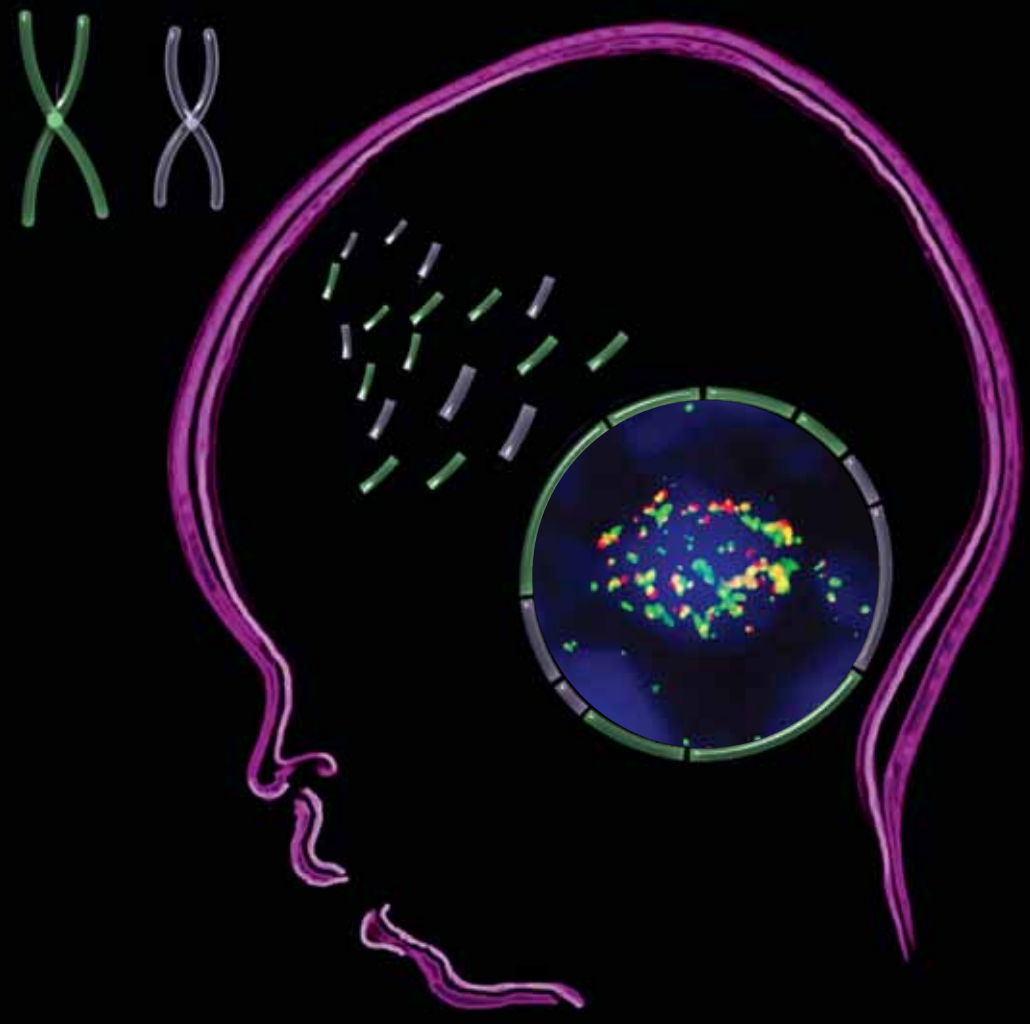
Cell News

Newsletter of the German Society for Cell Biology
Volume 38, 1/2012



Catastrophic events in brain tumor development

Deutsche Gesellschaft für Zellbiologie

Your Power for Health

greiner bio-one

Challenging Experiments?

Advanced TC™ Cell Culture Vessels from Greiner Bio-One

- Innovative polymer modification improves cellular adhesion
- Positive effects on cell functionality and performance
- Enhanced propagation of fastidious cells
- Improved cell expansion under limited growth conditions
- Better assay consistency
- Long-term stability and storage at room temperature

Germany (Main office): Greiner Bio-One GmbH, info@de.gbo.com I **Austria:** Greiner Bio-One GmbH, office@at.gbo.com
Belgium: Greiner Bio-One BVBA/SPRL, info@be.gbo.com I **Brazil:** Greiner Bio-One Brasil, office@br.gbo.com I **China:** Greiner Bio-One GmbH, info@cn.gbo.com
France: Greiner Bio-One SAS, infos@fr.gbo.com I **Japan:** Greiner Bio-One Co. Ltd., info@jp.gbo.com I **Netherlands:** Greiner Bio-One B.V., info@nl.gbo.com
UK: Greiner Bio-One Ltd., info@uk.gbo.com I **USA:** Greiner Bio-One North America Inc., info@us.gbo.com

www.gbo.com/bioscience

CONTENT

Mitteilungen der Deutschen Gesellschaft für Zellbiologie

Cover image: Sequencing of a medulloblastoma brain tumor from a patient with an inherited TP53 mutation in the context of the German contribution to the International Cancer Genome Consortium (ICGC) revealed catastrophic chromosome rearrangements (also termed 'chromothripsis'), visualized on the cover as countless chromosome parts that are lost to the cell, or randomly glued together. A subset of the shattered pieces formed small circular chromsomes ('double minute chromosomes') in the tumor cells, as the one depicted in the center of the image. The co-localization of distant pieces in a double minute chromosome was verified by fluorescence in situ hybridization (FISH), with green and red probes positioned on chrX and chr3 in the image. (FISH performed by Andrey Korshunov, University of Heidelberg, and Clinical Cooperation Unit Neuropathology, German Cancer Research Center [DKFZ]. Graphical design by Jelena Tica, Tobias Rausch, and Thomas Zichner, European Molecular Biology Laboratory, Heidelberg.)

Prime of the Year: The Annual Meeting

After lots of preparations, some of you may have a clue what it is like, the annual meeting is ready to start. As you will learn on the following pages, the Dresden team – Elisabeth Knust, Ewa Paluch and Marino Zerial – several members of the DGZ as well as the invited chairs have succeeded to organize a terrific program. This time, the non-scientific part of the organization was operated by MCI, a company we have relied on a lot in the past. Their help was absolutely essential as this time we will hold the meeting in a commercial congress centre. Moreover, MCI is very experienced, for instance with registration and abstract management, nevertheless we want your feedback in case problems arose such that we can improve these processes further. Here the author has a "flash back" how laborious and frustrating registration could be only 10 years ago when you did for example online registration for the ASCB Annual Meeting. Remember, in the middle of the process the net "froze" and you could start anew. Yet, our big sister society did a lot of development for meeting culture and organization and it is fair to say that we indeed learned from them.

Going to Dresden

Talking to several people, I was surprised that they did not notice an essential service that was installed by MCI. On the homepage of the meeting (www.zellbiologie2012.de) you will find a button – the DB Bahn logo – that connects you to a page where you will see that going to Dresden with the DGZ is cheap: A roundtrip is Euro 99,00 for second class and Euro 159,00 for first class. Hence, I encourage all attendees to make use of this very strong offer. It is valid for March 21st to 25th.

The New Board Members

The members for the new Executive Board have been elected. Hence, Eugen Kerkhoff (Regensburg) will be the new President, Ralph Gräf (Potsdam) the Vice-President, Oliver Gruss (Heidelberg) the Chief Operating Officer and Klemens Rottner (Bonn) the Secretary of the Society. Doris Wedlich, Eckart Lammert and Volker Gerke have been re-elected to the Advisory Board. The former Past-President Reinhard Fässler is rotationally leaving the Advisory Board just as Klemens Rottner who moves to the Executive Board. Anja Bosserhoff, the former Vice-President, will step rotatively on the Advisory Board just like myself, being the new Past-President. We cordially welcome this young crew of scientists that is distributed all over Germany, and we hope that these four will develop new efficient activities to integrate more of our colleagues in cell biology and neighbouring sciences into the activities of the DGZ.

Yesterday's Gone

This is how an old Fleetwood Mac song was called. So what about tomorrow? In the last two years the two Boards tried to accomplish several things. First of all, to continue at the high standards that previous board members had established, in particular with the annual meetings. However, as every meeting is actually an experiment, new things were tried out, in particular, new meeting formats such as the two very successful "International Meetings". The first of this series, "Actin dynamics" of 2010, is held again this year, due to its enormous success. In addition, we supported the biophysical community in the organization of their latest "Physics of Cancer" symposium in Leipzig in October 2011. Secondly, we changed the publication frequency for our newsletter *Cell News* and now have four issues per year. Hence, we can provide prompt information for upcoming meetings.

Focus on Dresden

As an example of how *Cell News* can be used for providing comprehensive information, we presented several groups from the site of this years' annual meeting in the last three issues: One article in 3/2011, six in 4/2012 and now three in this issue. With these ten articles we have installed a strong platform for the institutes located in Dresden to communicate their activities. Actually, it was even eleven articles: Jochen Guck, sending his article for the 3-2011 issue still from Cambridge/UK, is from the beginning of this year on residing in Dresden at the *Technische Universität*. In the centre of the eleven articles was the *Max Planck Institute of Molecular Cell Biology and Genetics* (MPI-CBG). Other affiliations appearing were The *Max Planck Institute for the Physics of Complex Systems*, *Institut für Genetik at Technische Universität*, the *DFG Research Center for Regenerative Therapies Dresden* (CRTD) and the *Biotechnology Center* (BIOTEC) of *Technische Universität*.

