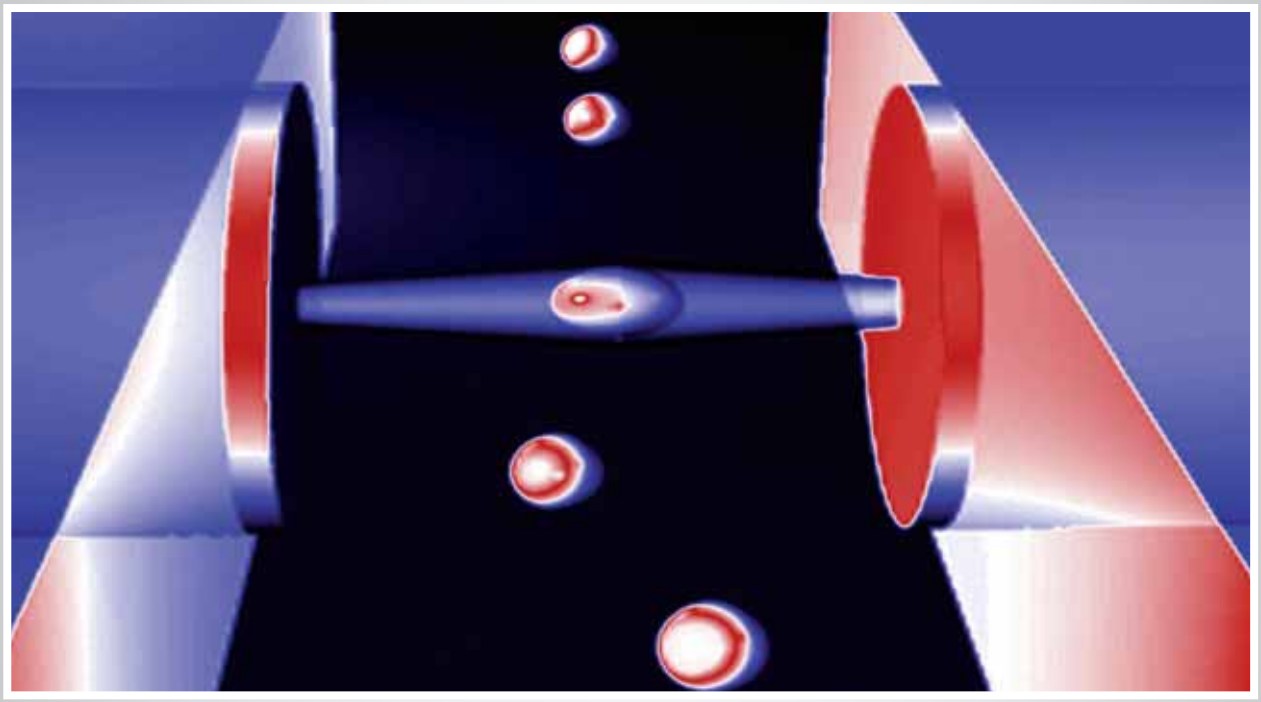
Cell News

Newsletter of the German Society for Cell Biology
Volume 37, 3/2011



Focus on Biophysics

Program for the Annual Meeting 2012 in Dresden

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Mitteilungen der Deutschen Gesellschaft für Zellbiologie

Cover image: The optical stretcher. Cells (red spheres) are flowed through a microfluidic system, where they are individually trapped by two counter-propagating laser beams (blue) for measurement of their elasticity. For more details see article by Kevin Chalut and colleagues (p. 29 - 33).

The next annual meeting in Dresden

The program for the next annual meeting in Dresden, March 2012, has taken shape and a day-by-day summary, as it stands now, is depicted on the next page. The organizers have done a terrific job, and in my opinion they have assembled, like in recent years, a very attractive program with a broad spectrum of topics that cover the "hottest" fields cell biologists are engaged in at present. Therefore, we hope that it will address a reasonable number of our members and attract, like in previous meetings, many new master and PhD students to present their work at a major conference for the first time. As in previous years, several chairs of sessions are from abroad, and the same holds true for the speakers invited such that our meeting will have indeed a strong international touch.

Two highlights of the meeting are the special lectures. The first lecture, the ***Carl Zeiss Lecture*** 2012, will be given by Fiona Watt from Cambridge. Fiona is Deputy Director of the Wellcome Trust Centre for Stem Cell Research, Herchel Smith Professor of Molecular Genetics and Deputy Director of Cancer Research UK - Cambridge Research Institute. Notably, she is conducting research with two groups of researchers: one located in the Wellcome Trust Centre for Stem Cell Research (University of Cambridge, CSCR), and the other in the Cancer Research UK -Cambridge Research Institute (CRI). As announced on the Watt Group website, Fiona is indeed committing herself to quite some change: "We are excited to announce that the lab is relocating to King's College London in 2012. Fiona Watt will be the director of a new centre for stem cells and regenerative medicine". Hence, from these few personal data you may imagine that you will meet one of the most eminent and influential scientists in stem cell research. Actually, if you go to Pubmed, you will learn that Fiona's first paper in 1978 (according to Pubmed) listed her as first author together with H. (Henry) Harris from Oxford and with Mary Osborn and Klaus Weber from Göttingen: *The distribution of actin cables and microtubules in hybrids between malignant and non-malignant cells, and in tumours derived from them*. She then went on to publish with Howard Green (MIT and Harvard University) pioneering work on epidermal cells. As soon as she had started her independent career, papers were published continuously. Early on, she wrote together with Peter A. Hall one of the high impact reviews on stem cells (Development 106, 619-633 (1989) *Stem cells: the generation and maintenance of cellular diversity*). And, as most of you will know, Fiona has been Editor-in-Chief of the Journal of Cell Science since 1992. Last but not least, she is married and has three children.

The second special lecture, the ***Frontiers in Science Lecture***, will be given by Kai Simons, who probably does not need any introduction for German scientists, except maybe for young researchers, as he is a dominant figure in cell biology in Germany. Kai is presently a group leader at the Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, and he was indeed one of the founding directors of this institute, subsequently serving as one of the directors from 1998 until 2006. Born and educated in Helsinki, Finland, where he got his MD in 1964; he worked as a post-doctoral fellow at Rockefeller University and came to Heidelberg in 1975 to serve as a group leader at EMBL until the year 2000. In addition, from 1982 to 1997 Kai was the coordinator of the *Cell Biology Programme* at the EMBL, a very important issue for the development of cell biology in Germany and Europe. In the centre of Kai's work is the study of membranes and their properties for the organization of a living cell, in particular the role of so-called lipid rafts as dynamic platforms for the generation of functional membranes.

On stage, 115 speakers

The five plenary sessions will present five invited speakers each, starting with "Nuclear organization" on Wednesday followed by two afternoon sessions each on the next two days. In the morning, from Thursday to Saturday, we will have five symposia each, for which three speakers are invited and three will be selected from the submitted abstracts. Hence, there will be in total 45 attendees selected from the submitted abstracts to present their work as a *Short Talk*.

Posters sessions are central

Based on the experience we made at the 2011 annual meeting in Bonn, with more than 260 posters being presented with very vivid discussions of participants, we decided to have the meeting this time not in a university building but in a congress centre, the *International Congress Center Dresden*, that provides enough space so that we will have the opportunity to display posters side by side with the commercial exhibition and have enough room for proper discussion and interaction.

The Congress Center is in walking distance to the centre of town. From its terrace participants will have a gorgeous view and good opportunity to gather with colleagues for a chat and a breath of fresh air. As other East-German cities too, Dresden is not expensive compared to Bonn, Heidelberg or Hamburg. Inexpensive accommodation is available, for instance, the many comfortable hostels offer unbeatable rates. As during lunch time food will be served, this meeting should not need a large travel budget but will be affordable for students from groups with small travel funds. Last but not least, as every year the DGZ will provide upon application a limited number of travel scholarships (see p. 4).

Physics of cancer

From October 13th to 15th, the DGZ Special Interest Meeting "Physics of Cancer" will be held in the centre of Leipzig. In anticipation of this meeting, this current issue of *Cell News* turned into a "Focus on Biophysics". Articles by Ewa Paluch (Dresden), Sarah Köster (Göttingen), Jochen Guck (Cambridge, UK) and Michael Beil (Ulm) give an impression how physics changed the way modern cell biology is treated. The program for this meeting is available at

http://www.softmatterphysics.com/.

Even though the deadline for application is over, last minute visitors will be able to register at the site. We hope that this meeting will bring biophysicists and cell biologists closer together, and eventually we may witness the emergence of something like a "Physical Cell Biology".

Harald Herrmann

Program

March 21-24, 2012 in Dresden

Organized by Elisabeth Knust, Ewa Paluch and Marino Zerial
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden

Carl Zeiss Lecture:

21.3. Wednesday:

Fiona Watt (Cambridge)

Frontiers in Science Lecture:

23.3. Friday:

Kai Simons (Dresden)

Plenary Sessions and Chairs:

21.3. Wednesday:

Nuclear organization, Ivan Raska (Prag)

22.3. Thursday:

Cell and tissue morphogenesis, Elisabeth Knust (Dresden)

Cell adhesion and migration, Maria Leptin (Heidelberg)

23.3. Friday:

Cilia, Lotte Pedersen (Kopenhagen)

Frontiers in microscopy, Petra Schwille (München)

Symposia and Chairs:

22.3. Thursday:

Modelling in cell biology, Ewa Paluch (Dresden)

miRNA and cancer, Marcus Peter (Chicago)

Cell metabolism and cell homeostasis, Mike Hall (Basel)

Regeneration and stem cells, Rüdiger Simon (Düsseldorf)

Microtubules and motors, Zeynep Ökten (München)

23.3. Friday:

Asymmetric division – Mechanics of cell division,

Daniel Gerlich (Zürich)

Autophagy and cross-talk between organelles,
Zvulun Elazar (Rehovot)

Cell biology of the immune response, Jack Neefjes
(Amsterdam)

Control of cell and organ size, Aurelio Teleman (Heidelberg)

Cytoskeleton mechanics, Andreas Bausch (München)

24.3. Saturday:

Evolution of the cell, Gaspar Jékély (Tübingen)

Meiosis, Wolfgang Zachariae (München)

Cell biology of therapeutic delivery, Lennart Rome
(Los Angeles)

Neuronal network, Gaia Tavosanis (München)

Protein aggregates, Zoya Ignatova (Potsdam)

www.zellbiologie2012.de

Travel grants for young DGZ members

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

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 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
   DGZ annual meeting
4. Information about your income
5. Travel expenses for a second class train return ticket to Dresden
6. Your bank account data for reimbursement

Please send your application by mail or e-mail to:

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

Deadline for applications: **January 31, 2012**

Applications received after the deadline cannot be considered anymore.

Walther Flemming Medal 2012

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 EUR 4000, partly sponsored by the European Journal of Cell Biology.

Applications have to consist of a cover letter, a CV and a list of publications. The subject of the application should relate to one distinct field of research. In addition, a short summary of the work and a compelling description of the importance of the work for cell biology should be presented.

Both individual applications and nominations are accepted. 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 on March 21-24, 2011 in Dresden.

Please send your application by e-mail (and in parallel one hard copy by mail) to the DGZ office:

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

Deadline for applications:
January 15, 2012

Binder Innovation Prize 2012

The Binder Innovation Prize is founded by BINDER GmbH in Tuttlingen and awarded by the German Society for Cell Biology (DGZ). It is endowed with EUR 4000 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. DGZ membership is desired but not required.

Applications have to consist of a cover letter, CV and a research profile.

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 on March 21-24, 2012 in Dresden.

Please send your application by e-mail (and in parallel one hard copy by mail) to the DGZ office:

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

Deadline for applications: January 15, 2012

Werner Risau Prize 2012 for Outstanding Studies in Endothelial Cell Biology

Together with the German Society for Cell Biology (DGZ) the prize comittee will award a prize 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 EUR 4000.

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. rer. nat. Rupert Hallmann
Institute of Physiological Chemistry and Pathobiochemistry
Westfälische Wilhelms-Universität Münster
Waldeyerstr. 15
D-48161 Münster, Germany
E-mail: hallmanr@uni-muenster.de

Deadline for applications: January 15, 2012

The prize will be awarded during the Annual Meeting of the German Society for Cell Biology (March 21 – 24, 2012) in Dresden, Germany.

Hanswalter Zentgraf, Mitglied des Gründungsvorstands der Deutschen Gesellschaft für Zellbiologie (DGZ), am 18. Juli 2011 verstorben

Er hatte diese kleine komödiantische "Schrulle" drauf – auf dem Flur, im Labor oder sonstwo: Wenn er etwas Neues oder besonders Schönes gefunden und belegt hatte – als Elektronenmikroskop- oder Immunfluoreszenz-Bild etwa oder als Western Blot–-, winkte er einen – bühnenreif – verschmitzt-lächelnd und mit pseudo-konspirativem Gehabe zu sich heran, das Lächeln wurde zum Grinsen und er holte irgendwo hinter seinem Rücken oder sonstwoher das Dokument, zeigte es und sagte gedehnt: "Jahaha! Das isses!" So in dem Sinne von: Das haben wir jetzt erwischt! Und weidete sich mit dem Betrachter an der technischen Schönheit oder der Bedeutung des Fundes.

Hanswalter ist nicht mehr unter uns. Oder halt: Das stimmt so nicht, es wird ihn noch sehr lange geben – in seinen Entdeckungen, seinen Bildern, seinen Reagenzien, vor allem den in den letzten Jahrzehnten geschaffenen – unglaublich guten und unglaublich schnell – über Lymphknotenpräparationen – gewonnenen – Antikörpern, viele davon weltweit in der Diagnostik eingesetzt. Und er lebt natürlich in seinen 254 Veröffentlichungen* und Patentschriften weiter. Das ist ja das Tröstliche bei uns Naturwissenschaftlern! So wird er weiter zu uns sprechen. Das Schwarz-Weiß-Bild zeigt ihn wie er leibt und lebt und vorträgt.

Hanswalter Zentgraf wurde am 2. Februar 1944 (mein Gott, was für eine Zeit!) in Georgenthal (Thüringen) geboren. Nach dem Abitur 1963 im hessischen Lauterbach studierte er Biologie und Chemie an der Universität Freiburg i. Br., was er 1969 mit dem Staatsexamen und 1974 mit der Promotion abschloss.

Nach einem Jahr in der Abteilung für Membranbiologie und Biochemie am Deutschen Krebsforschungszentrum nahm er 1975 eine Stellung als Leiter der "Einheit Elektronenmikroskopie" im Institut für Virusforschung (später Angewandte Tumorvirologie) an. Nach seiner Habilitation an der Fakultät für Biologie der Heidelberger Universität 1989 mit Bildern atemraubender Qualität (Titel: "Evidence for the existense of globular units in the supranucleosomal organization of chromatin") erhielt er die venia legendi für das Fach Genetik und wurde 2001 zum apl. Professor ernannt.

Hanswalter war für die Gründung der DGZ am 28. April 1975 wesentlich und erfolgreich als Sekretär – als "Schaffer" wie man in der Kurpfalz doppeldeutig gern sagt – und wurde deshalb zweimal wiedergewählt, bis er 1979 und 1980 mit besonderem Einsatz bei der Vorbereitung und der Durchführung des "Second International Congress on Cell Biology" im September 1980 in Berlin zum Erfolg dieses weltweiten Treffens beitrug. Das mittlere Bild zeigt ihn im Diskussionsgespräch mit den Pathologen Fritz Miller (München) und Helmut Denk (Graz, links).

Hanswalter war besonders bekannt für seine effektiven und angenehmen wissenschaftlichen Kooperationen, wie auch schon seine Publikationen ausweisen, wobei eine außerordentliche "Treue" über Jahrzehnte hin auffällt. So gibt es z.B. gemeinsame Veröffentlichungen mit Ada und Don Olins von 1980 bis 2011 (ein Bild dieser drei mit den Olins-Kindern beim Kongress in Berlin 1980 zeigt Hanswalter in dieser seiner menschlichen Persönlichkeit).

Hanswalter Zentgraf starb an den Spätfolgen eines Unfalls. Er hinterlässt eine Tochter und einen Sohn. 1994 heiratete er seine langjährige Kollegin und Ko-Autorin Ulrike Müller.

Werner W. Franke

* Das Veröffentlichungsverzeichnis von Hanswalter Zentgraf wird vom 1. Oktober 2011 auf der "Web-Site" des Autors dieses Nachrufs zu sehen sein.

