation of senescence and organismal aging (Baker et al., 2005). However, it remains to be determined whether altered expression of the proteins summarized in Table 1 regulates not only the onset of the postmitotic senescence response but also modulates its severity. Interestingly, whereas the proliferation arrest upon treatment with the Aurora-A kinase inhibitor MLN8054 was irreversible (Huck et al., 2010), we observed indications for a reversible nature of the senescence phenotype caused by TACC3 depletion (Schmidt et al., 2010).

| Protein(s) | Localization & function | Mode of targeting function/expression | Effector mechanisms & senescence markers | References |
|---|---|---|---|---|
| **Aurora-A** | mitotic kinase | small drugs, siRNA | p53, $p21^{WAF}$, pRb, SA-β-Gal | Huck et al., 2010, Görgün et al., 2010 |
| **TACC3** | CT, MT dynamics | siRNA | p53, $p21^{WAF}$, pRb, SAHF, SA-β-Gal | Schmidt et al., 2010 |
| **PCM1** | CT integrity | siRNA | p53, $p21^{WAF}$, pRb, SA-β-Gal | Mikule et al., 2006; Srsen et al., 2006 |
| **Pericentrin** | CT integrity | siRNA | p53, $p21^{WAF}$, pRb, SA-β-Gal | Mikule et al., 2006; Srsen et al., 2006 |
| **$p31^{Comet}$** | SAC | overexpression | p53, $p21^{WAF}$, SA-β-Gal, PAI-1 | Yun et al., 2009 |
| **CENP-A** | KT organisation | OIS, RS, siRNA | $p16^{INK4a}$, p53, $p21^{WAF}$, SAHF, SA-β-Gal | Maehara et al., 2010 |
| **Bub1** | KT / SAC | RS, siRNA | p53, $p21^{WAF}$ | Gjoerup et al., 2007 |
| **Bub3/Rae1** | KT / SAC | **haploinsufficiency (*in vivo*)** | $p16^{INK4a}$, p53, $p21^{WAF}$, SA-β-Gal | Baker et al., 2006 |
| **BubR1** | KT / SAC | **hypomorphic alleles (*in vivo*)** | $p16^{INK4a}$, p53, $p21^{WAF}$, SA-β-Gal | Baker et al., 2004 |
| **Mad2** | KT / SAC | siRNA | p53, $p21^{WAF}$, SA-β–Gal, SASP | Prencipe et al., 2009 |

This table lists proteins of the mitotic spindle apparatus whose functional inhibition and/or the indicated changes in expression result in p53 activation and a cellular senescence response. Abbrevations: Bub, budding uninhibited by benzimidazoles; CENP, centromeric protein; CT, centrosomal; Mad, mitotic arrest deficient; KT, kinetochore; MT, microtubule; OIS, oncogene induced senescence; PAI, plasminogen activator inhibitor; PCM, pericentriolar material; pRb, phosphorylated Retinoblastoma protein; RS, replicative senescence; SAC, spindle assembly checkpoint; SAHF, senescence-associated heterochromatic foci; SASP, senescence-associated secretory phenotype; TACC, transforming acidic coiled coil.

***Table 1***: Proteins of the mitotic spindle apparatus linked to premature senescence.

## Perspective – Exogenous noxae, mitotic spindle stress, and aging

Genomic and mitochondrial DNAs are classical cellular targets for the detrimental and senescence inducing effects of various intrinsic and extrinsic noxae, including ROS, ionizing radiation, and UV light (**Figure 3**). In contrast to these cellular macromolecules, surpisingly little is known about DNA damage-independent molecular effects and protein targets of exogenous noxae at centrosomes and mitotic spindle apparatus. Mitotic dysfunction has a major impact on proliferative homeostasis and tissue regeneration, processes, which are progressively impaired during normal aging and characterized by the accumulation of senescent cells in proliferative tissues from aged primates (Jeyapalan et al., 2007; Campisi & Sedivy, 2009). Moreover, tetraploidy and polyploidy are biomarkers of aging as polyploid vascular smooth muscle cells with a senescent phenotype accumulate during aging (Yang et al., 2007). Given these observations it is conceivable that altered expression and/or functional inhibition of proteins of the mitotic spindle apparatus through the influence of intrinsic or extrinsic noxae contribute to aberrant mitosis and cytokinesis, tetraploidy and cellular senescence, and lastly dysfunction of major proliferative organs. In this regard, the proteins listed in **Table 1** represent bona fide candidates for decisive stress sensors which regulate the commitment of dividing cells to undergo premature senescence.