A further activity was to connect to colleagues from specific fields: Biophysics, Genetics, Stem Cells. Maybe, the young generation uses Twitter or Facebook to team up. However, being somehow old-fashioned I do believe that it is important to sit together and chat. And for this you have to meet. Skype is not as full an experience as a conversation face to face is. Most importantly, the young generation has to have the possibility to mix with the big shots. Sometimes it is helpful to listen, ask and think for a while, before running out to invent the wheel or whatever just another time.

Annual Meeting 2013

Inevitably, we have to organize next years' meeting right now. The next meeting will be a joint meeting with the *Gesellschaft für Entwicklungbiologie* (GfE). Joachim Wittbrodt *Department of Developmental Biology, Centre for Organismal Studies Heidelberg* (COS), *University of Heidelberg*, and myself will organize this event. Please hold the date: March 20 to 23, 2013 in Heidelberg. We are still open for suggestions relating program topics and would be glad to receive suggestions from our members. As you may expect, we will use *Cell News* to prepare for this joint venture. Therefore, please inform your colleagues about this event, think about contributing yourself and hand over your copy of *Cell News* to non-member colleagues or ask for extra copies that you want to hand out in lectures or seminars.

Last but not least I want to invite the members to the **DGZ Member Meeting**, Thursday March 22nd 2012, 12:15 to 13:15. Here we will introduce the new Executive Board and discuss how to proceed with the activities of our society.

Harald Herrmann

DGZ Member Meeting 2012

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

Thursday, March 22, 2012, 13:15 - 14:15 (room: Konferenzraum 3)

at the 35th DGZ Annual Meeting in Dresden (MARITIM Hotel & Internationales Congress Center Dresden, Ostra-Ufer 2).

Agenda:

1. Confirmation of the minutes of the last year's DGZ member meeting 2011
2. The president's annual report
3. Financial report
4. The auditors' report
5. Approval of the executive board
6. DGZ election 2012-2014
7. Change of the DGZ bylaws
8. „Other"

We are looking forward to seeing you in Dresden.

Travel Information

The Maritim Hotel & International Congress Centre Dresden is located in a tranquil spot right on the banks of the Elbe and not far from the attractions of the historic old city. The "Semperoper", the "Frauenkirche" ("Church of Our Lady"), the Green Vault and much more are just a few minutes away on foot. The attractive and architecturally unique building is the ideal starting point for all travel occasions.

For more details see **www.zellbiologie2012.de** (Travel Information)

Location of the Congress Centre

Source:
MARITIM Hotel & Internationales Congress Center Dresden

Scientific Programme

Wednesday, March 21

14^{00}–14^{15} **Opening Ceremony**

14^{15}–17^{00} **Plenary Session 1: Nuclear organization**
Chair: Ivan Raska (Prag)
Wendy Bickmore (Edinburgh): Chromatin organization in the nucleus and gene regulation – from cells to embryos
William Earnshaw (Edinburgh): Repo-Man coordinates chromatin modifications with nuclear reassembly during mitotic exit
Karla Neugebauer (Dresden): Organizational principles for nuclear pre-mRNA processing: is each gene a mini-organelle?
Ivan Raska (Prag): Are the Polycomb group proteins bodies gene silencing factories?
Eric Schirmer (Edinburgh): Tissue-specific nuclear envelope proteins contribute to differentiation by influencing gene expression through spatial organization of the genome

17^{00}–17^{15} Break

17^{15}–18^{15} **DGZ Award Ceremony**

Walther Flemming Medal
Martin Beck (Heidelberg): Compositional remodeling of the human nuclear pore complex
Binder Innovation Prize
Sven Diederichs (Heidelberg): microRNA & long non-coding RNA in cancer
Werner Risau Prize
Jorge Ivan Alvarez (Montreal): The hedgehog pathway sustains blood-brain barrier properties and promotes CNS immunoquiescence

18^{15}–19^{00} **Carl Zeiss Lecture**
Fiona Watt (Cambridge): Studying stem cells in mammalian epidermis

19^{00} **Poster Session and Welcome Reception**

Thursday, March 22

09^{00}–12^{00} **Symposium 1: Modelling in cell biology**
Chair: Ewa Paluch (Dresden)
Martin Howard (Norwich): Construction of a robust intracellular concentration gradient in fission yeast
Achim Kramer (Berlin): Dynamics and synchronization of circadian clocks
Ewa Paluch (Dresden): Actin cortex mechanics and cell shape control in cytokinesis
and speakers selected from the abstracts

09^{00}–12^{00} **Symposium 2: miRNA and cancer**
Chair: Marcus Peter (Chicago)
Reuven Agami (Amsterdam): Cancerous microRNAs and regulatory RNA binding proteins
Thomas Brabletz (Freiburg): EMT, microRNAs and cancer stem cells
Marcus Peter (Chicago): The role of microRNAs in tumor progression
and speakers selected from the abstracts

09^{00}–12^{00} **Symposium 3: Cell metabolism and cell homeostasis**
Chair: Mike Hall (Basel)
Johan Auwerx (Lausanne): Integrating metabolic control by NAD+ sensors
Mike Hall (Basel): TOR signaling in growth and metabolism
Tobias Huber (Freiburg): Role of mTOR signalling in Diabetic Nephropathy
and speakers selected from the abstracts

09^{00}–12^{00} **Symposium 4: Meiosis**
Chair: Wolfgang Zachariae (München)
Scott Keeney (New York): Controlling the number and timing of meiotic double-strand breaks
Marie-Helene Verlhac (Paris): Error prone mammalian female meiosis from silencing the SAC without interkinetochore tension
Wolfgang Zachariae (München): Regulation of meiotic prophase
and speakers selected from the abstracts

09^{00}–12^{00} **Symposium 5: Microtubules and Motors**
Chair: Zeynep Ökten (München)
Joe Howard (Dresden): Motors and microtubule dynamics
Carsten Janke (Paris): Regulation of microtubule functions by posttranslational modifications
Zeynep Ökten (München): Neck structure determines the path of processive kinesin motors along microtubules
and speakers selected from the abstracts

12^{00}–15^{30} **Poster Session / Lunch**

12^{15}–13^{15} **Lunch Symposium: Carl Zeiss MicroImaging GmbH**
Imaging with smart software

13^{15}–14^{15} **DGZ Member Meeting**

15³⁰–18³⁰ **Plenary Session 2: Cell and tissue morphogenesis**
Chair: Elisabeth Knust (Dresden)
Darren Gilmour (Heidelberg): Coordinating cell movement within migrating collectives
Elisabeth Knust (Dresden): Cell polarity – pattern formation at the cellular level
Thomas Lecuit (Marseille): The subcellular mechanics of tissue morphogenesis
James Nelson (Stanford): Regulation of cadherin cell-cell adhesion and actin dynamics: An evolutionary perspective
Benjamin Podbilewicz (Haifa): Conserved eukaryotic cell-cell fusion proteins function in membrane sculpting

15³⁰–18³⁰ **Plenary Session 3: Frontiers in microscopy**
Chair: Petra Schwille (Dresden/München)
Jan Huisken (Dresden): Visualizing zebrafish development in real-time with high-speed SPIM
Petra Schwille (Dresden/München): How to fill the voids: in situ characterization of reaction-diffusion systems
Holger Stark (Göttingen): Visualizing dynamics – The ribosome in motion!
Philip Tinnefeld (Braunschweig): Testing superresolution microscopy with DNA origami
Andreas Zumbusch (Konstanz): CARS microscopy of intracellular lipid droplet dynamics

19⁰⁰–20⁰⁰ **Distinguished Lecturer**
Kim Nasmyth (Oxford)

Friday, March 23

09⁰⁰–12⁰⁰ **Symposium 6: Asymmetric division – Mechanics of cell division**
Chair: Daniel Gerlich (Zürich)
Daniel Gerlich (Zürich): Imaging abscission, the last step in cell division
Matthieu Piel (Paris): ESCRT assembly and cytokinetic abscission are delayed by tension in the intercellular bridge
Melina Schuh (Cambridge): An actin-dependent mechanism for long-range vesicle transport
and speakers selected from the abstracts

09⁰⁰–12⁰⁰ **Symposium 7: Autophagy and cross-talk between organelles**
Chair: Zvulun Elazar (Rehovot)
Christian Behl (Mainz): BAG3-mediated selective macroautophagy of aggregation-prone proteins
Zvulun Elazar (Rehovot): Different roles of Atg8s in autophagosme biogenesis and cargo selection
Jon Lane (Bristol): Autophagy and mitochondrial quality control in differentiation and disease
and speakers selected from the abstracts

09⁰⁰–12⁰⁰ **Symposium 8: Cell biology of the immune response**
Chair: Jack Neefjes (Amsterdam)
Jack Neefjes (Amsterdam): Making sense of mass siRNA data to generate new biology for systems in the immune system
Tim Lämmermann (Bethesda): Real-time imaging of chemotactic and adhesive events at the wound site
Paul Lehner (Cambridge): Novel E3 ligases in the MHC class I antigen presentation pathway – a role for ubiquitin in post-transcriptional regulation
and speakers selected from the abstracts