Keeping kinetochores on track

Patrick Meraldi

Background:

In every cell cycle the genome must be duplicated and equally distributed into two future daughter cells. Each chromatid is replicated during S-phase and the duplicated sister chromatids are segregated during mitosis. Chromosome segregation is controlled in eukaryotes by the microtubule-based mitotic spindle and kinetochores, which are microtubule binding sites built on specialized chromosomal regions called centromeres (Santaguida and Musacchio, 2009; Walczak and Heald, 2008). The mitotic spindle has a bipolar structure, as microtubules are anchored with their minus ends at both spindle poles, and bind the kinetochores via their plus-ends. Faithful chromosome segregation requires that each sister-kinetochore pair binds microtubules emanating from opposite spindle poles (amphitelic or bipolar attachment). In case of errors, or absence of attachments, kinetochores engage the spindle checkpoint, leading to a delay in anaphase onset (Khodjakov and Pines, 2010). This safeguard mechanism guarantees that anaphase only occurs once all kinetochore pairs are attached in a bipolar manner. This set of events is essential to prevent genetic instability at the chromosomal level and the development of chromosomal imbalances (aneuploidy), a phenomenon that is present in about 85% of solid human tumours (Weaver and Cleveland, 2006).

Kinetochores are not only the centromeric microtubule attachment sites and key sensors for the spindle checkpoint, they also form the machinery that is responsible for mitotic chromosome movements, in particular congression to the metaphase plate and separation of the sister chromatids at anaphase onset (Kops et al., 2010). Kinetochores regulate chromosome movements both through microtubule motor proteins (kinesins and dynein) and by regulating the dynamics of the microtubule plus-ends they are bound to. Kinetochores also stabilize the microtubules they are bound to, ensuring that chromosomes remain attached to the spindle during all phases of chromosome segregation. The importance of kinetochores is illustrated by the fact that in unicellular organisms, such as the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, or in multi-cellular organisms such the nematode *Caenorhabditis elegans* or the fruit fly *Drosophila melanogaster*, most kinetochore proteins are essential. This is also true in vertebrates, where many kinetochores are essential, even at the cellular level.

The multiple functions of kinetochores are reflected in their complex composition, with over hundred different proteins, which self-associate in several functional subcomplexes. Most of these kinetochore proteins were identified over the last 10-12 years using a combination of genetic, cell biological, biochemical, and bioinformatic approaches in various model organisms. The key challenge since then has been to determine the structural architecture of kinetochores, define the functions of its different subcomponents, and understand its regulation, both in response to the rapid changes in microtubule dynamics or to sense erroneous attachments for spindle checkpoint signalling. Here, we present some of the key advances obtained in the last six years on the biology of kinetochores, both through our work and through the work of many other groups studying this exciting structure.

Kinetochores, a highly conserved structure only at second look

The study of kinetochores proteins has been difficult for a long time for two main reasons: firstly the proteins are only present in low abundance, and until very recently the purification of entire kinetochores was elusive (Akiyoshi et al., 2010). This delayed the functional study of kinetochore proteins, as only a few of them were known. The second difficulty is that the protein sequences of most kinetochore subunits are evolutionarily highly divergent. For a long time, the sequences of fungal kinetochore proteins were not sufficient to identify metazoan kinetochore proteins and vice-versa in a BLAST search, a fact that considerably delayed comparative functional studies. As an example, in 2005 only two structural proteins were known in *D. melanogaster*, compared to over 60 in *S. cerevisiae* (Heeger et al., 2005). To make things more complicated, it was also known that the structure and size of kinetochores vary strongly from organism to organism (Przewloka and Glover, 2009). The kinetochores of most eukaryotes are built on so-called regional centromeres, long stretches of repetitive AT-rich sequence that lack any sequence-specificity. Such kinetochores can be built on centromeres that are as small as a few kilobases and only bind 3 microtubules (*S. pombe*), or sit on centromeres of several megabases and bind up to 25 microtubules (mammalian cells). The range is even wider if one considers the kinetochores of certain budding yeasts (including *S. cerevisiae*), which are built on short (125bp) and sequence-specific point centromeres and only bind one microtubule, or to the other extreme, certain nematodes (including *C. elegans*), which possess holocentric centro-

meres that span the entire length of the chromosomes and are bound by a multitude of microtubules. Given the high diversity in structure and sequence, it was unclear to which extent the composition of kinetochores in eukaryotes would be conserved.

However, a parallel steady increase in fully sequenced genomes and in the number of annotated kinetochore proteins over time allowed a more precise bioinformatic analysis, which revealed that most kinetochore proteins are conserved amongst eukaryotes. In particular, our group developed an algorithm to identify novel kinetochore proteins both in fungi, plants and metazoans using known *S. cerevisiae* or *S. pombe* kinetochore proteins as a starting point (Meraldi et al., 2006). Since the sequences of kinetochore proteins are often only conserved in very short stretches, we generated multiple-sequence alignments of fungal kinetochore proteins to identify these short stretches and use them as search patterns against metazoan or plant databases. This approach identified seven novel orthologous kinetochore proteins in mammals, three novel proteins in *D. melanogaster* and two novel proteins in plants. These results implied that most kinetochore proteins are conserved in eukaryotes, a finding that was confirmed by biochemical and genetic studies identifying those same proteins as *bona fide* kinetochore proteins (Cheeseman et al., 2004; Foltz et al., 2006; Obuse et al., 2004; Okada et al., 2006; Schittenhelm et al., 2007). The overall conclusion of these studies was that the core of eukaryotic kinetochores is composed of two large conserved protein networks: on one side the KMN network, which consists of Knl-1, the hetero-tetrameric MIND/Mis12 subcomplex, and the hetero-tetrameric-NDC80 subcomplex, and on the other side the CCAN network (Constitutive Centromere Associated Network, which is also called CENP-A NAC/CAD or CENP-H/I complex), which consists of 15 subunits. Soon after the NDC80 subcomplex was recognized as the key microtubule attachment site at kinetochores, which is essential for the binding to spindle microtubules in all eukaryotes (Cheeseman et al., 2006; DeLuca et al., 2006).

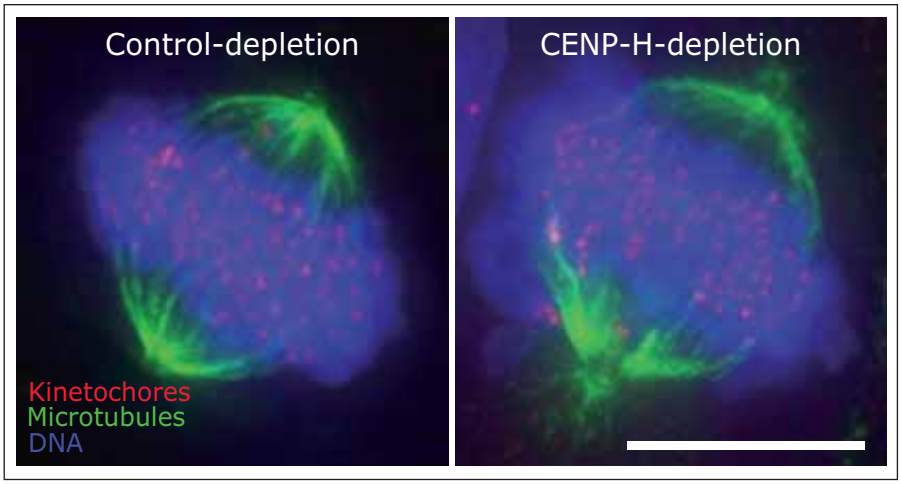

Fig. 1.: Disruption of the CCAN network impairs correct chromosome movements. Shown are representative immunofluorescence pictures of CENP-H- or Control-depleted HeLa metaphase cells stained with antibodies against CREST (red; kinetochores), antibodies against α-tubulin (green; microtubules) and 4,6-diamidino-2-phenylindole (DAPI for DNA; blue; courtesy of A.C. Amaro). Note the tight metaphase plate in control-depleted cells, and the multiple unaligned chromosomes in cells lacking the CCAN network (CENP-H depletion; white arrows). Scale bar = 10 µm

Kinetochores, key drivers of chromosome movements

Once kinetochores have established a stable microtubule-attachment through the NDC80 complex, one of their essential functions is to drive the chromosomes to the metaphase plate, a process that is less well understood. In mammalian cells, one can distinguish two phases in this process: first kinetochores establish lateral attachments to microtubules (in an NDC80 independent manner), allowing them to congress to the centre of spindle to form stable bipolar, end-on attachments via the NDC80 complex (Cai et al., 2009). Microtubule motors, in particular the kinesin CENP-E and dynein, control the movements in this initial phase (Kapoor et al., 2006; Vorozhko et al., 2008). In a second step, once kinetochore pairs are attached in a bipolar manner, chromosome movements are driven by changes in the dynamics of kinetochore-microtubules (Jaqaman et al., 2010; Tirnauer et al., 2002). However, the mechanisms by which kinetochores control plus-end microtubule dynamics have only starting to emerge recently. One difficulty is that these dynamics cannot be easily measured since kinetochores are not bound to a single microtubule, but rather are attached to 25 microtubules, which are bundled into a kinetochore-fibre, all of which have different inherent dynamics (VandenBeldt et al., 2006). Therefore, one key step in the study of kinetochore movements was the joint development (in collaboration with the Danuser, McAinsh and Swedlow groups) of a kinetochore-tracking assay in human cells (Jaqaman et al., 2010). This assay, which was based on the computational analysis of rapid and high-resolution recordings of GFP-labelled kinetochores in dividing human cells, allowed for first time to quantify and characterize the exact movements of human sister-kinetochores in 4-dimension, and to screen for perturbations that affect these movements. It revealed that metaphase sister-kinetochore pairs undergo semi-regular oscillations along the spindle axis, a phenomenon that had been previously

described in a qualitative manner (Skibbens et al., 1993). Our automated assay further showed that the speed of kinetochore movements is controlled antagonistically by two microtubule-depolymerases located at kinetochores, the kinesins MCAK and Kif18a, consistent with previous studies on Kif18a (Jaqaman et al., 2010; Stumpff et al., 2008). However, the depletion of either depolymerase did not affect the semi-regularity of sister-kinetochore oscillations, indicating that other components must be controlling the regularity of chromosome movements. Soon after, using the same assay we could show that the CCAN network is essential for sister-kinetochore oscillations and that it plays an essential role in the control of microtubule dynamics (Amaro et al., 2010). Indeed, CENP-H depletion, which disrupts the entire CCAN complex, abolishes regular sister-kinetochore oscillations, leading to rapid and erratic kinetochore movements and a disorganized metaphase plate (Figure 1). This phenotype is due to the ability of the CCAN network to control the turnover of kinetochore-microtubules. While free mitotic microtubules have a half-life of 10-15 seconds, kinetochore-microtubules have a much slower turnover of 4-6 minutes (Zhai et al., 1995). However, in the absence of CENP-H kinetochore-microtubule plus-ends show a turnover of 10-15 seconds, indicating that kinetochores have lost the ability to suppress the rapid turnover of mitotic microtubules (Amaro et al., 2010). Importantly, the CCAN network is most likely directly controlling microtubule dynamics: at least one CCAN subunit, CENP-Q, can efficiently bind microtubules *in vitro* and our live cell imaging of GFP-CENP-I, another CCAN subunit, indicates that it preferentially accumulates on the sister-kinetochore bound to growing microtubules, a behaviour that is only known for a few microtubule-binding proteins (Amaro et al., 2010). Interestingly, the CCAN network directly binds to the centromeric CENP-A nucleosomes and contributes to the assembly of the centromeric nucleosomes, suggesting that it acts as a link between centromeric DNA and the microtubule plus-ends (Carroll et al., 2009; Foltz et al., 2006; Okada et al., 2006). One critical challenge for the future will be to determine the molecular mechanisms by which the CCAN network controls the turnover rate of kinetochore-microtubules, and the precise function of the individual components of this large protein network in this process.

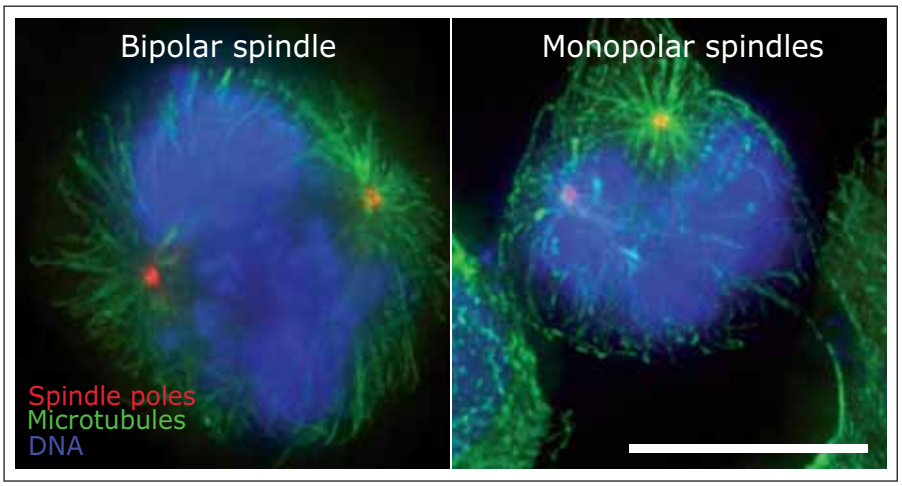

Fig. 2.: Depletion of the CCAN kinetochore protein CENP-L leads to the formation of transient monopolar spindles in 30% of the cells. Shown are representative immunofluorescence pictures of CENP-L-depleted HeLa cells in prometaphase stained with antibodies against γ-tubulin (red; spindle poles), antibodies against α-tubulin (green; microtubules) and 4,6-diamidino-2-phenylindole (DAPI for DNA; blue; courtesy of N. Mchedlishvili). While the majority of CENP-L depleted cells display a normal bipolar spindle (left panel), about 30% of cells lacking CENP-L show a monopolar configuration. This population of cells with monopolar spindles reflects the inability of CENP-L depleted cells to rapidly separate the two spindle poles during prometaphase. This shows how a local dysfunction at kinetochores can affect the global architecture of the mitotic spindle. Scale bar = 10 µm

Kinetochore function has a global effect on the dynamics of the mitotic spindle

Our investigation of CCAN kinetochore proteins suggested early on that kinetochores are not just controlling chromosome movements, but that in addition they globally affect spindle morphology and dynamics. Indeed, when we depleted the CCAN proteins CENP-O or CENP-L, we found that those depletions led to an accumulation of transient monopolar spindles in about 30-40% of the cells (McAinsh et al., 2006; McClelland et al., 2007; Mchedlishvili et al., 2011; Toso et al., 2009). This suggested that kinetochores are not only driving chromosome movements at the local level, but have a more global effect on spindle architecture. Our live-cell imaging analysis of CENP-O or CENP-L-depleted cells revealed that this effect was linked to the respective history of centrosome separation at the single cell level. If cells had been able to separate their centrosomes before nuclear envelope breakdown, CENP-O or CENP-L depletion did not affect spindle formation, chromosome alignment or chromosome segregation (Mchedlishvili et al., 2011; Toso et al., 2009). In contrast, if cells had not yet separated their centrosomes at nuclear envelope breakdown – a phenomenon that has been observed in many cancerous or primary cell lines – CENP-O or CENP-L depletion delay centrosome separation by 6-9 minutes, leading to a prolonged monopolar spindle conformation (Figure 2; Mchedlishvili et al., 2011; Toso et al., 2009). Our recent data show that even such a small delay will result in a very severe delay of chromosome

alignment and a 2.5-fold increase in the error rate of chromosome segregation, indicating that the rapid formation of a bipolar spindle with the help of a kinetochore-based pushing force plays a crucial role in faithful transmission of chromosomes (Mchedlishvili et al., 2011). At the more mechanistic level, our investigations further revealed that this kinetochore-pushing force in early prometaphase depends on the ability of kinetochores to incorporate tubulin subunits at the microtubule plus end, causing bipolarly-attached kinetochores to accelerate the separation of the two spindle poles (Toso et al., 2009). Both CENP-O and CENP-L depletions reduce this force, by weakening kinetochore-microtubules, reflecting the influence of the CCAN complex on kinetochore-fiber dynamics. These studies therefore indicate that kinetochores are not just local players that control the movements of individual chromosomes, but that they play a crucial global role in the dynamics of the entire mitotic spindle. These studies also open up a new exciting field for the future where we will not only have to understand the function and composition of a single kinetochore, but we will increasingly have to dissect how local microtubule dynamics at kinetochores at one end, and spindle poles at the other end are coordinated during cell division, to ensure the correct systemic function of the mitotic spindle.

Acknowledgments

I thank Jason Swedlow (Univerisity of Dundee), Gaudenz Danuser (Harvard University) and in particular Andrew McAinsh (University of Warwick) for our fruitful collaborations, all my co-workers for their exciting research, and the Swiss National Fund, the ETH Zurich, EURYI, and the Swiss Cancer league for financial support.