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## The DGZ welcomes the following new members:

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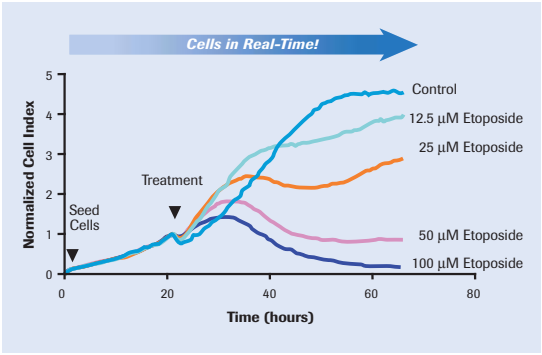


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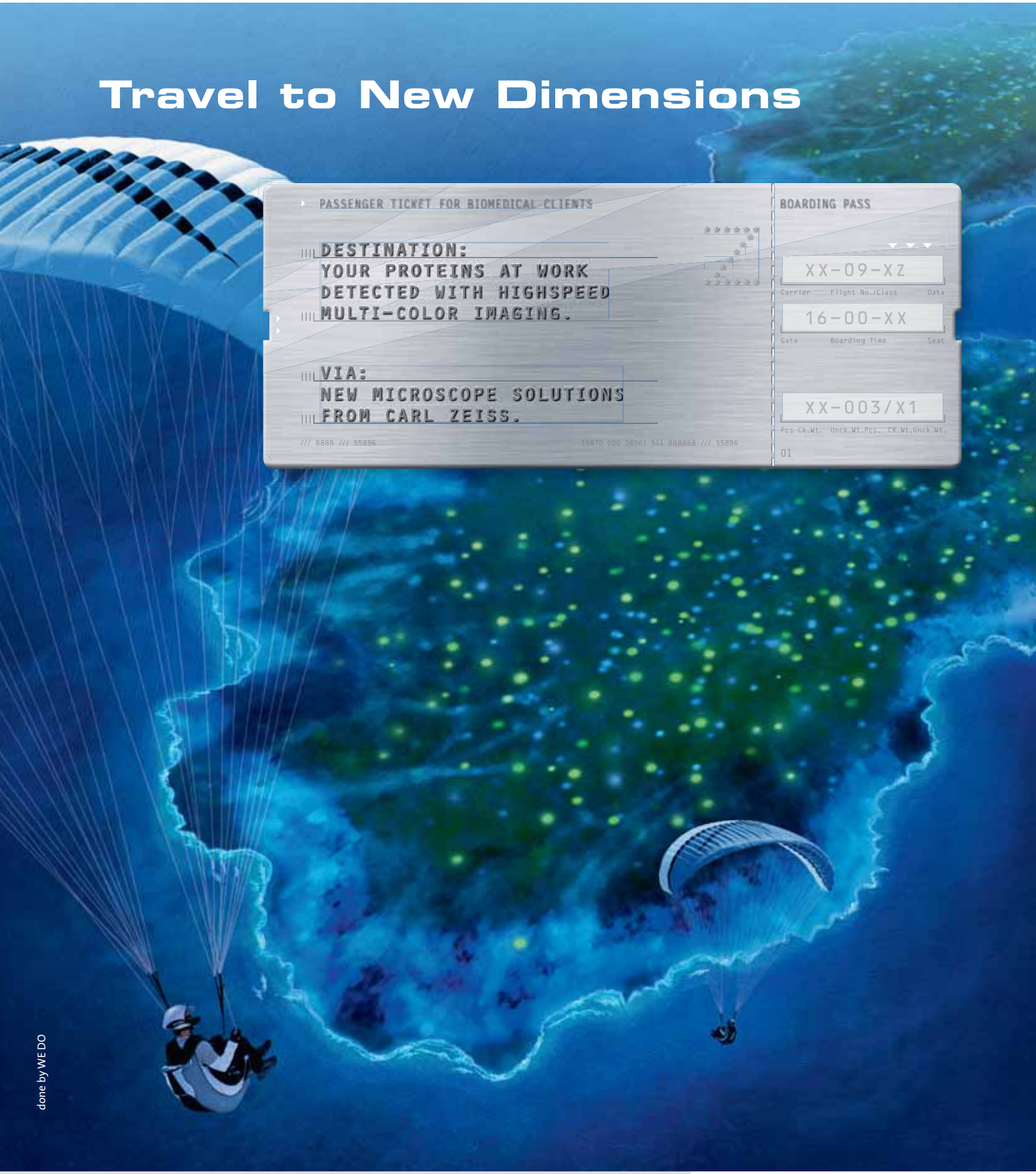


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