09⁰⁰–12⁰⁰ **Symposium 9: Control of cell and organ size**
Chair: Aurelio Teleman (Heidelberg)
Ernst Hafen (Zürich): From genetics to systems genetics of growth control
Alison Lloyd (London): The control of mammalian cell size
Aurelio Teleman (Heidelberg): Regulation of growth by insulin signaling in Drosophila
and speakers selected from the abstracts

09⁰⁰–12⁰⁰ **Symposium 10: Cytoskeleton mechanics**
Chair: Andreas Bausch (München)
Andreas Bausch (München): Cytoskeletal pattern formation: Self organization of driven filaments
Stephan Grill (Dresden): Mechanics meets Biochemistry: PAR polarization in C. elegans
Xavier Trepat (Barcelona): The forces behind collective cell guidance
and speakers selected from the abstracts

12⁰⁰–15³⁰ **Poster Session / Lunch**

12¹⁵–13¹⁵ **Lunch Symposium: ibidi GmbH**
Cell Culture Assays

15³⁰–18³⁰ **Plenary Session 4: Cilia**
Chair: Lotte Pedersen (Kopenhagen)
Karl Lechtreck (Athens, USA): Chlamydomonas as a model for ciliary disease: Bardet-Biedl syndrome
Heymut Omran (Münster): Molecular defects of motile cilia and flagella
Lotte Pedersen (Kopenhagen): Primary cilia and kinesin-3 motor proteins
Nathalie Spassky (Paris): Development and roles of ciliated cells during mammalian neurogenesis
Peter Swoboda (Stockholm): RFX transcription factors, ciliogenesis and more?

15³⁰–18³⁰ **Plenary Session 5: Cell adhesion and migration**
Chair: Maria Leptin (Heidelberg)
Ralf Adams (Münster): Molecular regulation of angiogenic blood vessel growth
John Condeelis (New York): Invadopod-dependent tumor cell migration in breast tumors
Maria Leptin (Heidelberg): Genetics and cell biology of complex cell shapes
Laura Machesky (Glasgow): Signaling to actin dynamics in invasion and migration
Manuel Thery (Grenoble): Centrosome positioning in response to cell-cell and cell-ECM adhesions

18⁴⁵–19⁰⁰ **Poster Awards**

19⁰⁰–20⁰⁰ **Frontiers in Science Lecture**
Kai Simons (Dresden): Moving cell biology beyond boundaries of today

20⁰⁰ Get Together

Saturday, March 24

09⁰⁰–10⁰⁰ **Matthias Schleiden Lecture**
Günter Blobel (New York): Molecular design of nature's largest and most versatile channel anchored in the center of the nuclear pore

10⁰⁰–13⁰⁰ **Symposium 11: Evolution of the cell**
Chair: Gaspar Jékély (Tübingen)
Martin Embley (Newcastle): Evolutionary and functional relationships between eukaryotes and mitochondria
Gaspar Jékély (Tübingen): Origin of the first neurons as sensory-motor and sensory-neurosecretory cells
José Pereira Leal (Lissabon): The translational applications of an evolutionary cell biology
and speakers selected from the abstracts

10⁰⁰–13⁰⁰ **Symposium 12: Regeneration and stem cells**
Chair: Rüdiger Simon (Düsseldorf)
Jochen Rink (Dresden): Organogenesis during planarian regeneration
Rüdiger Simon (Düsseldorf): Plant stem cell systems
Elly Tanaka (Dresden): Epithelial organization during axolotl spinal cord regeneration
and speakers selected from the abstracts

10⁰⁰–13⁰⁰ **Symposium 13: Cell biology of therapeutic delivery**
Chair: Leonard Rome (Los Angeles)
Enrico Mastrobattista (Utrecht): Cellular barriers to nanocarrier-mediated delivery of proteins and nucleic acids
Leonard Rome (Los Angeles): Vault Nanoparticles: A Platform Technology for Delivery of Therapeutics
Ernst Wagner (München): Polymers for DNA and RNA delivery: Inspired by viruses to be targeted, dynamic and precise
and speakers selected from the abstracts

10⁰⁰–13⁰⁰ **Symposium 14: Neuronal network**
Chair: Gaia Tavosanis (Bonn)
Caspar Hoogernaad (Rotterdam): Control of neuronal polarity and plasticity - the role of dynamic cargo trafficking
Beatriz Rico (Alicante): On the assembly of neural circuits: from axon development to synapse formation
Gaia Tavosanis (Bonn): Activity shapes a central circuit in Drosophila
and speakers selected from the abstracts

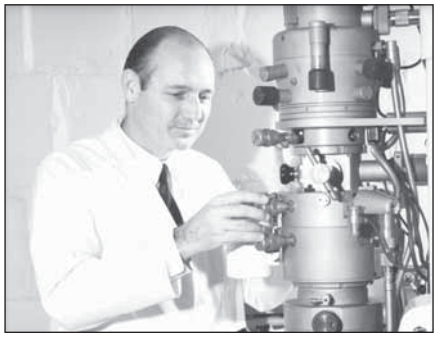
10⁰⁰–13⁰⁰ **Symposium 15: Protein conformation diseases: cellular mechanisms and consequences**
Chair: Zoya Ignatova (Potsdam)
Zoya Ignatova (Potsdam): Translational frameshift and dynamics of intracellular aggregates: consequences for the pathology of polyglutamine-repeat diseases
Martin Vabulas (Frankfurt): Structural determinants of intracellular proteotoxicity
Ina Vorberg (Bonn): Cytosolic protein aggregates as infectious entities
and speakers selected from the abstracts

13:00 Closing Ceremony

www.zellbiologie2012.de

Dr. Oscar Lee Miller, Jr.,

who showed us genes in transcription, died on January 28, 2012 at the University of Virginia Hospital



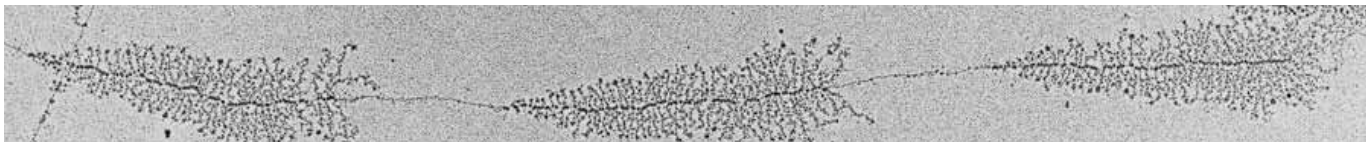
Dr. Miller was born on April 12, 1925 in Gastonia, North Carolina. He was preceded in death by his parents, Dr. Oscar Lee Miller and Rose Evans Miller and by his older brother, Dr. Robert E. Miller. He is survived by his loving wife of sixty-three years, Mary Rose Miller; his sister, Caroline Miller McClintock; his younger brother, the Rev. John Miller; his daughter, Sharon Miller Bushnell; and his son, Oscar Lee Miller, III

Oscar Miller served in the US Navy from 1943 through 1946. After earning a bachelor's and master's degrees in agronomy from NC State, he was a farmer for six years and then enrolled in the University of Minnesota where he earned his PhD in plant genetics. He joined the research staff at Oak Ridge National Laboratory in 1961 and began the work that earned him worldwide renown as a molecular biologist. Prior to this time, the existence of genes and of the double helix structure of DNA were accepted in the scientific community as sound hypotheses that were, unfortunately, beyond the scope of actual observation. He developed a technique – now known as "Miller spreading" – that enabled scientists to visualize individual genes through electron microscopy and led to many important advances in our view and understanding of chromatin and transcription (see the picture below, showing fully transcribed genes of ribosomal RNA separated by non-transcribed "spacer" regions).

Oscar Miller was elected to the National Academy of Sciences in 1978. He was named a Fellow of the American Association for the Advancement of Science in 1980. He was Chairman of the Department of Biology and held the William R. Kenan and the Lewis and Clark Professorship at the University of Virginia. He was visiting professor of biology at the Center of Investigation and Advanced Studies in Mexico City, the California Institute of Technology, the Max-Planck-Institut für Zellbiologie in Heidelberg, Germany and the University of California at Irvine. He was the Senior Fulbright Scholar at the Division of Molecular Biology, CSIRO at New South Wales, Australia. Among his many honors from around the world, he received the Life Achievement Award in Science from the Commonwealth of Virginia in 1997. Oscar Miller was an inspiring teacher and a thought provoking companion, admired by his colleagues and adored by his family.

In the name of all German "Miller spread" colleagues we again thank Oscar for what he showed us and the world, and that electron microscopy can sometimes really meet the statement: "Seeing is believing, and understanding, too."

Werner W. Franke, Ulrich Scheer, Michael Trendelenburg, Herbert Spring



Selected Publications

Miller, OL Jr, Beatty, BR, 1969. Visualization of nucleolar genes. *Science* **164**:955-957.

Miller OL Jr, Hamkalo BA, Thomas CA Jr, 1970. Visualization of bacterial genes in action. *Science*. **169**:392-395.

Miller OL Jr, Hamkalo BA, 1972. Visualization of RNA synthesis on chromosomes. *Int Rev Cytol*. **33**:1-25.

Hamkalo BA, Miller OL Jr, 1973. Electronmicroscopy of genetic activity. *Annu Rev Biochem*. **42**:379-396.

Miller OL Jr, 1973. The visualization of genes in action. *Sci Am*. **228**:34-42.