Selected Publications

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WALTHER FLEMMING MEDAL

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10 years of the human genome: so what?

Stefan Wiemann

Starting with the sequencing of the human and a plethora of other genomes (http://www.ncbi.nlm.nih.gov/sites/genome), a number of fields have been transformed from small-scale into high-throughput science. A potential benefit of the technologies, their application and, in particular, of high-throughput research and its results are not always obvious. Here I would like to demonstrate, with help of some examples, how omics' kind of research has impacted other disciplines, including cell biology.

What are -omes and -omics?

"The suffix *-ome* as used in molecular biology refers to a totality of some sort" (http://www.wikipedia.org/wiki/Omics; accessed August 9, 2011) and "Omics is a general term for a broad discipline of science and engineering for analyzing the interactions of biological information objects in various omes" (http://omics.org/index.php/What_is_omics; accessed August 5, 2011). Consequently, omics type of research aims to be comprehensive in the analysis of some kind of biomolecules or their activities. Starting from genomics, the number of -omes that are currently studied has constantly expanded, the sense of some being somewhat questionable. All these fields of research have in common that they exploit technology developments within large-scale applications.

Technology development stimulates research - and vice versa

The sequencing of the first human genome was done with Sanger technology and took about 10 years at a cost of several billion dollars [1]. With next generation sequencing both the time and cost have dropped by several orders of magnitude since. Large projects like the 1000genomes project [2], The Cancer Genome Atlas [3], or the International Cancer Genome Consortium [4] sequence many genomes, epigenomes and transcriptomes in order to catalog the natural as well as the disease-inducing variation. These projects became only feasible owing to breathtaking advances in sequencing technologies, however, the projects also stimulate(d) further technology development. Similar dependencies of technology development and application are apparent also in other omics sciences: In the "proteomics" field, this term had been mostly associated with 2D gel electrophoresis some 10 years ago. However, nowadays mass spectrometry [5] and other qualitative or quantitative technologies, like protein arrays, allow for a much improved sensitivity and/or throughput and reproducibility. Relative and absolute quantification of thousands of proteins in complex lysates are now feasible, allowing proteome-wide analyses of effects that diverse kinds of diseases or experimental perturbations induce in the studied systems.

Some lessons learned

Up until 2001 the number of (protein coding) genes was a hot debate. For example, a so-called "GeneSweep" contest was carried out at a Cold Spring Harbor genome conference in 2000, where estimates for the number of protein-coding genes in the human genome were collected [6]. Votes ranged from about 25,000 to well over 100,000 with a mean of some 60,000 genes# (Fig. 1.).

Only when the genome sequence had been unraveled (note: the genome has still not been completed as several hundred million base pairs cannot be determined with current technologies) the number could be narrowed down to a much smaller range (current estimates range from something between 19,000 up to 24,000) and could a proper annotation of the genetic elements begin. This rather small number of our protein coding genes came somewhat as a surprise as this is not much different from the number of genes in *Drosophila* or *Caenorhabditis elegans*. Consequently, the regulation and function(s) of our genes and their products need to be unraveled in order to (better) understand why we are different from, for example, the mouse. Along this line, international efforts were carried out in parallel to the sequencing of the genome aiming to systematically clone and sequence cDNAs. These projects created a wealth of information as

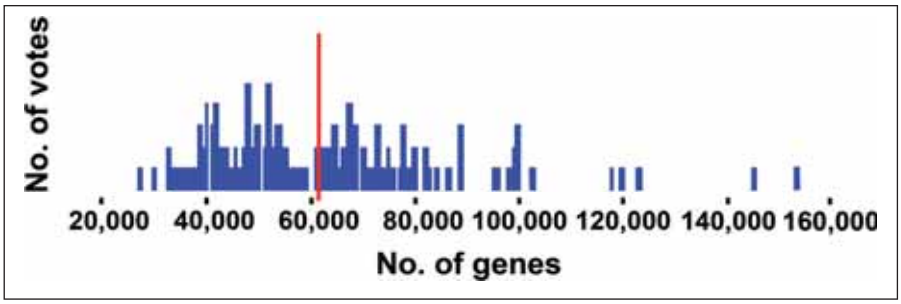

Fig. 1: *Distribution of votes in the "GeneSweep" contest that was conducted by Ewan Birney at the Cold Spring Harbor Genome Meeting in 2000. Red line indicates the mean of all estimates.*

to the expressed fraction of the genome as well as to the variability of the "transcriptome". Information on the alternative usage of promoters, splicing, and polyadenylation helped to identify "genes" (I am unsure if there is yet a clear definition of this term that would correctly describe all flavors the 'units of heredity' may comprise) and to generate comprehensive catalogs of the coding part of the genome [7, 8]. Based on these findings the roles of non-protein coding genes and of other transcribed sequences were begun to be appreciated. Building on this information, technologies were developed, like gene expression profiling and tiling arrays, that helped to unravel differentially expressed genes in disease conditions and to discover that a much larger fraction of the genome appeared to be transcribed than had previously been anticipated, respectively. Furthermore, the genome sequence was basis for an explosion of research on miRNAs, permitted systematic analysis of promoter regions and other regulatory elements e.g. via nucleosome positioning and its impact on transcription [9], the detection of new types of DNA modifications [10] as well as unexpected mechanisms for genome maintenance [11], just to name a few. Recent data even suggests that the 'final' RNA in many cases would not be a direct copy of the master DNA but that rather a large number of sequence differences would exist in the same cell [12]. The genome is likely to still hide secrets that await discovery. Having available huge amounts of data, however, is no guarantee that this can be readily interpreted. For example, there has been a recent dispute whether or not the available data would indeed support extensive so-called pervasive transcription of the human genome [13, 14].

Tools – the missing link?

In a comment published in Nature this year, Aled M. Edwards and colleagues [15] argued that, despite the human genome was sequenced 10 years ago, still most protein research focus would be on the same genes that had been studied before. They illustrate this notion in a graph showing the number of publications that have been published prior to and some years after release of the genome sequence on the more than 500 human protein kinases. Disturbingly, the number of kinases that have been the matter in these publications appears to not having substantially increased, most kinases seem to be still untouched (Fig. 2.).

Asking for the reasons for this lack of attention in the majority of genes the authors argue that the availability of proper tools would be prerequisite to extend research also to the yet orphan genes and proteins. There, the omics' type of research is ready to step in, as any large-scale applications require tools for these studies. Consequently, the technological developments of the past years have been accompanied by a revolution also in the generation of research tools. These developments would all not have been possible without the genome and transcriptome sequences of human, mouse and other model organisms, as these defined the gene sets and thus the targets for tool production. The article mentions antibody resources and chemical probes as examples for such missing tools. Academic projects [16] and a number of companies now generate and distribute antibodies for a rapidly increasing fraction of the human proteins; chemical libraries are increasingly screened for inhibitors of biochemical processes.

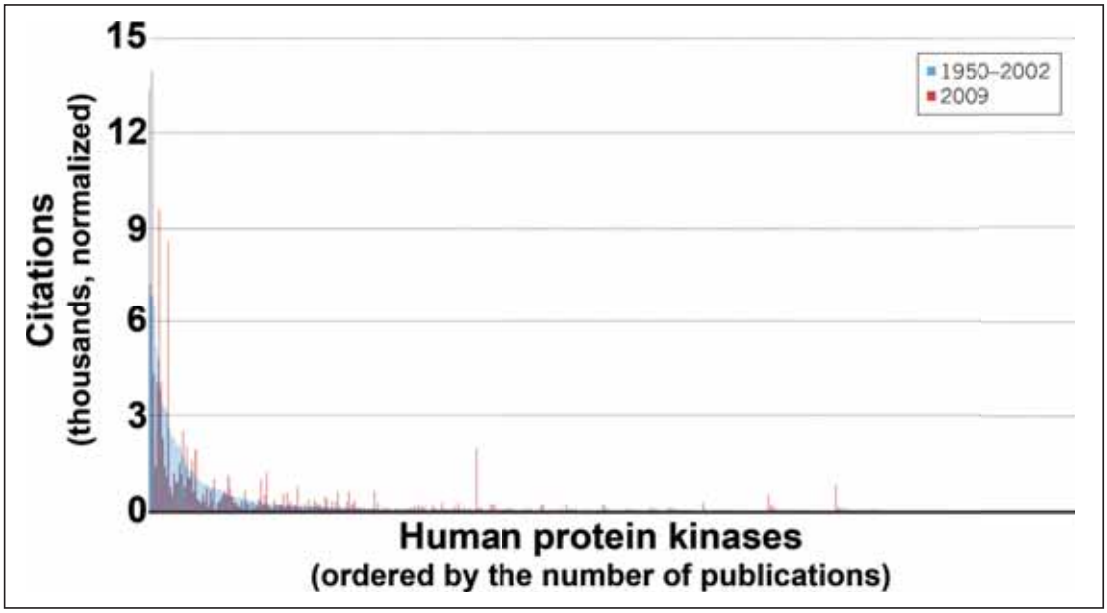

Fig. 2: *The same few protein kinases that had been in the focus of research between 1950 and 2002 (blue) have been worked on also several years (in 2009) after completion of the human genome (red), except for some kinases having since been linked to disease or that are of special interest to industry. Adapted by permission from Macmillan Publishers Ltd: Nature 470:163-165, copyright 2011.*

The toolbox is expanding – a shift of bottlenecks

In addition to antibodies, functional genomic tools, like cDNA libraries [17], reagents for RNA interference (si, sh, miRNAs…) and many other physical resources are becoming accessible to the community. Large and international projects have joined forces to establish genome-wide coverage of expression ready ORFeome clones (http://www.orfeomecollaboration.org) (Fig. 3.) and knock-out mouse strains [19]. In view of the human kinases, several studies have already exploited one or the other resource to systematically unravel functions of these proteins in normal and disease conditions [20-22]. Larger institutions invest in core facilities that, in part, offer services for the large-scale phenotypic screening utilizing, predominantly, RNAi resources. Building on such and other omics-types of projects, vast amounts of data, information and resources are becoming available that, if used extensively, can indeed support also research on smaller scale or of single genes. In my eyes, however, the ability to access and exploit the available public data is one major limiting factor that increasingly hampers progress in the utilization of this data in the community. While there is a large number of databases trying to present this data and that link out also to primary data sources, the knowledge on what and how this data could be properly analyzed, interpreted as well as exploited must be constantly improved. Bottlenecks thus need

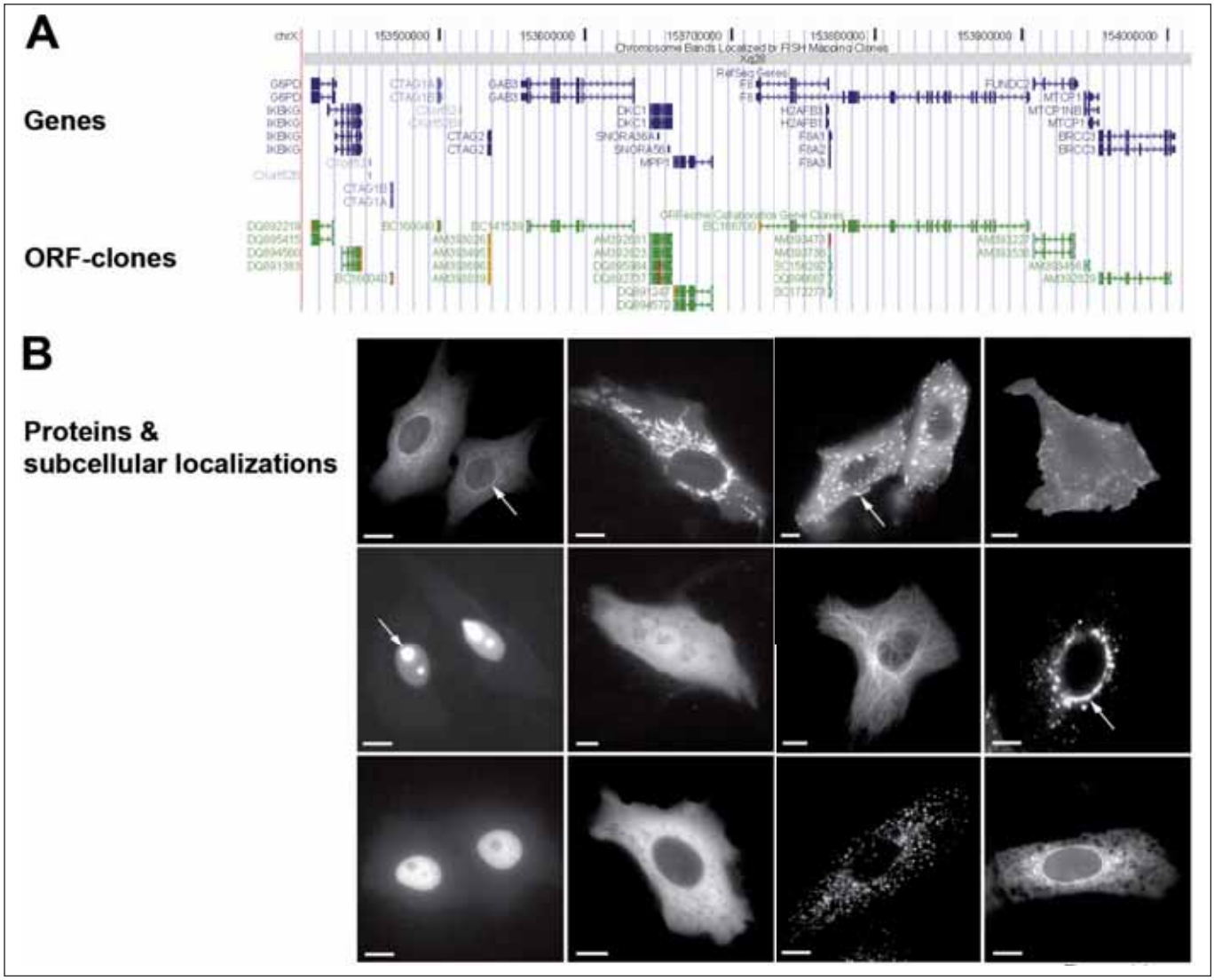

Fig. 3: *(A) Based on cDNA resources and the genome annotation (UCSC browser - http://genome.ucsc.edu) the International ORFeome Collaboration establishes and distributes ORF-clone resources. (B) These clones may be exploited in functional studies, such as to determine the subcellular localization of encoded proteins. Shown are examples of GFP-tagged proteins localizing to different subcellular compartments [18].*

to be minimized in technologies, their application as well as in the analysis and interpretation of the often highly heterogeneous data.

All international – how about Germany?

Thanks to some visionary scientists, like Annemarie Poustka, Hans Lehrach and several others, the German Human Genome Project (DHGP) was established in the mid 90s. These researchers had been among the pioneers of genome research, for example, by establishing methods for the mapping and analysis of large genomic regions [23, 24] and the systematic (positional) cloning of disease genes [25, 26]. In the DHGP, the groundwork for the integration of basic and disease-oriented high-throughput research in Germany was laid, aiming to understand the basic mechanisms underlying human disease. DHGP scientists contributed, among others, to the sequencing of the human genome [27, 28], identification of the human transcriptome [29] and interactome [30], analysis of differential gene expression in diseases, and creation of resources of transgenic and knock-out mice [31, 32]. Starting in 2001, this work could be substantially expanded in the National Genome Research Network (http://www.ngfn.de). There, disease oriented genome research has since addressed major common diseases like cancer, cardiovascular diseases, diseases of the nervous spectrum and environmental diseases. In this program, omics'-type technologies have been applied in order to discover disease genes and to improve the understanding of disease aetiologies and pathophysiological interrelations [33, 34]. To this end, high-throughput strategies are followed to improve prevention of disease by the development of predictive models as well as by improving risk assessment and preventive intervention. The development of diagnostic, prognostic and predictive markers will help for a better stratification of patients towards personalized medicine, as will the knowledge of disease mechanisms lead to the identification of novel targets for therapeutic intervention. For example, treatment of malignant melanoma with BRAF inhibitors is now decided also based on the mutation status of patients, and the prognosis of astrocytoma and oligodendroglioma brain tumor patients has been proven to be impacted by the sequence of the IDH1 gene. Such clinical improvements would not have been possible without progress in technologies, systematic gathering and analysis of genetic information as well as the development of diagnostic tools [35] that have all been made in the past few years.