McKnight SL, Miller OL Jr, 1976. Ultrastructural patterns of RNA synthesis during early embryogenesis of Drosophila melanogaster. *Cell* **8**:305-319.

McKnight SL, Miller OL Jr, 1977. Electron microscopic analysis of chromatin replication in the cellular blastoderm Drosophila melanogaster embryo. *Cell* **12**:795-804.

McKnight SL, Miller OL Jr, 1979. Post-replicative nonribosomal transcription units in D. melanogaster embryos. *Cell*. **17**:551-563.

Beyer AL, Miller OL Jr, McKnight SL, 1980. Ribonucleoprotein structure in nascent hnRNA is nonrandom and sequence-dependent. *Cell*. **20**:75-84.

Beyer AL, Bouton AH, Miller OL Jr, 1981. Correlation of hnRNP structure and nascent transcript cleavage. *Cell* **26**:155-165.

Miller, OL Jr, 1981. The nucleolus, chromosomes, and visualization of genetic activity. *J. Cell Biol*. **91**:155-27s.

Beyer AL, Bouton AH, Hodge LD, Miller OL Jr, 1981. Visualization of the major late R strand transcription unit of adenovirus serotype 2. *J Mol Biol*. **147**:269-295.

Francke C, Edström JE, McDowall AW, Miller OL., 1982. Electron microscopic visualization of a discrete class of giant translation units in salivary gland cells of Chironomus tentans. *EMBO J*. **1**:59-62.

Osheim YN, Miller OL Jr., 1983. Novel amplification and transcriptional activity of chorion genes in Drosophila melanogaster follicle cells. *Cell*. **33**:543-553.

Martin KA, Miller OL Jr, 1983. Polysome structure in sea urchin eggs and embryos: an electron microscopic analysis. *Dev Biol*. **98**:338-348.

Miller OL, 1984. Some ultrastructural aspects of genetic activity in eukaryotes. *J. Cell Sci. Suppl*. **1**:81-93.

Osheim YN, Miller OL Jr, Beyer AL, 1985. RNP particles at splice junction sequences on Drosophila chorion transcripts. *Cell* **43**:143-151.

French S, Martin K, Patterson T, Bauerle R, Miller OL Jr, 1985. Electron microscopic visualization of trp operon expression in Salmonella typhimurium. Proc Natl Acad Sci U S A. 82:4638-4642.

Saffer LD, Miller OL Jr, 1986. Electron microscopic study of Saccharomyces cerevisiae rDNA chromatin replication. *Mol Cell Biol*. **6**:1148-1157.

Osheim YN, Miller OL Jr, Beyer AL, 1988. Visualization of Drosophila melanogaster chorion genes undergoing amplification. *Mol Cell Biol*. **8**:2811-2821.

Mougey EB, O'Reilly M, Osheim Y, Miller OL Jr, Beyer A, Sollner-Webb B, 1993. The terminal balls characteristic of eukaryotic rRNA transcription units in chromatin spreads are rRNA processing complexes. *Genes Dev*. **7**:1609-1619.

100th birthday of George Emil Palade and Keith Roberts Porter

The third revolution in cell biology

Manfred Schliwa

Fields of scientific inquiry have numerous contributors, but more often than not they are truly shaped by only a few. This is especially true for the first revolution in cell biology. In the early 19th century, many microscopists had a diffuse notion of the existence of smaller building blocks in complex organisms that comprise organs and tissues, but it took the courage of Schleiden and Schwann to suggest *"that there is one universal principle of development for the elementary parts of organisms, however different, and that this principle is the formation of cells"* (Schwann 1839). The "cell theory", as it was called thereafter, was accepted enthusiastically by such future eminent scientists as Robert Remak, Johannes Müller, Max Schultze, Albert Kölliker, and Rudolf Virchow. This first revolution in cell biology was undoubtedly its boldest.

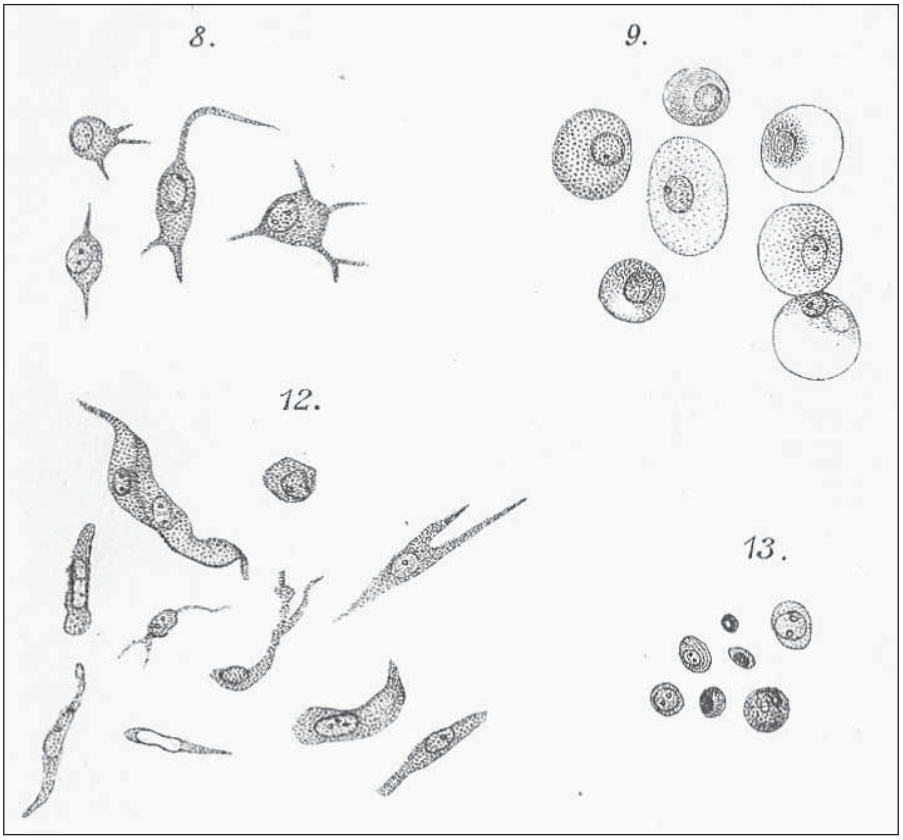


Figure 1: Cells as seen by Schwann (1939). All drawings are from pig fetuses as follows: region between chorion and amnion (Fig. 8); tissue of the eye cavity (Fig. 9); middle layer of the aorta (Fig. 12); interior of the musculus quadratus lumborum (Fig. 13).

In the decades that followed, the intricacies of these building blocks postulated by Schleiden and Schwann were probed with increasingly sophisticated microscopical, histological, and chemical techniques. When Palade and Porter were born in 1912, Martin Heidenhain had just published his monumental treatise *Plasma und Zelle* (Heidenhain 1907-1911), in which he summarized on over 1100 pages the current knowledge about cell structure and function.

And what a body of knowledge it was! The rather indistinct entities observed and drawn by Schwann, with nothing more than a nucleus as their characteristic feature (Fig. 1), now revealed an unprecedented complexity thanks to a combination of differential staining procedures, improved optics, and diligent observation and experimentation. For example, thread-like *bioblasten* (Altmann 1894), an *apparato reticolare intorno* (Golgi 1898), *granuläre inseln* (Nissl 1894), and *drüsenkörnchen* (Heidenhain 1868) were described as distinct –though not necessarily universal – cell constituents (Fig. 2). They are known today as, respectively, mitochondria, Golgi apparatus, rough endoplasmic reticulum, and secretory vesicles. It was a time when cell theory turned into cell biology. This was the second revolution in cell biology. It did not burst on the scene in a flash but rather evolved over a number of years, but it was a revolution nonetheless because it provided an entirely new view of the cell as an intriguingly complex entity.

All this was, of course, unknown to young George and Keith when they grew up in Iasi,

eastern Romania, and Yarmouth, Nova Scotia, Canada, respectively. Nevertheless, there is an invisible link between these discoveries around the turn of the 20th century and their own work on the very same cell components a few decades later. The work of Porter, Palade and their contemporaries lifted the study of cells to yet another higher level and heralded the third revolution in cell biology when the links between cell architecture and function emerged.

The initial phases of the academic careers of both Palade and Porter (Fig. 3) were indistinguishable from those of many other studious and intelligent students. Porter graduated from Acadia University in Wolfville, Nova Scotia, and then went to graduate school at Harvard University, receiving his Ph.D. in 1938. After a year in Princeton he joined the Rockefeller Institute for Medical Research (which later turned into Rockefeller University) in New York. Palade studied at the School of Medicine of the University of Bucharest, from where he received an M.D. in 1940. He then served in the medical corps of the Romanian Army until the end of the war. In 1946 he went to work with Robert Chambers at New York University, but a seminar given there by Albert Claude inspired him to join his group at the Rockefeller Institute. This is where Porter's and Palade's paths eventually crossed.

The environment at the Rockefeller Institute in the 1940s had a unique atmosphere and spirit. It sparked one of those rare moments where people with different backgrounds, perspectives and motives come together more or less by chance at the right time in the right place to change the course of science. Albert Claude, working with James Murphy, had succeeded in purifying by ultracentrifugation an agent that transmitted sarcomas from infected to healthy chicken (Claude 1940). That agent was a particle he called a microsome that was invisible in the light microscope. Porter, working in the same lab, studied the effects of carcinogens that transformed cultured cells into a malignant state, but he was dissatisfied with the limited resolution of the light microscope to reveal structural changes in the process. Thanks to fortuitous circumstances, the two of them were allowed to use one of the few existing (and functioning) electron microscopes, at the Interchemical Corporation in New York. The first electron microscopes had been built roughly a decade earlier by Ernst Ruska and his colleagues (Knoll and Ruska 1932), but these instruments were hardly ever employed in biology largely due to the lack of suitable preparative techniques. At the Interchemical Corporation, the microscope was used for profitable work on industrial materials, but Claude and Porter were allowed to "fool around" with their biological samples after hours under the supervision of the operator, Ernest Fullam (who later ran a successful company for electron microscopy supplies).

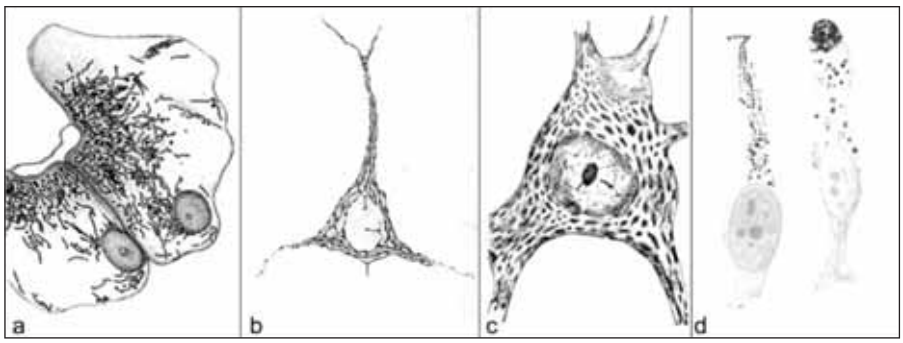


Figure 2: Visualization of organelles as seen by specific staining procedures at the end of the 19th/ beginning of the 20th century.
(a) Bioblasts (now mitochondria) in liver cells of Rana esculenta, differential staining with aniline red and picric acid. Altmann (1894).
(b) Internal reticular apparatus (now Golgi apparatus) in a nerve cell of the cerebral cortex of the mouse as revealed by Golgi's "black method". Unpublished original drawing preserved with Golgi's papers (around 1898).
(c) Anterior horn cell of the rabbit stained with Nissl's method (1894) using methylene blue showing the cell body to be filled with granular islands (now rough endoplasmic reticulum).
(d) Goblet cells in the intestine of the salamander stained with osmic acid and gentian violet, showing secretory granules. From Heidenhain (1907).

They literally had to start from scratch to view their samples. Embedding and thin sectioning techniques were unknown, but thin plastic or collodion films were already in use, as were 3mm mesh carriers for sample viewing. So Claude's particulate fractions were spread on the film and Porter's cells were cultured on it. Tests revealed osmium vapors to be most suitable both as a fixative and an electron-dense stain. Then the samples were simply viewed in their as-is state. The results of their efforts (Claude and Fullam 1945, Porter et al. 1945) were stunning images of isolated organelles and the first whole-mount preparation of a cell visualized in the electron microscope (Fig. 4). The cell images revealed, besides mitochondria and vesicle-like bodies, a lace-like tubular network that later was given the name endoplasmic reticulum by Porter (1953). It was nothing less than sensational.

It would be nice to know what went through Porter's mind when he held these very first electron micrographs in his hands. Did he envision already at that point that electron microscopy would revolutionize biology and develop into a big industry? He certainly did not think that this was the end of the

line, because he immediately started working on procedures that allowed to study other cells that are not as thinly spread as the fibroblasts of the first preparations. His efforts in the years that followed led to the development of fixation, embedding, and sectioning techniques suitable for preparing a wide variety of biological samples for electron microscopy. These developments included, in collaboration with Joseph Blum, the mechanic of the Rockefeller machine shop, the design of a thin sectioning machine. In the years to come, the "Porter-Blum Ultramicrotome" became the gold standard for microtomy.

When Palade joined Albert Claude at the Rockefeller in 1946, the cell biology revolution was already in full swing. Initially, Palade followed his own agenda, working on cell fractionation and biochemical studies of these fractions. Together with Hogeboom and Schneider he introduced the sucrose technique for the analysis of liver cell fractions (Hogeboom et al. 1948). After Claude left the Rockefeller in 1949, Palade turned part-time electron microscopist, and both he and Porter worked out improved fixation procedures. Eventually, the two also teamed up experimentally (Palade and Porter 1954) in a study of the lace-like network visualized in the very first electron micrographs of 1945. Then their paths of inquiry diverted again. Porter, who now headed the cell biology group at the Rockefeller, continued his tour de force through the intricacies of cellular and extracellular organization, exploring a wide spectrum of cells, organelles and macromolecular assemblies, literally setting up new fields of study in the process. Palade also did his share in exploring the fine structure of organelles such as mitochondria, chloroplasts, synapses and, significantly, the small particles that became known as ribosomes (Palade 1955). He then went back to cell fractionation and biochemical analysis, but he continued to include electron microscopy as a tool to understand the links between biochemical function and structural organization. Possibly the most significant contribution that emerged from these studies was the elucidation of the secretory pathway from the site of synthesis at the endoplasmic reticulum via the Golgi apparatus to secretory granules. This work functionally integrated the cell constituents that had been described as separate entities in the late 1900s, as outlined above, and thus reflects back on these discoveries of the second revolution in cell biology.

Figure 3: Palade and Porter at the beginning of their scientific careers.

The paths of Porter's and Palade's lives finally also separated spatially when Porter went to chair the Biology Department at Harvard in 1961. Palade stayed at the Rockefeller until 1973 and then became chair of Cell Biology at Yale University. Both of them remained imaginative, visionary and influential figures in the field they had revolutionized in the 1940s.

It was only a question of time when this revolution and their exponents should be honored with a Nobel Prize. It did indeed take a while, roughly 30 years, but it finally happened in 1974. Already then, and increasingly so in the decades that followed, it was becoming difficult to single out the exponents of a discovery or development that most deserved the prize because the Nobel statutes only allow a maximum of three laureates. One of the awardees of the Nobel Prize in Physiology or Medicine 1974 for discoveries in cell biology was Christian de Duve. He did not make an appearance in this homage of the Porter/Palade anniversary so far because he did not interact with either of the two birthday boys. Working in Louvain (Belgium), he discovered lysosomes and peroxisomes in the 1950s. (His seminal work certainly would be worth a separate feature in this journal.) This leaves two more potential awardees. Of course, it had to be the Rockefeller group – no doubt about that. The choice was made for Albert Claude and George Palade. Porter was left out, even though his contributions were no less important than those of the other two. He simply got the short end of the stick, in the view of many an egregious injustice. The October 2008 issue of *Scientific American* counts Porter

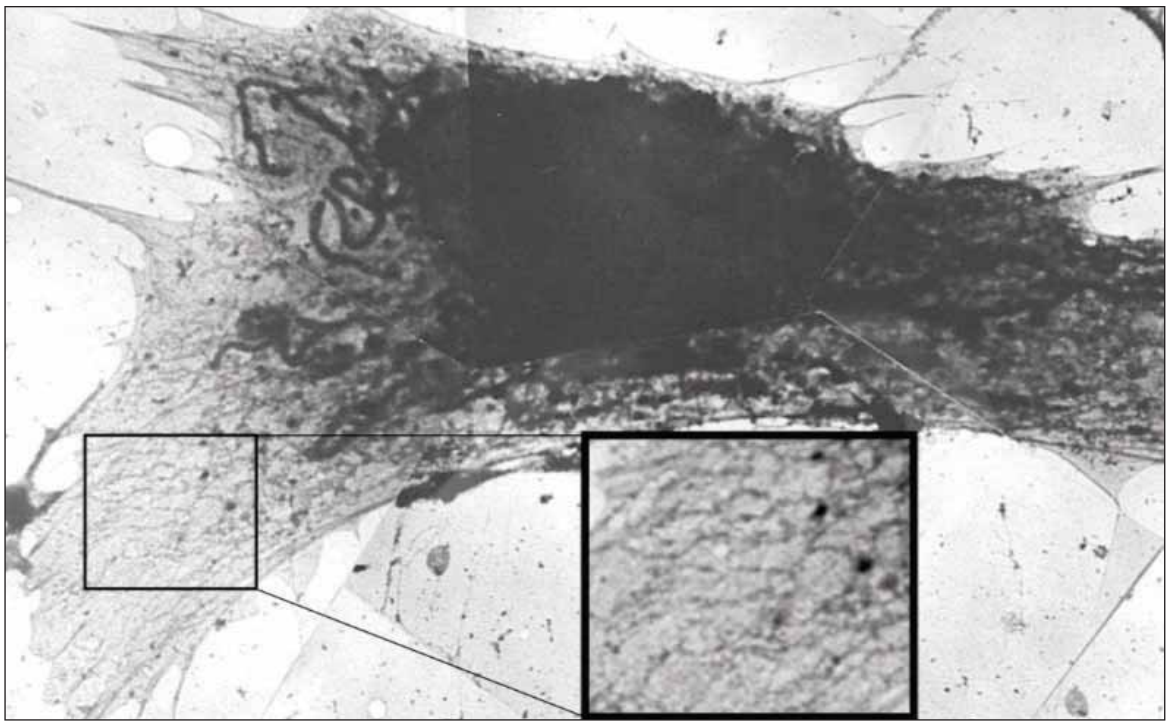


Figure 4: First published electron microscopic image of a fibroblast-like cell showing filamentous mitochondria, dense "elements" presumed to be Golgi bodies, and a "delicate lace-work" (left side and insert) later named endoplasmic reticulum by Porter. The nucleus is virtually obscured due to the high electron density in the cell center. Fixation in osmium tetroxide vapors, washing in distilled water, air-drying. From Porter et al. (1945). Magnification ~2000.

among the top ten Nobel "snubs", or award exclusions, even though he provided some of the most important contributions in the cell biology revolution of the 1940s.