Ten years of the human genome sequence and of the National Genome Research Network in Germany have thus led to a revolution in large-scale data acquisition and opened the arena for individualized patient treatment. Even the complete sequencing of patients' genomes comes into sight. Cancer and cardiovascular diseases will likely be at the forefront in the integration of these high-throughput technologies into the clinics. However, in particular the collection and utilization of personal genome information requires progress also in the legal and ethical discussion which is necessary to be carried out between academia, the society as well as politicians. Scientists and clinicians are required to contribute and help to develop safe and sound policies that aid in the protection of individuals' rights while maximizing the exploitation of technological options to the benefit of every patient. Along these lines, projects like that of the Marsilius Kolleg of Heidelberg University (http://www.marsilius-kolleg.uni-heidelberg.de/projekte/totalsequenzierung.html) have been established, aimed at supporting the different groups in society with expertise and advice. This debate should be finalized before genome sequencing may eventually become clinical practice. The genome era was defined to have ended with the "book of life" having been published a decade ago, however, all the developments in the past 10 years tell me that this genome era is not history but is rather still in its infancies.

In 2003, Lee Rowen was declared as the winner of the "GeneSweep" contest [36]. She had estimated the number of human genes to be 25,947. This number was the lowest estimate of all.

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Cell growth: signaling meets metabolism

Aurelio A. Teleman

Cells proliferate via a combination of two processes: 1) cell growth, characterized by accumulation of mass, and 2) cell division, which is regulated by the cell cycle. These two processes can be uncoupled, indicating that they are distinct from each other. For instance, early divisions of most embryos, including those of Drosophila, Xenopus and humans, are 'reductive': the cells divide without intervening cell growth, thereby becoming smaller. The opposite combination - cell growth and DNA replication without cell division, termed endoreplication - is also widespread throughout nature, and is found in plants, insects, and in some mammalian tissues [1]. That said, in order for a tissue to increase significantly in size, either during normal development of an animal, or during cancer development, the cells need to undergo both cell growth and cell division in a concerted fashion. The mechanisms regulating the cell cycle have received widespread attention in the past decades. In comparison, the mechanisms regulating cell growth are less well understood.

What does it mean for a cell to grow? The cell needs to synthesize proteins, lipids, and other macromolecules which constitute cell mass. This occurs via activation of biochemical and metabolic pathways that import and synthesize the necessary building blocks such as amino acids and fatty acids. Additionally, translation needs to be up-regulated to generate proteins. Therefore, to a large extent, cell growth is a metabolic problem. These processes, however, are regulated via signaling pathways such as the insulin, TOR and myc pathways. As a result, there is an intricate interplay between the metabolic pathways and the signaling pathways regulating cell growth (Figure 1). On the one hand, signaling pathways such as insulin and TOR regulate enzymes in the biochemical/metabolic pathways via mechanisms that are not completely understood. The "touch-points" between these signaling and biochemical pathways are critical for understanding how cell growth is regulated, yet they represent an important gap in our knowledge. On the other hand, the activity of these signaling pathways is regulated by availability of nutrients and energy. For instance, the kinase TOR, an important regulator of translation in the cell, is regulated by the availability of amino acids. This makes sense because a cell should not activate translation if amino acids are missing. Here too, however, the molecular mechanisms are not well understood and require further investigation [2]. Therefore, not only does signaling regulate metabolism, but metabolism also regulates signaling. Both metabolic pathways and signaling pathways can be envisioned as complex networks. These two sets of networks are interlinked (Figure 1), resulting in a challenge to either understand the important nodes of regulation, or to obtain a holistic understanding of the system and how it works.

These considerations on cell growth apply not only to normal development, but also to cancer. There have been two historical perspectives on cancer as a disease. In the early 1900s, cancer was seen as a metabolic disease, epitomized by the observations of

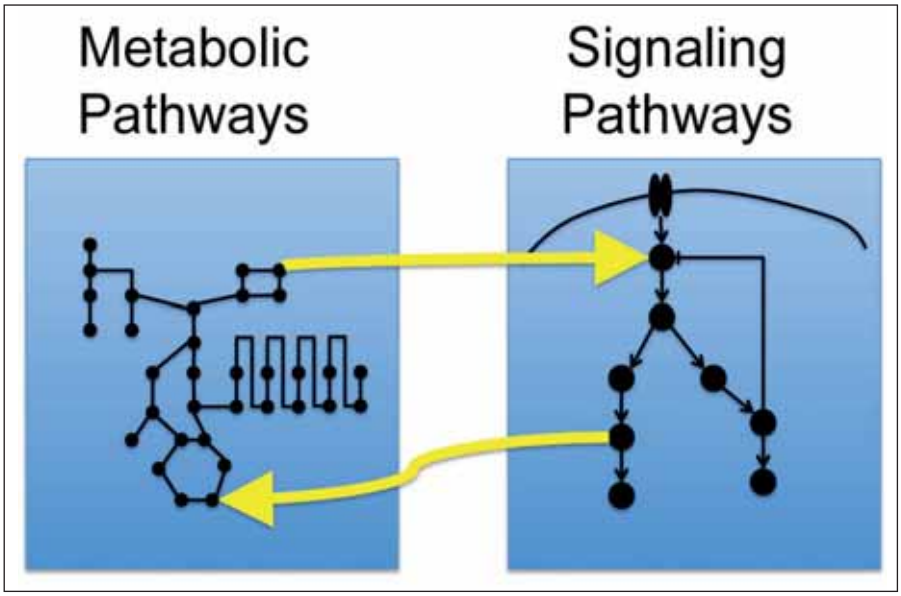

Fig. 1.: Graphic representation of the processes regulating cell growth. Cell growth results from activation of metabolic pathways responsible for import and biosynthesis of molecules such as lipids and proteins (left side). These processes are regulated by signaling pathways such as the insulin, TOR and myc pathways (right side). Each of these metabolic and signaling pathways can be thought of as a complex network. These complex networks regulate with each other, yielding an overlaid layer of complexity. Identifying the key nodes of regulation of this system is a major challenge.

Otto Warburg who noted that cancer cells frequently rely on elevated flux through glycolysis and reduced citric acid cycle activity. Subsequently, the discovery of oncogenes shifted the prevailing view towards a more genetic, signaling view on the etiology of cancer. The inter-linkage between the metabolic and signaling pathways regulating cell growth reconciles these two views.

Regulation of signaling by metabolism

I will present only a few anecdotal examples of how metabolic parameters regulate signaling in the single cell, without trying to be comprehensive, in order to highlight some recent exciting findings. Clearly, metabolic parameters affect signaling pathways in multicellular organism at the organismal level. For instance, in mammals the pancreas senses circulating glucose and responds by modulating insulin secretion, thereby affecting insulin signaling in all organs of the body. However, also at the single cell level metabolites affect signaling. One well-known example is the liver X receptor (LXR), a nuclear receptor expressed in many tissues of the body, which cell-autonomously senses derivatives of cholesterol to regulate expression of a panel of genes involved in lipid biosynthesis. These examples are not so 'surprising' since the functions of insulin and LXR are to regulate carbohydrate and lipid homeostasis. More surprising connections, however, are starting to emerge. For instance, a recent study from the lab of Benjamin Tu (Southwestern Medical Center, USA) found an unexpected mechanism by which yeast senses carbohydrate availability [3]. They showed that in yeast, as expected, carbon sources cause an increase in intracellular acetyl-CoA, a key metabolite linking glycolysis to the citric acid cycle. The surprisingly finding is that this results in acetylation of histones in the vicinity of genes involved in cell growth, thereby activating a program of gene expression which promotes growth. This is an unexpectedly direct connection by which a metabolite affects the inner working of a cell! Another recent study sought to systematically identify protein/metabolite interactions by large-scale analysis [4]. Unexpectedly, they found that many key regulatory proteins such as kinases bind metabolites in vivo, suggesting metabolites might play an important general role as regulators of protein activity. We currently know of many examples of how metabolites regulate the activation of signaling pathways. For instance, activation of TOR, an oncogenic kinase that powerfully promotes cell growth, is regulated by intracellular AMP levels via AMPK, and by availability of amino acids via an unknown sensor [2]. Nonetheless, these recent studies suggest many more mechanisms remaining to be discovered, and that the regulation of signaling by metabolites might be more intimate and more widespread than expected.

Regulation of metabolism by signaling

One of the most powerful anabolic signals in animal cells is the kinase TOR. Activation of TOR causes massive cellular growth, and for this reason TOR is hyperactivated in most cancers [5]. TOR achieves this by regulating cellular translation rates, as well as by regulating a large number of metabolic processes in the cell. For instance, TOR regulates lipid biosynthesis by affecting activity of sterol responsive element binding protein 1 (SREBP1), a lipogenic transcription factor which activates transcription of genes involved in lipid biosynthesis [6]. TOR also regulates expression of genes encoding components of glycolysis, and enzymes involved in amino acid metabolism, nucleotide metabolism and mitochondrial function [2], in part via regulation of myc and PGC1a activities [7, 8]. In addition, recent phospho-proteomic analyses have revealed an enormous number of metabolic enzymes which are differentially phosphorylated in response to TOR activation, including components of glycolysis, the citric acid cycle, and nucleotide and amino acid biosynthesis pathways [9, 10]. This suggests that in addition to its effects on the transcription of metabolic genes, TOR also has a powerful influence on metabolic pathways by regulating enzymes at the post-translational level. These are just a few examples of how one kinase, TOR, regulates cellular metabolism, to exemplify that many interconnections exist between signaling and metabolic pathways. Many more examples surely exist.

How much detail do we need to understand?

'Master regulators' such as TOR are rewarding to study because they have powerful effects on cellular biology, achieving this via regulation of a very large number of effectors. This poses a few questions: (1) If we consider the signaling pathways as one network, and the metabolic pathways as another network (Figure 1), which molecules are the equivalent 'master regulators' of growth for the metabolic pathways that we should study intensively? (2) Each of the effectors of TOR, such the biochemical enzymes identified in phospho-proteomic studies, mediates only a small fraction of the growth-promoting activity of TOR. Furthermore, the effect of TOR on these enzymes will only be modulatory. Hence, each individual regulatory interaction will only yield very mild phenotypic effects in vivo. To what extent do we need to understand each of these individual interactions? Is it enough to discover and understand the master regulators? If not, what kinds of high-throughput approaches will provide us with an efficient way to obtain a holistic yet in-depth analysis of the functioning of these networks? Considering the complexity of both the signaling and the metabolic networks, understanding how these two interface with each other will remain an exciting challenge for the future.

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Cellular blebs, or how to make use of intracellular pressure

Ewa Paluch

Introduction

In animal cells, and more generally in cells that do not have a cell wall, cell shape is usually determined by a cytoskeletal network lying immediately under the plasma membrane. This network constitutes the cellular cortex; with its main structural component being filamentous actin. Cortical actin forms a thin meshwork of cross-linked filaments, with meshsizes ranging from a few dozens to a few hundred nanometers depending on the cell type[1, 2]. The cortex is attached to the membrane by linker proteins, such as the ezrin, radixin, moesin family[3] and class 1 myosins[4]. Cortical actin undergoes continuous turnover and precise control of turnover dynamics is essential during cell shape changes, such as cytokinesis[5]. However, the mechanisms of cortex nucleation and assembly are poorly understood and fundamental questions, including the nature of cortical actin nucleators, have not been addressed. After actin, the most important cortical component is myosin 2. Myosin 2 motors assemble into mini-filaments and pull on the actin filaments in the meshwork. This generates a contractile tension in the network, which enables the cell to resist externally applied stresses and to exert mechanical work. Gradients of contractility can lead to local contractions and are responsible e.g. for cleavage furrow formation during cytokinesis, or for the cortical flows facilitating cell polarization in the C. elegans zygote[6, 7]. Precise control of cortical contractility is thus essential during cell shape changes. Misregulation of tension, and changes in cellular mechanics in general, are often associated with cancer progression[8].

A contractile cortex under tension generates an excess hydrostatic pressure in the cytoplasm. As long as the cortex and the membrane are tightly attached to each other, this pressure does not affect cell shape and is simply balanced by osmotic pressure[9, 10]. However, if the links between the cortex and the membrane are locally broken, or if the cortical network itself is ruptured, the intracellular pressure results in the growth of a

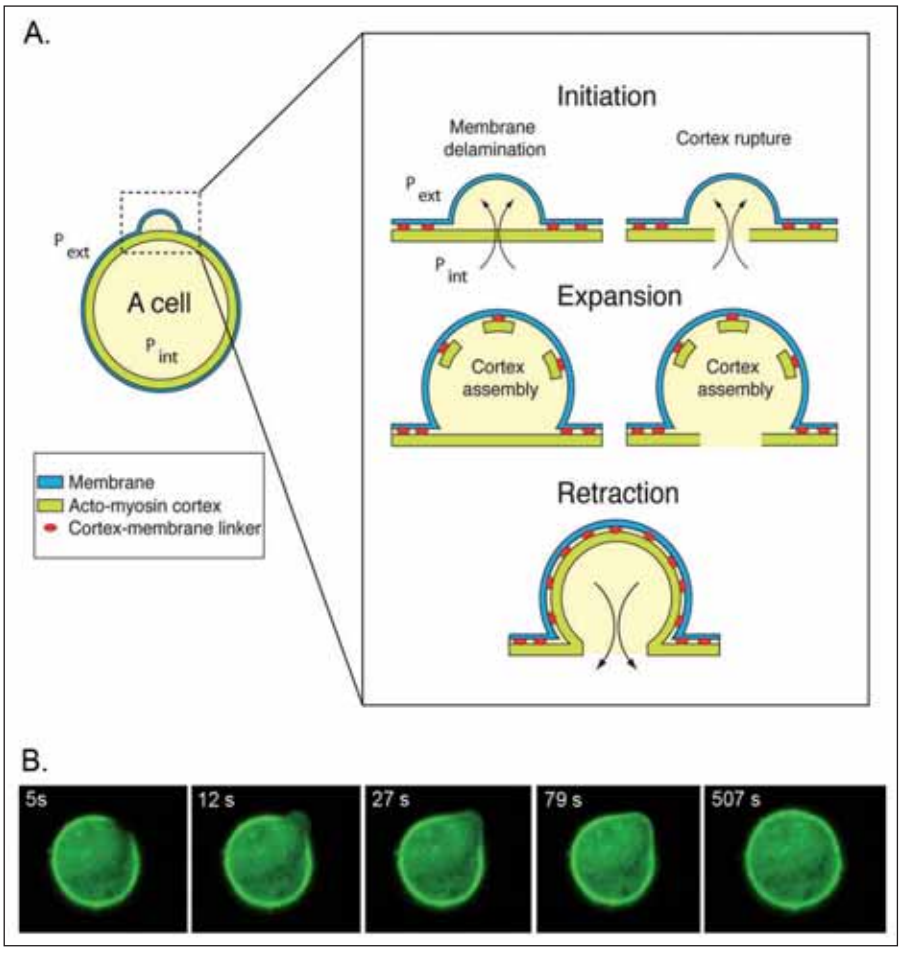

Fig. 1.: A. Schematic of the life cycle of a bleb. B. Example of a bleb in a L929 mouse fibroblast expressing myosin regulatory light chain tagged with GFP. The bleb is induced by laser ablation of the cortex shortly before the onset of the timelapse. Laser ablation locally disrupts the cortex, leading to the formation of a bulge of membrane initially devoid of cortex. After a few seconds, the cortex accumulates at the bleb membrane and ultimately leads to bleb retraction.

bulge of membrane, called a bleb[11] (Figure 1). Initially, the bleb membrane is devoid of actin filaments and only membrane tension limits the expansion of the bulge[12]. After a few seconds, actin starts reassembling at the bleb membrane, followed by the recruitment of myosin motors[2]. When this new cortex is sufficiently contractile, it drives bleb retraction into the cell body[13].

Over the past 30 years, any occurrence of blebbing has often been considered a hallmark of apoptosis. Indeed, a phase of dynamic blebbing is observed in apoptotic cells[14]. Apoptotic bleb formation is the direct result of over-activation of myosin 2 downstream of several apoptotic pathways[15, 16]. This in turn increases cortical tension, and thus favors the formation of blebs. Apoptotic cortex contraction and blebbing have been shown to contribute to nuclear disintegration and to the dispersion of the cellular genetic material into apoptotic bodies[17].

Interestingly, bleb formation also occurs in a number of non-apoptotic physiological processes[11]. These include cell division and cell migration (see below), cell spreading[18], and virus uptake[19]. The regulation and function of blebs in these various processes are, in most cases, very poorly understood. This is in great part due to the incorrect suspicion that blebs in cultured cells were apoptotic. In the following sections, I briefly summarize our current understanding of the function of blebs in two non-apoptotic processes where they play a prominent role: cytokinesis and cell migration.