It must have been a bitter moment for Keith Porter, too. However, being the gentleman he was, he took it with contenance, equanimity, and reverence to those selected – and possibly with a dash of his witty, deadpan humor. Showing a certain lack of tact and sensitivity, the editors of Science even asked him to write a laudation on two of the laureates, Claude and Palade (Porter 1974). It is amazing to see how he downplayed his own contributions, almost hiding them. And he had nothing but praise for his former labmates. Here is what he said about George Palade: "There is seemingly no letup in the pace at which new and important observations emerge from the Palade laboratory, and it is unlikely that this latest recognition of his achievements will affect this productivity. Obviously, original discovery is the native bent of some people, and the compulsion to go on and on seems not to diminish with age." Three years later, George Palade (1977) wrote on the occasion of Porter's 65th birthday: "So it is fitting to honor Keith Porter for his science: an ever-closer look, by increasingly stronger and diversified means, at the organization of living systems. And it is also fitting to recognize his ability to dare, move, and organize on a large scale." Evidently they held each other in high esteem. Both continued to make significant contributions well after regular retirement age. Keith Porter died in 1997, George Palade 11 years later. This birthday anniversary is a welcome opportunity to reflect on their life's work. They will not be forgotten.

References

Altmann, R. Die Elementarorganismen und ihre Beziehungen zu den Zellen. Leipzig, Veit & Co. 1894

Claude, A. Particulate Components of Normal and Tumor Cells. *Science* **91**, 77–78 (1940)

Claude, A. and Fullam, E.F. An electron microscope study of isolated mitochondria: method and preliminary results. *J. Exp. Med.* **81**, 51-62 (1945)

Golgi, C. Intorno alla strutura delle cellule nervose. *Boll. Soc. Med. Chirurg. Pavia* **13**, 1-14 (1898)

Heidenhain, R.. Beiträge zur Lehre von der Speichelabsonderung. *Stud. Physiol. Inst. Breslau* **4**, 1-124 (1868)

Heidenhain, M. Plasma und Zelle. 2 Volumes, 1110 pages. Jena, Gustav Fischer, 1907-1911

Hogeboom G.h., Schneider, W.C., Palade, G.E. Cytochemical studies of mammalian tissues; isolation of intact mitochondria from rat liver; some biochemical properties of mitochondria and submicroscopic particulate material. *J. Biol. Chem.* **172**, 619-635 (1948)

Knoll, M and Ruska, E. Das Elektronenmikroskop. *Z. Physik* **78**, 318-339 (1932)

Nissl, F., Über die sogenannten Granula der Nervenzellen. *Neurol. Centralbl.* **15**, 676-689 (1894)

Palade, G.E. A small particulate component of the cytoplasm. *J. Biophys. Biochem. Cytol.* **1**, 59-68 (1955)

Palade, G.E. Keith Roberts Porter and the development of contemporary cell biology. *J. Cell Biol.* **71**, 497-514 (1976)

Porter, K.R. Observations on a submicroscopic basophilic component of cytoplasm. *J. Exp. Med.* **97**, 727-750 (1953)

Palade, G.E. and Porter, K.R. Studies on the endoplasmic reticulum. I. Its identification in cells in situ. *J. Exp. Med.* **100**, 641-656 (1954)

Porter, K.R. The 1974 Nobel Prize for physiology or Medicine. *Science* **186**, 516-518 (1974)

Porter, K.R., Claude, A., Fullam, E.F. A Study of Tissue Culture Cells by Electron Microscopy. *J. Exp. Med.* **81**, 233–246 (1945)

Schwann, T. Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur und dem Wachstum der Tiere und Pflanzen. Berlin, Sander, 1839

Manfred Schliwa
Institut für Zellbiologie, LMU München
Schillerstr. 42, 80336 München

The cellular basis of constitutive and regenerative neurogenesis in the adult zebrafish brain

Volker Kroehne and Michael Brand

Introduction

In mammals, severe injury to the adult brain has catastrophic effects and significant brain regeneration does not occur. The inability of functional regeneration in mammals comprises the lack of cellular replacement of neurons in most types of injury paradigms, as well as the failure of surviving and newly generated neurons to re-establish correct neuronal connections (Horner and Gage, 2000; Ramón y Cajal and May, 1928). In contrast, non-mammalian vertebrates, like fish, reptiles and urodele amphibians can regenerate considerable portions of their central nervous system (CNS) even in adult stages (Becker and Becker, 2008; Fleisch et al., 2011; Font et al., 2001; Kaslin et al., 2008; Kirsche, 1965; Kizil et al., 2012; Tanaka and Ferretti, 2009). Pioneering work by Kirsche and Kirsche in the 1960s demonstrated that teleost fish are able to restore even whole brain parts, like the optic tectum, and that this remarkable regenerative capacity depends on the presence of constitutive proliferation zones (Kirsche and Kirsche, 1961). However, detailed knowledge about the cellular composition of adult neural stem cell niches in non-mammalian vertebrates remained elusive for decades. In our lab we use zebrafish, a model organism with well-developed genetics and molecular biology tools, to understand the cellular and molecular basis of adult neurogenesis and regeneration in the non-mammalian vertebrate brain.

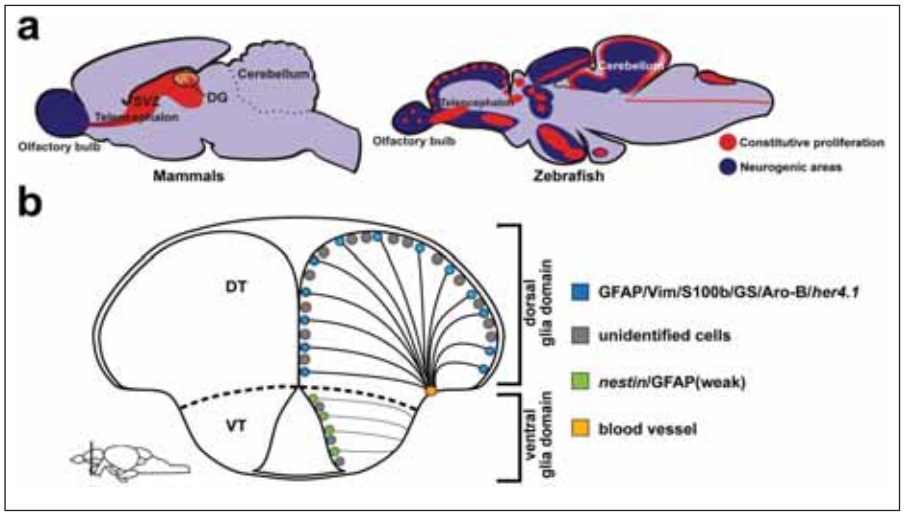


Figure 1: **Constitutive proliferation and adult neurogenesis in the mammalian and the zebrafish brain. a:** Areas of cell proliferation (red) are restricted to the telencephalic subventricular zone (SVZ) and the subgranular of the dentate gyrus (DG) in mammals, while in zebrafish mitotic cells are situated along the entire extent of the ventricular zones. Newborn neurons are constantly added to the neurogenic areas (blue). **b:** Heterogeneity of progenitor cells in the ventricular zone of the adult zebrafish telencephalon. The telencephalon is subdivided in a dorsal (DT) and a ventral (VT) domain. Two classes of progenitor cells are found in the ventricular zone in DT: radial glia that express canonical glial markers (blue) and lineage-marker negative, unidentified cells (grey). In vT some progenitors express nestin and also weakly glial markers (green), while others are not characterized by any lineage marker (grey). Adapted from (Kaslin et al., 2009 and Ganz et al., 2010).

Neurogenesis in the adult zebrafish brain

Adult neurogenesis, i.e. the constant addition of new neurons to the postembryonic brain, is a conserved trait found in all vertebrates examined (Chapouton et al., 2007; Kaslin et al., 2008; Kirsche, 1967; Zhao et al., 2008). In the adult zebrafish brain the first evidence for adult neurogenesis was already reported more than 40 years ago (Rahmann, 1968). However, detailed insight into the spatial organisation and the extent of proliferation zones in the adult zebrafish brain and the identity of newly generated cells has only been gained recently (Adolf et al., 2006; Ganz et al., 2010; Grandel et al., 2006; Kaslin et al., 2009; Zupanc et al., 2005). Life-long neurogenesis is much more widespread in adult zebrafish than in mammals. Sixteen distinct germinal niches, i.e. sites of proliferation, have been identified along the rostro-caudal brain axis in zebrafish, while in adult mammals proliferation of neural progenitors and neurogenesis are restricted to two specific zones in the telencephalon (Grandel et al., 2006; Kaslin et al., 2008; Kaslin et al., 2009 and Fig. 1a).