Blebs in cell division

Cell division is characterized by a succession of drastic shape changes orchestrated by the actomyosin cortex. Most animal cells, in tissue and in culture, round up as they enter mitosis. Mitotic rounding appears to result from a reinforcement of the actomyosin cortex, which becomes stiffer and more contractile at mitosis entry[9]. In spite of this increase in contractility, no bleb formation is usually observed in metaphase, possibly because the attachments between the cortex and the membrane are also reinforced at that stage[20]. In anaphase, as the chromosomes are partitioned into the two future daughter cells, the cell elongates and the actomyosin cortex progressively accumulates at the cell equator. In a number of cell types, bleb formation at the poles of the cells has been reported at this stage, and throughout cytokinesis. Polar bleb formation in cytokinesis was already described in the early 20th century[21]. This phenomenon has been reported for cells in culture[22, 23] (Figure 2A), but is also observed *in vivo*, during cell division in developing embryos (A Diz-Muñoz, CP Heisenberg, EP, unpublished observations). Whether polar blebs play an active role in division, or whether they are a mere side-effect or artifact is still debated in textbooks[24]. It has been proposed that polar blebs might help increase the amount of plasma membrane available for a cell to spread again after division[18]. In a recent study, we suggest that polar blebs could also act as tension valves, helping the cell balance hydrostatic pressure during cell division (Figure 2B). Indeed, the contractile cortex present at the poles of a dividing cell can destabilize the symmetric shape of the cell if one pole is significantly more contractile than the other, creating pressure gradients and cytoplasm flows between the two poles. Polar bleb formation could effectively release polar tension and pressure, avoiding the build-up or pressure gradients and thus helping stabilize cell shape[25].

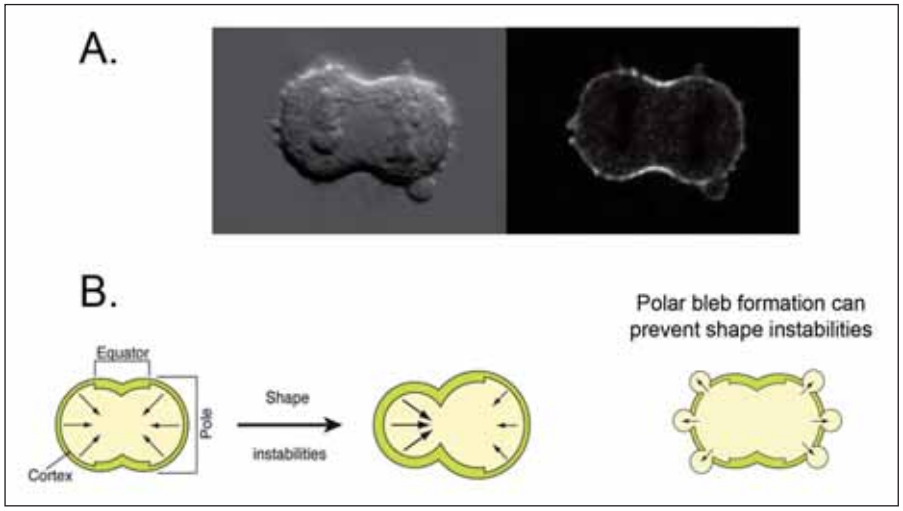

Fig. 2.: A. Polar blebs at the poles of a dividing L929 cell. Left: DIC, Right: Lifeact-GFP. B. Polar contractility makes the shape of the cell intrinsically unstable: unbalanced contraction of one of the poles can lead to shape instabilities and perturb the equatorial positioning of the cleavage furrow. Bleb formation at the poles constantly releases cortical tension and can help stabilize the shape of the cell. Drawings modified from Reference 25.

Blebs in cell migration

The occurrence of blebs at the leading edge of migrating cells has been extensively described in the early days of developmental biology. Johannes Holtfreter and later on, John Philip Trinkaus characterized bleb formation in various cell types within amphibian and fish embryos, or isolated from these embryos[26, 27]. In a seminal paper in 1973, Trinkaus described in detail the formation of blebs and lamellipodia at the leading edge of deep cells migrating in the early embryo

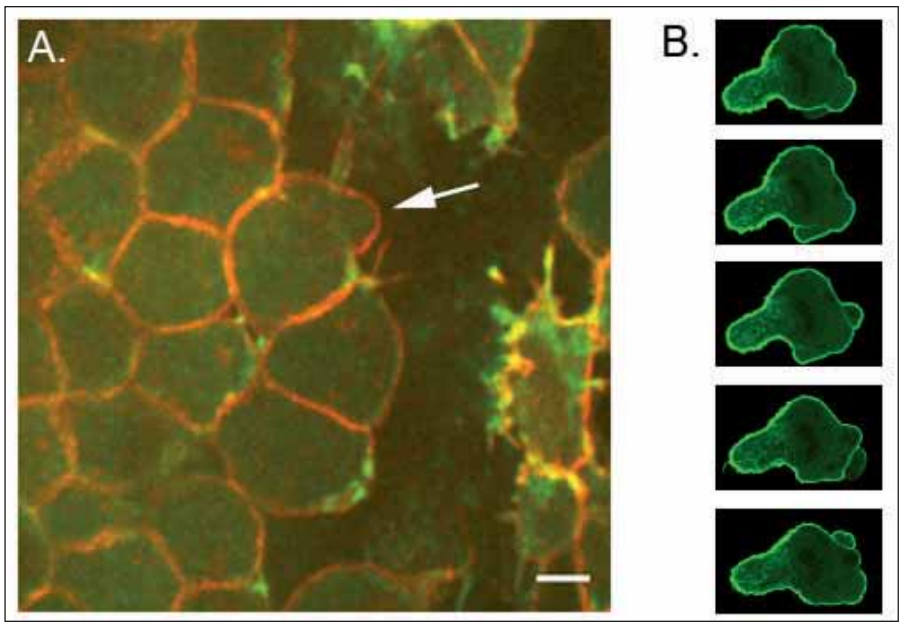

Fig. 3.: Examples of bleb formation during cell migration. A. Leading edge of the prechordal plate in a zebrafish embryo. Cells express Lifeact-GFP to mark filamentous actin and GPI-anchored-RFP to visualize the plasma membrane. The arrow points to a bleb where the separation between membrane and actin can be clearly seen. Scalebar: 10 µm. B. Timelapse of a Walker carcinosarcoma cell migrating under an agar pad. The cell expresses Lifeact-GFP to mark filamentous actin. The growing protrusions are devoid of actin.

of the killfish *Fundulus heteroclitus*[28]. However, in the three decades following this study, bleb formation in migrating cells has been largely overlooked. This is in part due to the growing suspicion that blebbing is a signature of apoptosis, as described above. Another reason is that in the 20 years from 1980 to 2000, migration studies mostly focused on cells migrating on glass coverslips, and blebs are almost never observed during migration in two-dimensional (2D) environments (with the notable exception of rat Walker carcinosarcoma cells[29] and *Dictyostelium*[30]). As a result, lamellipodia, flat actin-filled protrusions, which form at the front of most cells migrating on flat surfaces, became the paradigm of cellular protrusions[31].

Early in the last decade, observations from the cancer field suggested that bleb formation could be an alternative to lamellipodia during migration in 3D environments such as collagen matrices or live tissues[32, 33]. Later on, the group of Erez Raz reported that zebrafish primordial germ cells form protrusions initially devoid of actin, a characteristic of blebs[34]. Since these pioneering studies, the evidence for the occurrence of blebbing in 3D migration has been continuously accumulating[11, 35, 36] (see Figure 3 for some examples).

The mechanisms of formation of blebs and lamellipodia are very different. Lamellipodia grow because of actin polymerizing at the cell leading edge and pushing the membrane forward; blebs expand because of intracellular pressure pushing against the membrane. Some cells form exclusively one or the other protrusion type, but others can form lamellipodia, blebs, and other protrusions, such as filopodia, at the same time[28, 35]. How these two very different protrusive mechanisms are co-activated, sometimes in response to chemotactic cues, is poorly understood. It is also unclear whether the two protrusion types are interchangeable or if they play specific functions in migration. In a study in zebrafish, we suggested that blebs may allow the cell to rapidly explore their environment, while lamellipodia would favor a more focused and directional migration[35]. However, more studies with different cells in a variety of environments will be necessary to elucidate the regulation, mechanics and specificities of bleb- and lamellipodia-based migration modes. Importantly, plasticity in protrusion formation may favor tumor invasion by allowing cells to select the most efficient migration mode in a given environment[37].

Conclusion

Research on the regulation, function and biophysics of cellular blebs is currently undergoing a strong expansion. Many open questions remain for both biophysicists and biologists. To name a few, the mechanics of bleb growth, the signaling pathways leading to bleb formation, the mechanisms of cell body translocation in bleb-based migration, are mostly unknown. In a more general context, it will be interesting to investigate how evolution has given rise to blebs and other cellular protrusions, and to what extent their functions are redundant.

Acknowledgements

I thank my lab members and collaborators for insightful discussions, and J. Sedzinski, A. Diz-Muñoz and M. Bergert for providing images for the figures. Financial support from the Polish Ministry of Science and Higher Education, the Deutsche Forschungsgemeinschaft, the Human Frontier Science Program and the Max Planck Society is acknowledged.

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Threads of Life – the Physics of Biopolymers

Sarah Köster

Introduction

Polymers are an integral part of life. Be it the enormous elasticity of red blood cells that squeeze through the smallest capillaries, the stereocilia in auditory hair cells that act as force sensors and make hearing possible, the lightness and yet stability of bones, muscle contraction or blood clotting: all these physiological processes are based on a sophisticated interplay of polymeric components. As part of the cytoskeleton biopolymers define the shape of eukaryotic cells, provide mechanical stability and "tracks" for intracellular transport. Together with molecular motors, actin filaments and microtubules define active, dynamic systems. While actin filaments (also termed microfilaments), microtubules and intermediate filaments are found inside mammalian cells, the extracellular matrix outside the cells is made up of primarily collagen, but also some other polymers like elastin. These components provide the tissue surrounding the cells with a remarkable tensile strength. Even the genetic information is encoded in biopolymers - DNA and RNA, heteropolymers made up of nucleic acids. Some organisms take advantage of the remarkable properties of biopolymers and produce them as "building material". One prominent example is silk that has the highest elastic module of all natural fibers.

However different the chemical structure of polymers in general and biopolymers in particular may be, they share common traits: the diameter is in the range of several nanometers, while the length can be many micrometers or even longer. This high aspect ratio leads to fascinating behavior, which in recent years moved these systems into the focus of polymer- and biophysicists. The different mechanical functions in biological systems are mirrored in varying properties such as the persistence length, which is a measure for the stiffness of the polymers. The persistence length can range from nanometers (e.g. DNA, 50 nm) to millimeters (e.g. microtubules, ~ 5 mm). This large range of length scales shows the intriguing flexibly and a variability nature makes use of, while still sticking to very general physical principles.

Methods to study biopolymers experimentally are manifold. What is special about biological systems is that the length scales (nanometers to micrometers) are generally small; forces are weak (piconewton to nanonewton). Time scales, however, can range from subsecond to hours or days. These scales introduce particular challenges to the experimental study of biological systems many of which have been approached during recent years. Most widely spread are probably imaging techniques, ranging from bright field to fluorescence microscopy, including novel high-resolution methods[1]. The latter bridge the gap to nanoscale imaging techniques such as atomic force microscopy and electron microscopy and have the advantage that they can also capture dynamics of the studied systems.

Scattering techniques using different wavelengths (laser light, x-rays or neutrons) complement direct imaging and provide infor-

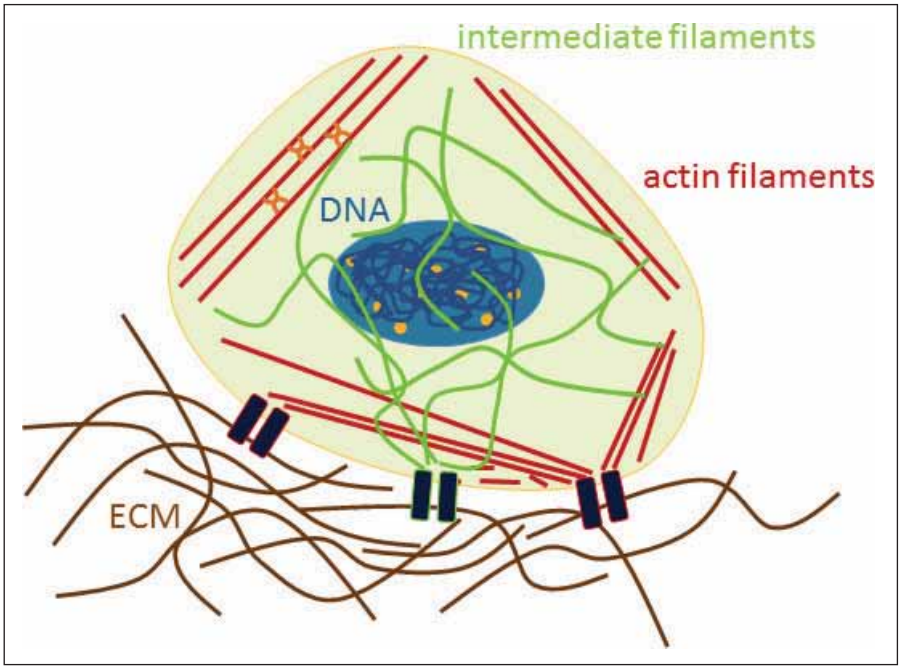

Fig. 1: *Schematic view of some typical biopolymers that are found in the intra- and extracellular space. Actin filaments and microtubules (not shown) are highly conserved even between different organisms. Intermediate filaments, by contrast, are unique for different cell types and form a large family of proteins. DNA, a heteropolymer with four different building blocks, carries the genetic information and is tightly packed in the nucleus. In multicellular organisms, most cells are embedded in connective tissue, formed of collagen and other extracellular matrix proteins. Despite strikingly different cellular functions and molecular architecture, all these molecules have in common that the aspect ratio (length to diameter) accounts up to several orders of magnitude, enabling us to employ polymer physics for their description.*

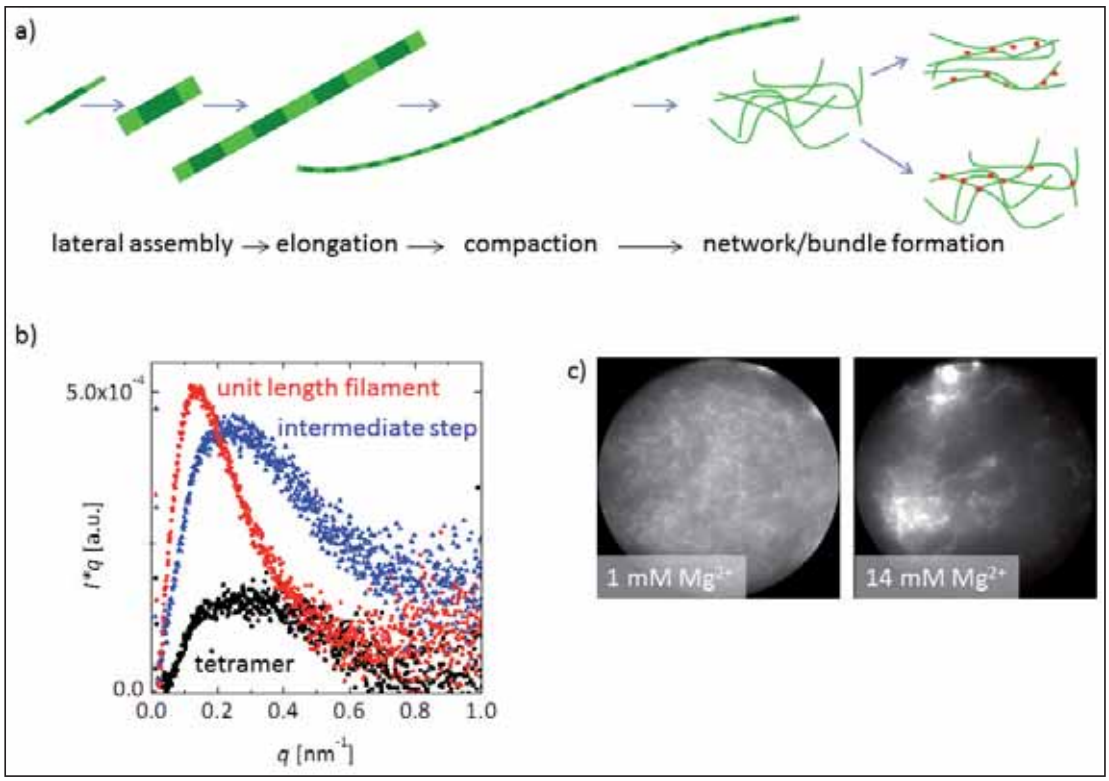

Fig. 2: Filament and network or bundle formation on the example of vimentin IFs. a) Tetrameric subunits first assemble laterally, then elongate (adapted from reference 6, not to scale). When multivalent ions are added (indicated by the red circles), bundles and networks form whose morphology strongly depends on the salt concentration. b) The lateral assembly step is investigated by small angle x-ray scattering (SAXS)[9]. c) Counterions clearly change the network morphology[13] (width of each fluorescence image about 40 µm).

mation about shape and size of components. Apart from measuring length scales and their change over time, force measurements complete the mechanical approach to biopolymers. For this purpose, *in vitro* systems and cell systems can be investigated using the above mentioned atomic force techniques or optical tweezers. Both these techniques are also widely used to manipulate biological systems and biopolymers in particular. The ability to apply small forces – also common to microfluidic techniques – is of great importance when a mechanical response of the biological systems shall be induced. Particular attention has been turned to the combination of techniques. Thereby biological systems (e.g., cells or individual polymers) are manipulated by external forces and the response is observed *in situ*.

From proteins to polymers – filament assembly

Many biopolymers are fibrous proteins built up of subunits, which can be individual monomers or assemblies thereof. The pathway by which this assembly occurs can vary a lot for different types of biopolymers. Apart from a "classical polymerization" where one subunit after the other is added (e.g. actin filaments, microtubules), hierarchical assembly is found, often in conjunction with distinct intermediates. Collagen, the most abundant protein in the human body, consists of peptide chains, which assemble into collagen "monomers" (made up of three peptide chains). These monomers form staggered pentamers and eventually collagen fibrils and thick fibers as they are found, e.g., in the connective tissue. The five-fold symmetry that is encoded in the amino-acid sequence of the molecule has long been theoretically predicted[2,3] and also experimentally verified[4,5]. Cytoskeletal intermediate filaments are another class of proteins that are known to assemble in a stepwise manner (see figure 2a and b). On the example of vimentin it has been shown, that the rod-shaped monomers first assemble laterally and then longitudinally[6-9].

The formation of silk fibers, by contrast, relies on a transition from globular protein (silk I) to a fiber consisting of both, ordered β-sheets (silk II) and globular proteins. The remarkable mechanical properties of silk are most probably rooted in this special secondary structure. A great challenge remains the controlled production of artificial silk fibers with tunable properties and first steps towards this direction are being taken, for example by employing microfluidics[10-12].

Condensation, aggregation and fiber formation

In their physiological context, biopolymers never occur isolated but always have to be viewed in the context of assemblies such as bundles or networks. The fibrous polymers inside mammalian cells – proteins in the cytoskeleton as well as DNA in the nucleus – form very distinct aggregates, which mirror their function in the cell. The genetic information is encoded in long DNA strands (in a

human cell the total length for all chromosomes adds up to about 2 m). The "trick" by which DNA is still packed to the size of the nucleus, which is in the range of a few tens of micrometers, and yet can be read off in specific positions in a very defined and controlled way, lies in counterion condensation. DNA is negatively charged and is wrapped around positively charged histone proteins. A suitable means to study this DNA packing by (natural or artificial) histones or multivalent kations are microfluidic diffusive mixers[14-16]. The advantage is, that two (or more) components can be mixed together in a very controlled way in the laminar flow and the flow conditions and concentrations in each position of the microfluidic device can be determined precisely by numeric modeling. This information can then be related to the observations concerning the aggregation phases.

Cytoskeletal proteins like actin or intermediate filaments of different kinds form bundles and networks mediated by other proteins or by multivalent ions. While crosslinking by proteins (e.g molecular motors) works via specific binding sites, in the case of ions electrostatic forces dominate. Interestingly, intermediate filaments are crosslinked even at very low concentrations of divalent kations (e.g. Magnesium, Calcium or Zink, see figure 3c) and show a similar mechanical response as actin networks crosslinked by specific actin binding proteins[17-19]. For some biopolymers, e.g. collagen or silk, it is not easy to clearly distinguish between fiber formation and aggregation or bundle formation since the two processes take place in parallel in a coordinated manner, eventually giving rise to tissues with specifically adapted mechanical properties.

Polymer mechanics

Cell and tissue mechanics are naturally very complex – due to the numerous components that are involved and their interactions, many of which are still unknown. In a bottom-up approach individual, *in vitro* reconstituted components are studied. The properties of the composite polymeric material are never just the sum of the individual properties (a phenomenon summarized in the term "emergence"). Nevertheless, the idea behind studying the component individually is that a profound knowledge on this level is needed before moving on to more complex, interacting and integrative systems.

The major fibrous proteins of the cytoskeleton – actin filaments, microtubules and intermediate filaments are not only interesting from a biological and medical point of view but also serve as model systems for polymer physics in general. This is mostly due to their length in the micrometer range and the possibility to label them fluorescently and therefore observe them in real time and at high resolution in optical microscopes. Theoretical models for polymers of different stiffness (i.e. different persistence length), polymers in confining "tubes"[20-24] or meshworks[25] have been experimentally verified. The reasoning behind studying individual biopolymers also in the context of geometric confinement is that the cell itself is a very crowded environment[26]. It has been shown that the mechanical behavior of these long, thin macromolecules does not only depend on the "material properties" of the polymer but also on the direct environment. One fascinating example is the "buckling" behavior of microtubules in cells[27]. The buckling wavelength that is observed does not correspond to the mechanical stiffness of a single microtubule, when Euler buckling (the simple "intuitive" bending of a rod that is fixed at both ends) is considered. However, when taking into account an elastic (actin) matrix that surrounds the microtubule and reinforces it laterally, the observations can be explained.

In cells, polymer networks like the IF and the actin network do not exist independently of each other, but are connected via crossbridges (e.g. binding proteins or molecular motors). The networks are connected via focal adhesions (actin) or hemidesmosomes (IFs) to other cells and to binding proteins in the surrounding tissue, the extracellular matrix. One feature that is shared by many different biopolymers is so-called strain-stiffening. This means that the polymer is "soft" when pulled with a small force, but the more the strain is increased the more the polymer stiffens. It is believed that this mechanism,

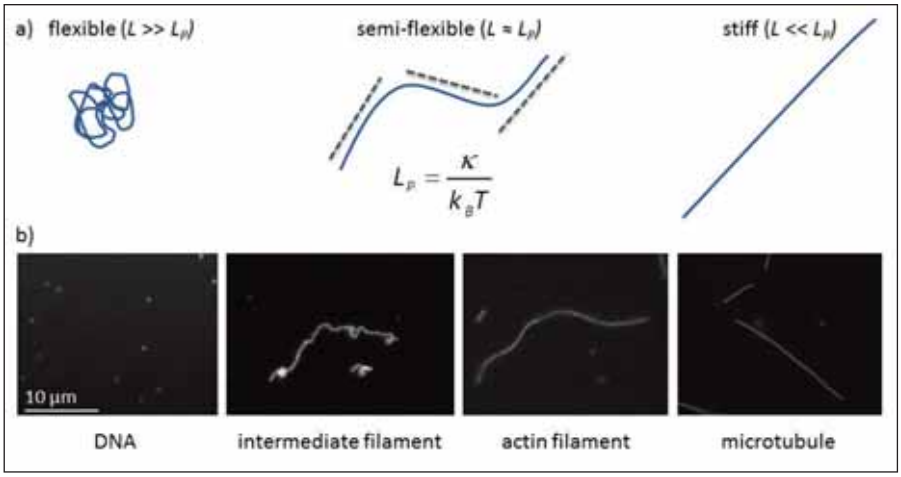

Fig. 3: *a) Polymers can be categorized according to the ratio of contour length L and persistence length L_p. The persistence length, the length over which the polymer is correlated (indicated by the dashed lines, is the bending rigidity κ in units of the Boltzmann factor k_BT. b) Fluorescence micrographs of example biopolymers for the different stiffness regimes (from left to right): DNA molecules (here in phage λ viruses) completely coil up on microscopic length scales and the contour is therefore not resolved; intermediate and actin filaments show clear bends while microtubules behave like straight rods. The scale bar applies to all micrographs.*

inside cells, helps to make them adaptable when external forces are small, but protects the cells from forces that become too strong.

Conclusion

Biopolymers are an excellent example for the interlinks between physics, biology, chemistry that govern modern research. To understand their structure and function beyond the biochemistry of the macromolecules, physical models are indispensible. *Vice versa*, polymer physics models, predictions and scaling laws have been experimentally verified because they could be applied to biological systems. The impact of biophysics on medical and biological question and the importance of understanding fundamental underlying principles has become clear though these studies.

Acknowledgement

I thank my lab members and collaborators for illuminating discussions and in particular Bernd Nöding, Jens Nolting, Christian Dammann and Martha Brennich for providing micrographs and data. Financial support by the German Science Foundation (DFG) in the framework of the SFB755, SFB937, CMPB, project KO3572/1, and the Excellence Initiative (Courant Research Centre *Nanospectroscopy and X-Ray Imaging*) is acknowledged.

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Systems Biology – A Physicist's View

Kevin Chalut, Kristian Franze, Moritz Kreysing, Franziska Lautenschläger, Pouria Moshayedi, Andreas Christ, Graeme Whyte and Jochen Guck

Most biological research to date is concerned with molecular aspects of the function of cells, while global cellular aspects seem as antiquated as using a rotary phone instead of a smart one. While this particular level of focus has been immensely successful so far, there are an increasing number of aspects to cellular function that require the consideration of other length scales and conceptual approaches if we really want to understand the function of biological systems. Here we review several examples where considering the global physical – mechanical and optical – properties of cells and their environment offers surprising new insight into cell physiology, sometimes revealing novel diagnostic and therapeutic approaches to pathological conditions.

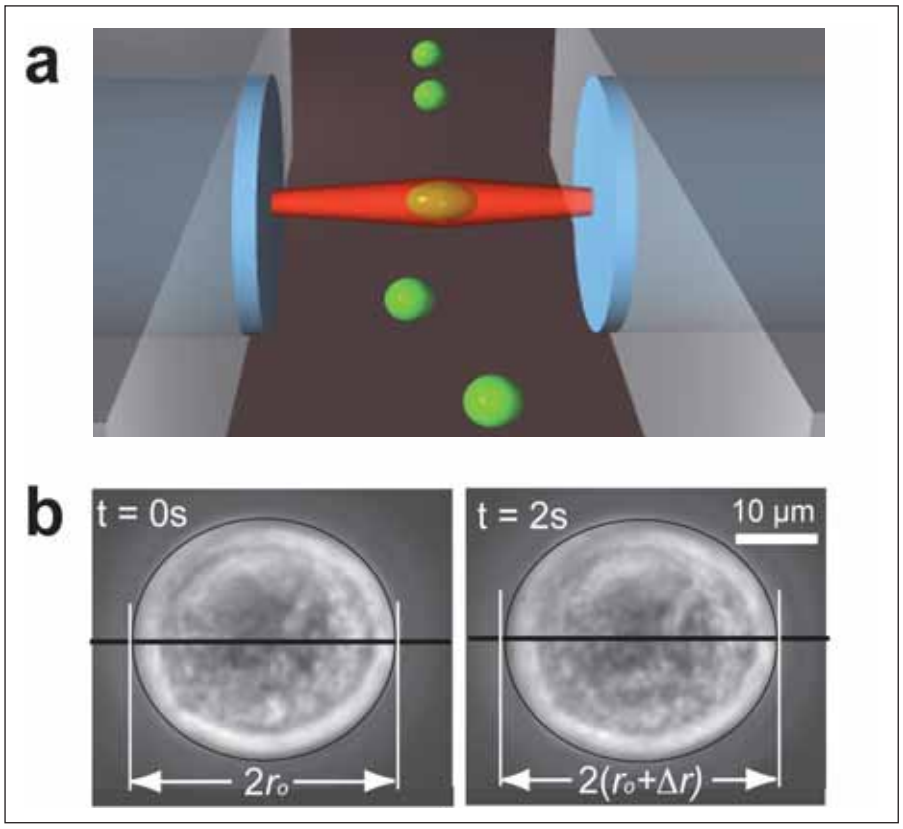

Fig. 1.: Schematic of the optical stretcher. a) Cells (green) are flowed through a microfluidic system to be trapped by two counter-propagating laser beams (red) emerging from single-mode optical fibers. b) When the light power is increased, the cell is visibly being stretched out along the axis of the laser beams over the course of a few seconds. The greater the relative deformation $\Delta r/r_0$, the more compliant the cell and the easier it can migrate in 3D environments.

What we have witnessed in biology in the past decades is quite impressive and nothing short of a revolution. The cracking of the structure of DNA and the genetic code, the advent of molecular biology, the mapping of the entire human genome and that of many other organisms. What we now seem to have at our disposal is full insight and control over cells on the molecular level – the entire blueprint of every cell. No wonder that the focus of biological study is on microarrays, sequencing, signaling pathways, gene knockouts and the language of biology is that of biochemistry. But is that it? Is everything known that we can possibly know about cells if we exclusively consider this molecular, biochemical aspect of cells?

The latest word in the quest for understanding cells seems to be systems biology. This is generally understood as using bioinformatics to deal with this wealth of molecular information. Grind the data through the computer, find a pattern, hope for some statistically significant change of a subset of genes. But does that help? Does this contribute any conceptual understanding of the system? Could there be more informative facets to understand cellular behavior? It is like fiddling with all the switches in the cockpit of an airborne plane in order to learn how to fly it. Surely, there is some chance that this might ultimately be successful – given enough tries. Then again, this does not answer the question: why does it work? The conceptual level of understanding involving the connection between speed, wing shape and the resulting lift is, in this way, inaccessible. The how and why – the essence – is

lost. It might also be time to take a few steps back and take a fresh look at the situation with living cells. Undoubtedly knock-outs can show which genes have causal links to the occurrence of specific phenotypes. The presence of the phenotype and the gene in nature, however, must be seen as a result of evolution, in which cells or entire organisms adapted to a given physical reality.

Physicists jump between length scales as appropriate to solve a particular problem. One would certainly not try to describe the motion of a bouncing rubber ball by solving Schrödinger's equation for all the constitutive atoms. It would be far better to realize that the emergent resulting effect of the interactions between all the atoms is captured in a single number – the elastic modulus – which is sufficient to describe the ball's motion. It is not a great leap to think that the elastic properties of cells in concert with their surrounding tissue might analogously guide the behavior of cells.

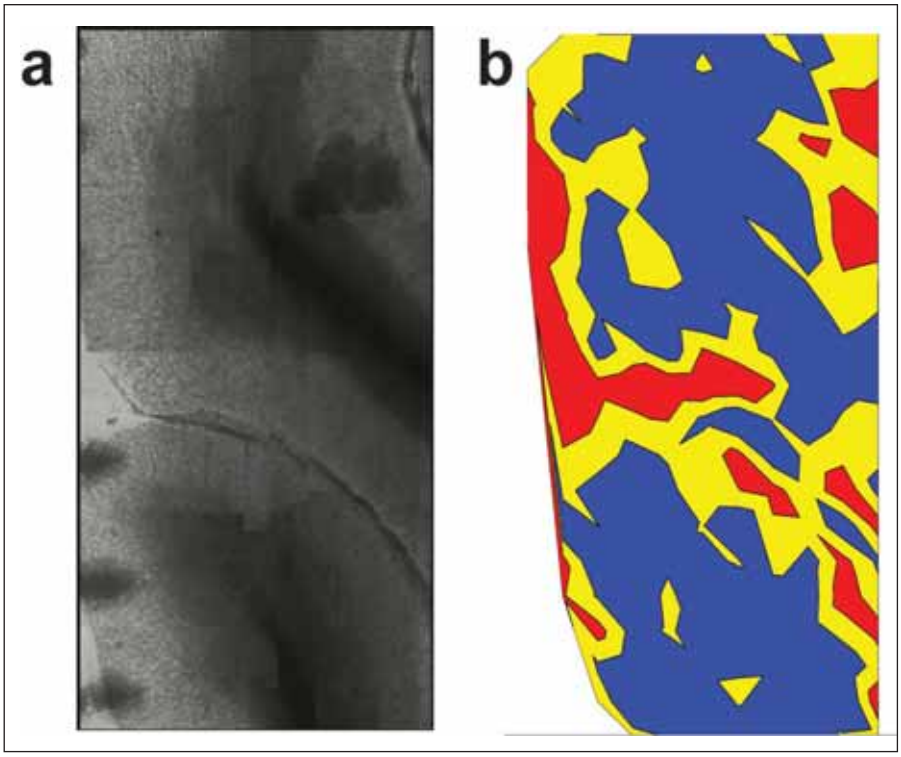

Fig. 2.: Mechanical mapping of brain stiffness using scanning force microscopy. a) Light microscopic image of a sagittal cerebellar slice in rat brain. The lighter regions are grey matter and the darker regions are white matter. The width of the image is about 1 mm. b) Corresponding elasticity map obtained with scanning force microscopy. The color code represents the elastic modulus of the material (blue = 100 – 450 Pa; yellow = 450 – 750 Pa; red = > 750 Pa). The tissue is mechanically heterogeneous, with grey matter being clearly stiffer than white matter.