Within the population of constitutively proliferating cells long-term label retaining cells, which are slowly cycling and long-term self-maintaining cells, have been found (Grandel et al., 2006). These cells remain in the ventricular zone (VZ) for a prolonged time period and have the capacity to self-renew.

Thus, they fulfil two important criteria of adult stem cells and are thus thought to be the origin of newly generated cells (Grandel et al., 2006). Interestingly, the majority of newborn cells in the adult brain are neurons of different neurochemical phenotype and structure, including interneurons and long projecting neurons (Grandel et al., 2006).
In the adult zebrafish cerebellum, we have recently shown that neural stem cells are bipotent and continuously produce granular neurons and a small number of glia. These progenitors do not show typical glial characteristics, as found in mammals, but instead retain neuroepithelial features (Kaslin et al., 2009).

Adult neurogenesis in the zebrafish telencephalon

Two distinct progenitor domains are found in the ventricular zones (VZ) of the adult zebrafish telencephalon, based on proliferation dynamics and marker expression profiles of the progenitor cells (Ganz et al., 2010 and Fig. 1b). In both regions, in the dorsal (DT) and ventral (VT) telencephalon, long-term label retaining cells, have been found (Ganz et al., 2010). The presumptive progenitors in DT cluster in different subpopulations: (i) cells with a morphology typical for radial glia that express canonical glia markers like Glial fibrillary acidic protein (GFAP), Vimentin (Vim), S100b, Glutamine synthetase (GS) and Aromatase B (Aro-B) and (ii) unidentified cells that do not show any expression of the these glial marker proteins (Ganz et al., 2010; Tong et al., 2009). Interestingly, radial glial cells display high Notch activity as determined by *in situ* hybridization analysis for components of the pathway and by the strong expression of the Notch-dependent *her4.1*:GFP transgenic line (Chapouton et al., 2010; Ganz et al., 2010; Kroehne et al., 2011).
In the second region, the VT, one sub-class of presumptive progenitors shows neuroepithelial characteristics, such as weak or no expression of glial markers, *nestin* expression, apical-basal polarity and interkinetic nuclear migration, while the other sub-class does not express canonical glial markers and has not been characterized further (Ganz et al., 2010 and Fig. 1b).

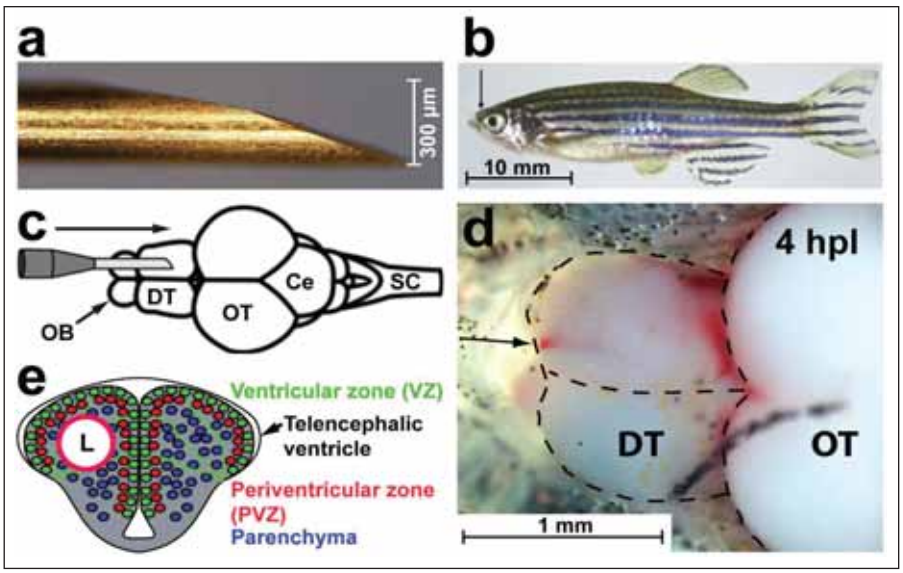


Figure 2: **The stab lesion assay. a:** The canula used for the stab lesion assay has an outer diameter of 300 µm. **b:** The bony nostrils (arrow) of adult zebrafish are used as landmarks to direct the canula to the dorsal telencephalon. **c:** A schematic sagittal overview shows the size and location of the canula in respect to the different brain parts: olfactory bulb (OB), dorsal telencephalon (DT), optic tectum, (OT), cerebellum (Ce), spinal cord (SC). **d:** Dorsal view of a stab lesioned brain in situ. 4 hours post lesion (hpl) the entry-point of the canula into the DT is marked by blood accumulation (arrow). The rostral part of the lesion canal is demarcated by a blood clot. **e:** In schematic cross-sections three layers can be functionally distinguished in the zebrafish telencephalon: Directly at the ventricle is the neural progenitor (radial glia) containing ventricular zone (VZ, green). During constitutive neurogenesis all newborn neurons integrate into the periventricular zone in the DT (PVZ, red, 1-2 cell diameters adjacent to the VZ). The central parenchyma (blue) is not a neuronal target area in constitutive neurogenesis. From (Kroehne, 2011).

Regeneration of the adult zebrafish telencephalon after traumatic injury

To study brain regeneration mechanisms in a non-mammalian vertebrate we developed a novel traumatic stab lesion assay in the forebrain of adult zebrafish (Kroehne et al., 2011 and Fig. 2). The stab lesion reproducibly injures the dorsal telencephalon, but spares the constitutive progenitor zones at the ventricle (Fig 2e). Initially, the lesion is demarcated by a blood clot and severe cerebral oedema that affects more than 40% of the injured hemisphere. Furthermore, the lesion triggers a distinct but temporally confined apoptotic cell death response and neurons die within the first days after lesion. Remarkably, the blood clot and oedema are resolved within 14 to 30 days post lesion (dpl) and the brain architecture is largely restored within 30 to 90 dpl. Distinct acute reactions to injury by glial cells and inflammatory leukocytes are detected early after lesion. However, in zebrafish both are resolved in the course of brain reconstitution and do not result in the formation of a permanent glial or fibrotic scar, like in mammals. In the adult zebrafish telencephalon no long-term accumulation of ectopic extra-cellular matrix components is detected and neuronal processes that are lost after lesion are re-established. A marked activation of proliferation of one sub-population of neural progenitors, i.e. radial glial

cells, is seen in the ventricular zone of the telencephalon from 3 to 14 dpl. To determine, if radial glia act as neuronal progenitors and generate new neurons after lesion, we applied a novel lineage tracing approach using the CreER^{T2}-loxP system in zebrafish (Hans et al., 2011; Hans et al., 2009) and Fig. 3). Genetic fate analysis using the inducible Cre recombinase system demonstrates that a sub-population of *her4.1*-expressing ventricular radial glia functions as a lesion responsive neuronal progenitor population: After injury radial glia increase proliferation, up-regulate neurogenic gene transcription and give rise to neuroblasts that migrate into the periventricular zone and deeper into parenchyma to the lesion site (Fig. 3 and 4). From 21 dpl on newly generated neurons are found that express mature neuronal and synaptic markers, like MAP2 and Parvalbumin. Furthermore, newly generated neurons in the lesion site are decorated with synaptic contacts and survive for more than 3 months, suggesting that they are functionally integrated (Fig. 4).