The mechanical properties of cells are mostly determined by their cytoskeleton, a hybrid polymer gel comprising several kinds of different filaments. These filaments are transiently cross-linked by specific proteins and their motion along each other is controlled by motor proteins. Soft-condensed matter physics, and specifically polymer physics, has illuminated the cytoskeleton and its mechanical properties.

But the cytoskeleton is no mere packaging material. It is maintained far out of equilibrium by the coupling of its polymerization to the hydrolysis of ATP. In certain cells, a significant portion of their available energy is actually spent in the regular turn-over of the cytoskeleton. This turn-over occurs even while the cells are at rest. Perhaps the cell does this for a purpose: to keep it primed for action when it is needed. Indeed, the cytoskeleton is involved in such vital tasks as motility, phagocytosis, and in the separation of chromosomes during mitosis – to name but few. To link to molecular biology, these processes are all mediated by signalling pathways, which often involve small GTPases such as Rac, Rho or CDC42 that also control the cytoskeletal state. But can we actually exploit the link between cell function and cell mechanics directly? Can we learn something about what cells are doing by measuring their mechanical properties? Recent research certainly points this way.

One of the most drastic functional changes that might occur in the life of a cell is when a normal, mature cell, with a cytoskeleton specific for its task, turns into a cancer cell. This rogue cell ceases what it should be doing and grows without bounds, quite often to the detriment of the entire organism. It has long been known that this malignant transformation comes alongside a drastic cytoskeletal restructuring, which is key for, and even augurs, cancer progression. Therefore, it is unsurprising that cancer cells also manifest different mechanical properties; they typically become more compliant, and their increasing compliance is actually correlated with an increased metastatic competence [1, 2]. Cells become increasingly compliant as the cancer progresses; this increased compliance correlates with the likelihood that it will spread and form metastatic settlements elsewhere in the body. An anthropomorphic explanation for this tendency is that cells must be soft to squeeze through

the surrounding tissue, to intravasate into and circulate through the blood or lymph system, and to eventually extravasate. A rigid cell would not be capable of these acrobatics: it would be stuck.

So, rather than *looking* for suspect cells, it seems sensible to *feel* for them. This insight can be exploited for diagnosis, for example, using a microfluidic optical stretcher – an optical trap where two non-focused and counter-propagating laser beams trap and localize cells from a flowing suspension and subsequently deform them by purely optical forces (see Figure 1) [3-5]. This optical stretcher can be combined with microfluidic delivery of cells and automated control of the measurement process for high-throughput [6, 7]. Such a microfluidic optical stretcher allows the analysis of many individual cells in suspension, which can be obtained non-invasively from a patient by fine-needle aspiration from internal tumors or by brushing of suspect lesions [2]. As few as a few hundred cells is sufficient to obtain statistically significant data. Such a mechanical analysis could help identify primary tumors that are likely to spread. This type of probe could have a big impact on therapy.

The classification of cells of interest by their mechanical fingerprint also allows sorting and subsequent culture for further detailed investigation. In turn, measuring mechanical properties of drug-treated cells could also help identify treatments to stiffen potentially dangerous cells, thereby preventing them from infiltrating other tissues. Of course, using cell mechanical measurements for characterizing and sorting cells is not limited to pathological changes but can be done for any cell functional change that influences the cytoskeleton, such as cell division or differentiation. In fact, we have recently confirmed sufficient cellular compliance of cells as physical prerequisite of cell migration through small pores in the hematopoietic system. When promyelocytic precursor cells (NB4 cells, HL60 cells) are differentiated into neutrophils or monocytes, their compliance increases and allows them to migrate through small pores that they are not able to enter before differentiation [8, 9]. Consequently, considering cell mechanics, in addition to cell adhesion and proteolysis, could be an important, previously underappreciated aspect in studies of cellular migration in 3D environments.

The importance of mechanics goes beyond individual cells. It has long been known that some cells are mechano-sensitive, meaning they measure and respond to mechanical stimuli from their environment. One only need think of bone loss experienced by astronauts after they have been deprived of the earth's gravitational pull. It is also conceivable that cells lining the blood vessels, throat or lungs respond to the mechanical stimulus provided by blood pulsation, passage of food or breathing, respectively. This mechano-sensitivity has forced itself into the spotlight of biophysical research in recent years [10]. Many cells migrate up stiffness gradients – a phenomenon aptly termed durotaxis [11] – or change their morphology, appearance, proliferation or growth depending on the stiffness of the substrate on which they sit. In fact, Dennis Discher's group at the University of Pennsylvania showed that stem cells, cultured on substrates of a particular stiffness, would spontaneously differentiate into cell types that would be found in tissues with the same mechanical properties of the substrate [12]. For example, when they grew mesenchymal stem cells, which can differentiate into a variety of cell types, on soft substrates, which mimicked the softness of the brain, these cells turned into neurons. When the same cells, in the same medium, were grown on intermediate stiffness (similar to heart tissue), they became muscle cells. And on very stiff substrates, comparable to bone, the cells differentiated into bone cells. Further, once these cells had committed to becoming a particular cell type, the standard biochemical method used to induce differentiation by growth factors was incapable of transforming them into other cell types. Mechanical cues, in this case, can clearly even supersede biochemical ones. This finding also insinuates why a layer of "feeder cells" – cells necessarily grown in the same dish with the cells of interest – is required to maintain non-differentiated stem cells in culture. The soft feeder cells mechanically shield the sensitive stem cells from the hard culture surface underneath and prevent mechanically triggered differentiation.

Even in our softest tissues, the central nervous system, cells seem to react to mechanical cues in addition to adhesive and soluble guidance cues. We have recently shown that the growth cones of neurons react to mechanical gradients, which steers them away from stiffer areas [13]. There are mechanical heterogeneities in the central nervous tissue that would provide a landscape for such mechanical orientation, as shown by mapping of cerebellar tissue and retina using scanning force microscopy (see Figure 2) [14, 15]. This avoidance of stiffer areas could also be a previously unexplored reason for the lack of regeneration of damaged axons through glial scars, which are putatively stiffer than unscarred tissue. And astrocytes and microglia stage a *bone fide* inflammatory reaction when in contact with surfaces slightly stiffer than what they are used to [16]. Again, this could be the underlying phenomenon that causes foreign body reactions against neural implants – with obvious possibilities to prevent these adverse reactions.

How cells convert these mechanical signals into one compatible with known internal biochemical pathways is currently an open question, and it is a very hot topic in this field [17]. Still, considering that cells are generally cultured and imaged on hard dishes

or slides (these are essentially infinitely stiff when compared to anything found in the body – even bone), one cannot help but wonder if what seems to be known in cell biology – what is currently dogma – might ultimately turn out to be artifacts of these non-physiological conditions.

It doesn't stop with mechanics. There are a multitude of further examples where cells betray a concern for physics; examples where their behavior can't be explained on the molecular level alone. For instance, the optical properties of cells found in the retina of vertebrates, including humans, seem to be optimized for the transmission of light. The retina is a layer of tissue acting as a screen covering the backside of the eye where light is converted into electrical signals, processed and sent on to the brain. The retina has a striking anatomical peculiarity: it is inverted. Astonishingly, the light-sensing photoreceptor cells are located on the wrong side – turned away from the eye lens. This means that any image projected onto the front of the retina has to travel through hundreds of microns of tissue prior to reception. Even if the tissue is transparent – which it is not – it still consists of many individual cell bodies and internal organelles, all possessing a wide variance of refractive indices. This wide refractive index distribution should lead to scattering, which will lead to distortion of any image projected through it. The situation is similar to placing a thin diffusing screen in front of the CCD chip of a camera, which seems absurd. This strange arrangement has long puzzled scientists.

But two recent extraordinary findings by us and colleagues at the University of Leipzig, the Max-Planck Institute for Brain Research in Frankfurt, and the Ludwigs-Maximilian-University Munich now revealed how nature optimized this seemingly unfortunate situation: by tweaking the arrangement and refractive indices of cells and organelles in the retina. The first discovery involves the long cylindrical glial cells of the retina, so-called Müller cells, which span two thirds of the thickness of the retina. These cells have a higher refractive index than the surrounding tissue and serve as optical fibers to guide the light through the retina [18]. To reduce potential scattering by these cells, their cytoskeleton is densely packed, arranged along the light direction, and otherwise contain very few internal organelles such as mitochondria. Even the nucleus is located outside the cell-waveguide and clings to it like a backpack to reduce detrimental scattering and light loss. The dense parallel array of all these Müller cells is reminiscent of artificial fiber-optic plates that are used to relay images over distances with low loss and distortion, which suggests a similar function in the retina.

If this is already a surprising adaptation, then the second discovery is nothing short of stunning. Below the layers that the Müller cell array bridges, there is the outer nuclear layer of the retina, through which the light must still traverse. It turns out that, in nocturnal animals, the nuclei in these layers have an internal structure unlike that of the nuclei of any other cell type in the body; for that matter these nuclei are unlike the same cells in diurnal animals. The DNA contained in a single nucleus is about 2 meters long when stretched out. To fit into a 10 micron nucleus it is tightly wrapped around proteins and coiled up into chromatin. The chromatin with the genes presently not needed, called heterochromatin, is especially densely packed and stowed away at the nuclear periphery, while euchromatin, containing often used genes, is more accessible and found at the center. This arrangement of chromatin is so universal that it is usually called 'conventional'. However, in the outer nuclear layer in nocturnal animals, the heterochromatin is in the center and the euchromatin is on the outside [19]. It turns out that denser heterochromatin has a higher refractive index than euchromatin. This unique inversion, with the heterochromatin on the outside, transforms the nuclei from scattering obstacles into micro-lenses that focus the light

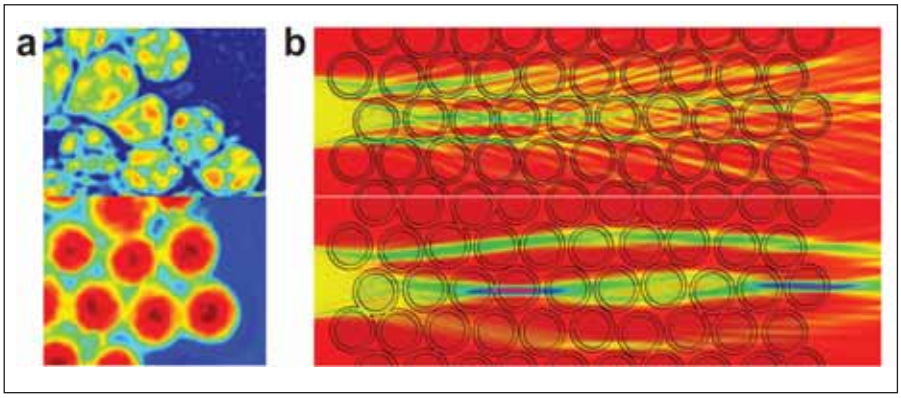

Fig. 3.: Inverted nuclei as micro-lenses. a) Quantitative phase maps of photoreceptor cell nuclei of (top) a pig as s typical diurnal animal with normal chromatin distribution and (bottom) mouse as a nocturnal animal. The phase-dense regions are indicated in yellow and red, corresponding to heterochromatin. While in pig, heterochromatin is preferentially found towards the periphery, in mouse all the heterochromatin is condensed into the center. b) Corresponding simulations of the light propagation from left to right through the outer nuclear layer. The position of the nuclei is indicated by the circles. While the diurnal photoreceptor nuclei with the conventional chromatin distribution largely scatter the light, in the nocturnal nuclei with their inverted chromatin distribution the light is focussed from one nucleus to the next, effectively creating a light guide. This reduces scatter and increases the signal-to-noise ratio detected by the photoreceptor segments located to the right.

through the outer nuclear layer – without much scattering – while maintaining a high signal-to-noise ratio (see Figure 3) [20]. The improved transmission leads to an optical advantage for seeing at low light levels, which has apparently caused this massive rearrangement to occur during evolution. The complete restructuring is even more surprising considering that the relative position of genes with respect to the location of hetero- and euchromatin is heavily implicated in the way the cell regulates gene expression. This means that these nuclei have thrown the entire conventional nuclear arrangement (tried and tested for hundreds of millions of years and conserved in all other cells) over board, in order to optimize their optical properties.

There is no light-guiding gene in cells. The refractive index is a collective property emerging at a different conceptual level — physics – and we are increasingly finding out that the same applies to many other case studies of the cell.

It shouldn't be surprising that very relevant contributions to biology are not coming from the crowded and very competitive mainstream area of this discipline but from its fringes. As Thomas Kuhn argued in his "The Structure of Scientific Revolutions", science does not progress linearly within certain paradigms but by changing paradigms. Physicists have always played an important role in opening up the view to new possibilities and new angles of investigation. Maybe it is not a coincidence that contemporary biology, originally conceived in the Cavendish Laboratory – a Physics department, will require physical approaches and considerations to take the next conceptual steps in advancing our understanding of biological systems. Physics may well be the new way to think about biology.

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Cells on the move - complex and uncontrollable?

Stephan Paschke, Elke Wolff-Hieber, Iris Repple and Michael Beil

Background

Despite the fundamental role of cell motility for many biological processes, the mechanisms governing the actual movement of cells within multicellular organisms are far from being understood. Most studies in this field have been focused on signaling pathways to elucidate the biochemical regulation of this process (Nguyen et al, 2009). However, the master molecule that fully controls cell motility is yet to be identified. It seems to be unlikely that the regulation of a biological function which is pivotal for survival would depend on a single molecule. Recent investigations rather suggest that the regulation of migration is characterized by functional redundancies (Chiang & Massagué, 2008; Mogilner & Keren, 2009). Cells are also able to adapt to variable environments along their migration path by changing the mode of migration, e.g. from adhesion-dependent to adhesion-independent (amoeboid) migration (Friedl & Weigelin, 2008). Only recently, research into cell motility started to pay attention again to the fact that a cell moves as a physical entity. Consequently, the morphological and mechanical characteristics of cells are major determinants of cell migration (Mogilner & Keren, 2009; Guck et al., 2010). This applies especially to three-dimensional environments imposing topological restrictions on cell movements (Nourshargh et al., 2010). In fact, the movement through tissue pores that are significantly smaller than the cell's diameter represents the rate-limiting step of many migration-related processes, e.g., extravasation of leucocytes. While proceeding towards their destination in confinement, cells are continuously forced to adapt their shape (Fig. 1) mostly by bending, bulging and elongating (Alonso-Latorre et al., 2009). Of note, proteolysis was shown to be dispensable in this situation (Wolf et al., 2003; Rowe & Weiss, 2008).

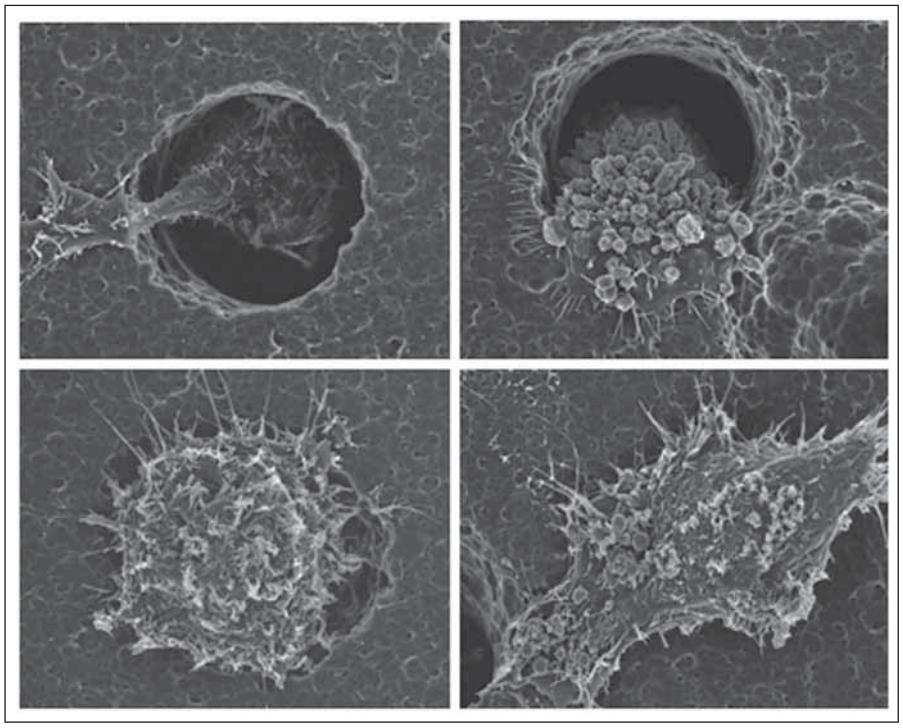

Fig. 1.: Changes of shape and membrane morphology of epithelial cancer cells migrating through 12 µm pores imaged by scanning electron microscopy.

The gap in the understanding of biochemical events on the one hand and the physical process of cell movement on the other hand is now beginning to close (Keren et al., 2008; Renkawitz & Sixt, 2010). Interestingly, interactions between these different scales do not only rely on mechanisms with well defined hierarchies of control (Iglesias, 2009), but also involve self-organization of macromolecular compartments without central bookkeeping (Bretschneider et al., 2009; Cardamone et al., 2011). Thus, biochemical signaling might be regarded as a modulator rather than a regulator of these processes. In view of these observations, Binamé et al. (2010) recently suggested considering migration as a default activity in some cell types with a preference for amoeboid, i.e. adhesion-independent, migration. Thus, it may be fair to ask what prevents these cells from moving instead of what stimulates them to move.

Cell mechanics

The cellular compartments defining the mechanical and morphological properties of cells during migration are the cell membrane (Keren, 2011), the biopolymer systems of the cytoskeleton (Fletcher & Mullins, 2010; Fig.

2) embedded in the porous cytoplasm (Mitchison et al., 2008) and the nucleus (Friedl et al., 2011). To complicate matters, these compartments are not only mechanically connected but are also exposed to signaling pathways whose complex interactions are still not fully understood. Consequently, the theoretical models proposed so far to describe cell mechanics cover only limited aspects of cell behaviour (Trepat et al., 2007; Mitchison et al., 2008). Moreover, it has been difficult to develop experimental models for each of these cellular compartments in a way that duplicates the behaviour of their intracellular counterpart with sufficient accuracy. Although such simplified model systems may provide insights into the mechanisms of self-organization (Soares Silva et al., 2011), they cannot reproduce the substantial heterogeneity of cell architecture that is, for example, due to spatio-temporal variations in biochemical signaling. Thus, whole cells still remain a valuable source of information to investigate cell mechanics.

During migration, modulation of cellular mechanics and morphology can be observed at various scales ranging from membrane compartments, e.g., blebbing (Tinevez et al., 2009), to parts of the cell (Rösel et al., 2008) and to the whole cell body (Guck et al., 2005). The cytoskeleton is crucially involved in all of these processes through generation of forces and modulation of deformability (Rauzi & Lenne, 2011). The specific contribution of actin microfilaments and microtubules has been investigated in great detail. Cell deformation during passage through small pores was found to be associated with rapid re-organization of cortical actin networks (Yap & Kamm, 2005). Forces are generated within the actomyosin system (Renkawitz & Sixt, 2010) and fine-tuned by septin during amoeboid migration (Tooley et al., 2009). The role of microtubules for the regulation of cell shape and mechanics is emphasized by the fact that stathmin, which modulates the assembly of microtubules, influences morphology and motility of tumor cells (Belletti et al., 2003; Li et al., 2011). Consequently, pharmacological inhibition of microtubule dynamics by paclitaxel was found to impair cell migration (Ganguly et al., 2011).

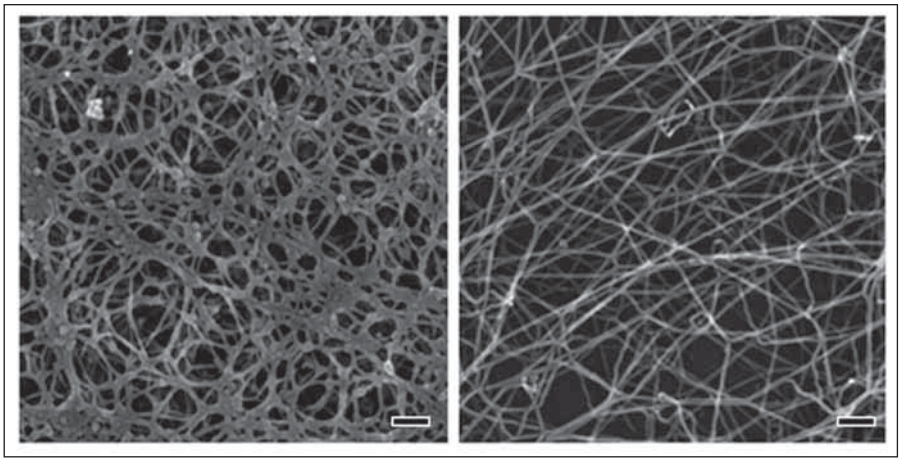

Fig. 2.: Morphology of the cytoskeleton in epithelial cells visualized by scanning electron microscopy. Cells were treated with detergents to remove the cell membrane and soluble fractions of the cytoplasm. The left panel depicts the cortical filament system that was adjacent to the cell membrane. The right panel shows mostly intermediate filaments filling the cytoplasm between the cell membrane and the nucleus. (scale bar: 200 nm)

In contrast to these two filament systems, the impact of the third system - intermediate filaments (Fig. 2) - on cell migration is much less understood. However, some findings strongly indicate that this system plays an important role for cell motility in multicellular organisms. For example, deficiency of specific keratin intermediate filaments impairs wound healing in mice, which is crucially dependent on cell migration (Mazzalupo et al., 2003). Survival of patients with colorectal carcinoma that is at least partially determined by metastasis is related to the expression of keratin intermediate filaments in cancer cells (Knösel et al., 2006). However, the lack of specific agents targeting intermediate filaments is a major obstacle to verify the impact of this biopolymer system on cell shape and mechanics. In the meantime, cells which are devoid of intermediate filaments can provide the opportunity to study this system at least in a qualitative way (Vijayaraj et al., 2009). For example, SW13 epithelial cancer cells which do not express intermediate filaments (Wöll et al., 2005) exhibit a decreased deformability when transfected with keratins 8 and 18 (Fig. 3). This change in cell mechanics only affects motility through pores which are smaller than the cell itself. Migration through substantially larger pores, which require cell body deformations to a much lesser extent, is not impaired by the presence of keratins 8 and 18 (Fig. 3). These data confirm previous findings that intermediate filaments are in control of cell mechanics when large deformations are required whereas small shape changes mostly involve actin microfilaments (Beil et al., 2003). On the analogy of the treadmilling activity of actin microfilaments, a continuous cycle of intermediate filament remodeling has recently been described, which may also have an impact on the mechanics of moving cells (Kölsch et al., 2010).

Outlook

Controlling cell motility is beneficial for managing a broad spectrum of medicial condi-

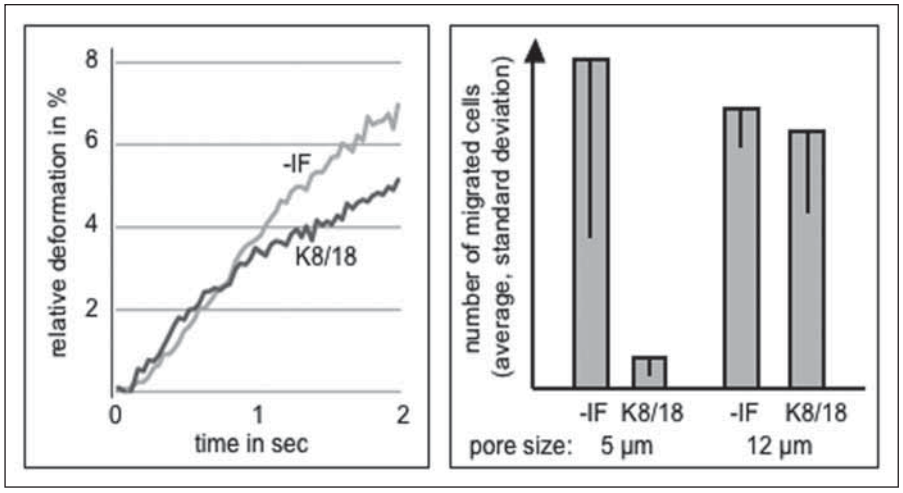

Fig. 3.: Mechanics and motility of SW13 cells with and without intermediate filaments (Wöll et al., 2005). The left panel depicts the deformation of these cells in response to optical stretching (Zink et al., 2010). The gray line represents cells without intermediate filaments (-IF). The black line shows the shape changes of cells expressing keratins 8 and 18 (K8/18). The presence of keratin intermediate filaments reduces cellular deformability in the late phase of deformation.
The right panel depicts the results of cell migration assays (Boyden chamber). Migration through 5 µm pores, which requires deformations of the cell body that are substantially larger than those during migration through 12 µm pores, is significantly impaired by the presence of keratin intermediate filaments.

tions ranging from cancer to inflammatory disorders. Current efforts to interfere with cell migration by inhibiting adhesion receptors (Goodin et al., 2008) or blocking proteolysis (Chiappori et al., 2007) have yielded only partial success so far. Despite the current knowledge about the cytoskeleton there are still very few treatment strategies targeting intracellular filament systems. This is mainly due to the lack of cell-type specificity with agents that interfere with the self-organization and regulation of actin microfilaments and to intolerable adverse events which can be expected in patients on account of it. However, two examples provide evidence for the efficacy of targeting the cytoskeleton notably with respect to modulating the microtubule system. First, paclitaxel-eluting coronary artery stents were shown to inhibit cell motility in a situation where it would otherwise cause re-stenosis within the stent (Liuzzo et al., 2005). Systemic side effects are prevented by a limited and local application of the drug. Second, colchicine inhibits leucocyte extravasation in inflammatory disorders without causing severe side effects (Nuki, 2008). This effect has been used in medicine for 3000 years (Amital & Ben-Chetrit, 2004). However, the spectrum of mechanisms by which colchicine modulates cell motility is still not fully clarified although a significant impact on transcriptional regulation at colchicine dosages administered to patients appears to be unlikely (Ben-Chetrit et al., 2006). These examples from clinical medicine emphasize the feasibility of controlling cell migration by interfering with the organization of cytoskeletal filament systems. Nonetheless, more has to be done to elucidate the complex interplay between biochemical and physical processes to fully decipher the mechanisms of cell motility.

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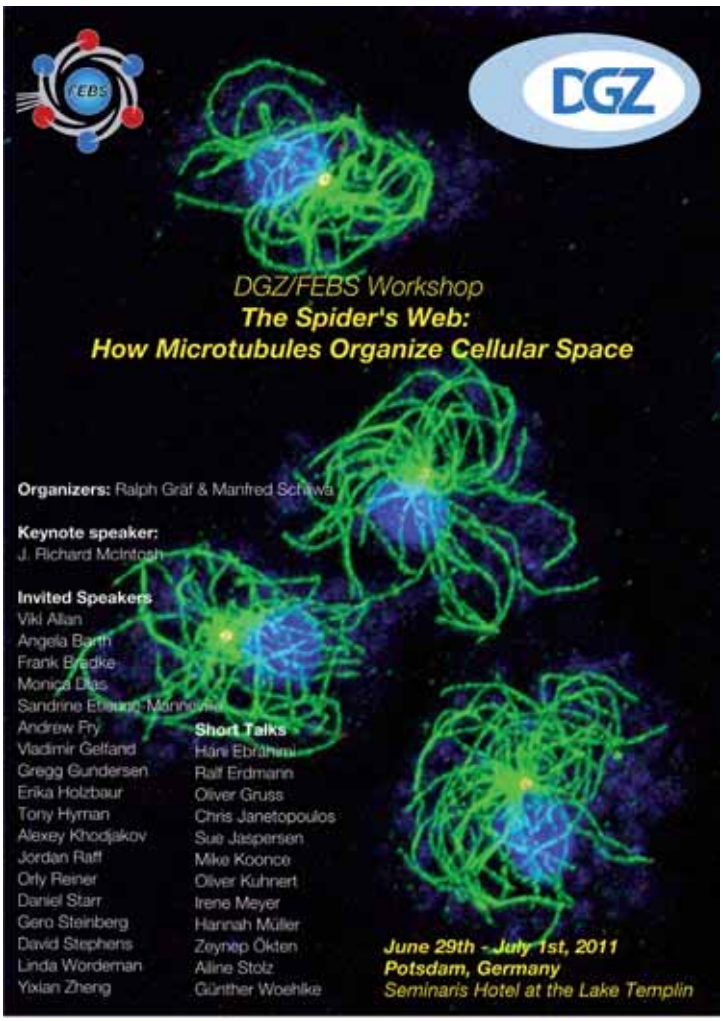
A big overview of tiny tubes

When John Gerhart's and Marc Kirschner's outstanding book **„Cells, Embryos, and Evolution"** was published in 1997, Marc Kirschner inscribed my copy with the dedication *„Dear Manfred: The secret of evolution lies in microtubules (Don't tell anyone!)"*. I followed his advice and kept the secret to myself. Others couldn't, and so a towering body of evidence now points to a central role of microtubules, their organizing centers, and their associated proteins in a wide variety of basic cellular and organismal processes, including those central to evolution. To highlight the many facets of microtubule functions, a workshop jointly organized by the German Society for Cell Biology and the Federation of European Biochemical Societies was held in Potsdam *. In 6 sessions of 5 talks each the workshop covered microtubule-organizing centers, dynamics, polarity, motors, trafficking, organelle positioning, mitosis, and the role of microtubules as cellular integrators. Naturally, due to the workshop character of the meeting that includes ample discussion time, not all microtubule functions could be exhaustively covered, but the themes presented gave an excellent overview of the breadth and depth of the field. That was intended because, much to the delight of the organizers, over 80% of the attendees were students, graduate students and postdocs. The meeting was kicked off by a splendid keynote lecture delivered by J. Richard McIntosh. Since the other invited keynote speakers cancelled their talks at the last minute, Dick McIntosh expanded his presentation at the last minute and started off with a partly historical, succinct overview of developments in the microtubule field that was greatly appreciated by the younger researchers. He then spent most of his time on a fascinating discussion on the biophysical properties of the microtubule polymer and how it serves as a pushing/pulling engine by assembly/disassembly alone. These aspects were probably not as familiar to many of the attendees as the biochemistry of microtubule function, so it offered novel perspectives. His outstanding presentation style left the audience spellbound so that the hour and a half of his presentation passed in a flash. The consistently excellent presentations by the invited speakers offered many insights into novel functions of microtubules, including: the intimate relations to the nuclear envelope, links between microtubules and the global organization of the brain, the intricacies of microtubule/motor interactions, how asymmetric cell divisions are generated, and a first fascinating look at the biogenesis and evolution of centrosomes. Moreover, the quality of the short talks selected from the abstracts was outstanding. The meeting impressively underscored that modern research on microtubules requires a breathtaking array of cutting-edge techniques. Interestingly, it still includes live-cell microscopy, confirming Yogi Berra's timeless dictum that "you can observe a lot just by watching". The overview character of the meeting was much appreciated by audience and speakers alike. The uniformly positive feedback after the meeting culminated in the request expressed in several subsequent emails that it should be repeated in two years time at the same venue, which was highly esteemed for its laid back atmosphere and beautiful surroundings at the Lake Templin.



Manfred Schliwa and Ralph Gräf

* *"The Spider's Web: How Microtubules Organize Cellular Space"*. Seminaris Hotel at Lake Templin near Potsdam, Germany, June 29 – July 1, 2011

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Publisher:
Deutsche Gesellschaft für
Zellbiologie e.V. (DGZ)
(German Society for Cell Biology)

Editorial Board:
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Production/Press:
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Waldhofer Str. 19 · 69123 Heidelberg
E-mail: info@abcdruck.de
Web: www.abcdruck.de

Media Creation:
Heike Fischer
E-mail: h.fischer@abcdruck.de

Copies:
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Frequency of publication:
4 issues yearly

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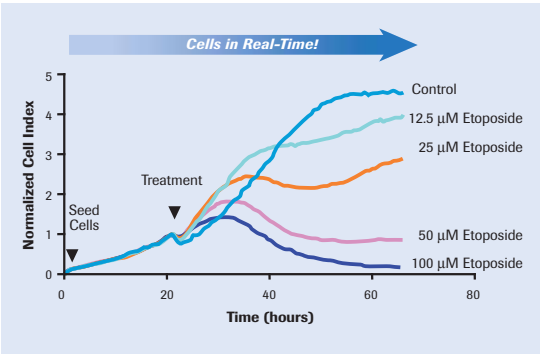

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