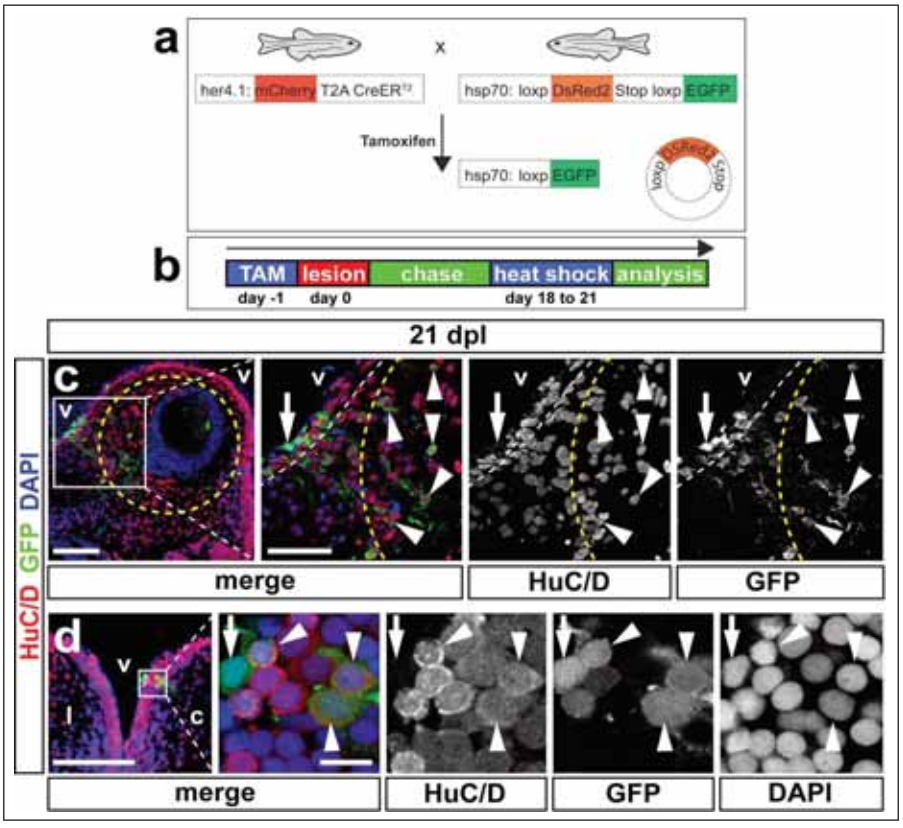


Figure 3: **Newly generated neurons derive from a her4.1-expressing radial glia sub-population.** **a**: Generation of double-transgenic fish used for cell fate analysis: fish expressing CreER^{T2} controlled by the *her4.1*-promotor were crossed to the red-to-green reporter fish. In double-transgenic fish GFP-expression can be specifically induced in *her4.1*-expressing radial glia and their progeny by Tamoxifen application and heat-shock. **b**: Recombination was induced specifically in *her4.1*-expressing radial glia by Tamoxifen injection 1 day before lesion. 18 until 21 dpl fish were heat-shocked to induce GFP expression in cells of the *her4.1*-lineage only. **c**: 21 dpl many recombined, GFP$^+$ (green)/HuC/D$^+$ (red) double-positive neurons/neuroblasts (arrowheads) are found within the lesion site (yellow dashed outline) and in the PVZ (white dashed outline). In the VZ recombined GFP$^+$/HuC/D$^-$ cells (arrow) are found. **d**: Recombined neurons (arrowheads) derived from *her4.1*$^+$ radial glia (arrow) are exclusively found in the constitutive neuronal target area (PVZ) in the dorsal part of unlesioned control hemispheres 21 dpl. v: ventricle; l: lesioned hemisphere; c: control hemisphere. Scale bars represent 100 µm in c, inset 50 µm; 100 µm in d. v, ventricle. Adapted from (Kroehne et al., 2011).

Taken together, these results show that severe traumatic lesions are efficiently regenerated in the adult zebrafish brain. The regenerative capacity is based on two major features of the zebrafish telencephalon: (i) It harbours a constitutive neuronal progenitor population, i.e. radial glia that can be induced to increase proliferation and neurogenesis. (ii) It provides a permissive environment for regeneration. Glial and fibrotic scarring does not occur and newly generated neurons are maintained for long times and acquire a mature state.

Conclusions and outlook

Injury to the mammalian CNS is devastating and significant regeneration does not occur. In contrast, adult non-mammalian vertebrates, like e.g. urodele amphibians, reptiles and bony fishes, have the capacity to regenerate a multitude of organs. A central question in regeneration research is the origin of the cells that replace tissue lost by injury. Different mechanisms and cellular sources, including de- and trans-differentiation and activation of adult stem/ progenitor cells have been suggested to orchestrate the regeneration of appendages, heart and the CNS. However, the cellular basis of neuronal regeneration in the brain and spinal cord has been elusive. By using a novel genetic lineage tracing strategy based on the conditional CreER^{T2}-loxP technology we identified a *her4.1*-positive ventricular radial glial progenitor population as a major stem/progenitor population reacting to injury. Therefore, further analysis of the *her4.1*-positive radial glia will help to understand the molecular mechanisms involved in compensatory proliferation and regenerative neurogenesis in vertebrates. To this end, efforts have been started to analyse the response of neurogenic *her4.1*-positive radial glia to stab lesion injury of adult zebrafish brains by transcriptome analysis, and to functionally interfere with gene expression in these cells by cerebroventricular microinjection of vivo mor-

pholinos (Kizil and Brand, 2011; Kizil et al., 2012).

Major barriers to CNS reconstitution in mammals are reactive gliosis and inflammation that lead to the formation a compact glial scar that inhibits regeneration. Reactive gliosis and inflammation are detected shortly after lesion, but signs of long-term glial and fibrotic scarring are not evident. Therefore our results suggest that the adult zebrafish brain provides a permissive environment for regeneration, and raise the possibility that reactive gliosis and inflammation do not necessarily result in the formation of permanent scar tissue.

Low degree reactive neurogenesis can occur in mammals after non-traumatic brain injury, but the vast majority of newly generated neurons are not maintained for long-term. Many newly generated neurons in the adult zebrafish brain persist for long times after stab lesions. Our studies indicate that these neurons are mature and give evidence that they could be integrated into the circuitry. Understanding how new neurons can be integrated quantitatively into the network of the adult vertebrate brain is of fundamental importance and could help to develop new therapeutic strategies for the diseased or injured human brain.

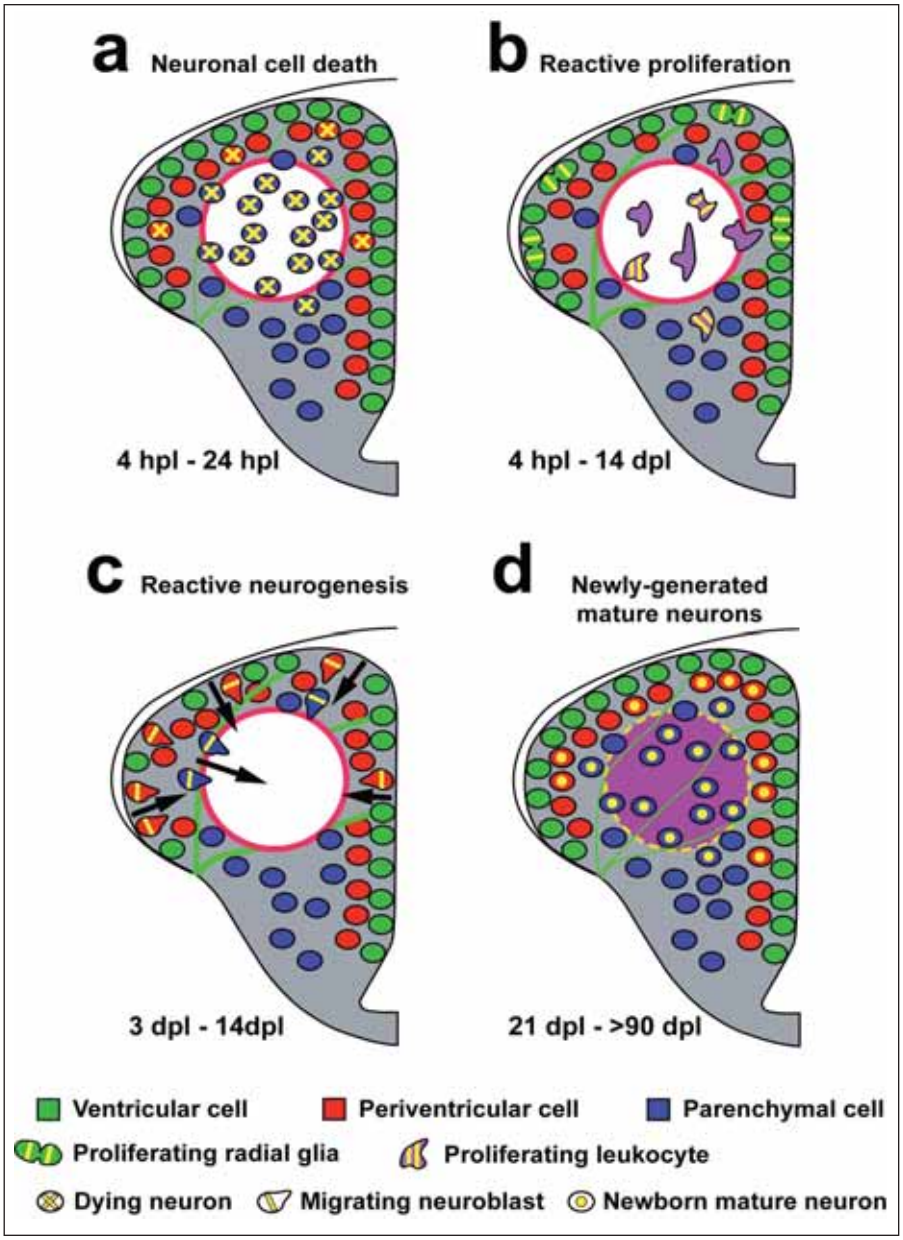


Figure 4: **Key events in regeneration of the adult zebrafish telencephalon after stab lesion.** **a**: Initially (4 to 24 hpl) neurons in the parenchyma and the PVZ of the injured hemisphere enter apoptotic cell death. **b**: Resident microglia, invading leukocytes and ventricular radial glia enter a phase of reactive proliferation from 4 hpl to 14 dpl (peaking at 3 dpl). Radial glia show characteristics of reactive gliosis. **c**: From 3 to 14 dpl radial glia up-regulate proliferation and subsequently undergo neurogenesis (reactive neurogenesis). The newborn neuroblasts leave the VZ and migrate towards the lesion. **d**: From 21 to at least 90 dpl many newly-generated mature and active neurons are detected within the lesion site in the parenchyma and in the PVZ of the lesioned hemisphere. From (Kroehne et al., 2011)

Acknowledgments

We would like to acknowledge that the work described here from our lab was funded by the DFG SFB 655, the European Union (Zf-Health) and a CRTD seed grant.

References

Adolf, B., Chapouton, P., Lam, C. S., Topp, S., Tannhauser, B., Strahle, U., Gotz, M. and Bally-Cuif, L. (2006). Conserved and acquired features of adult neurogenesis in the zebrafish telencephalon. *Dev Biol* **295**, 278-93.

Becker, C. G. and Becker, T. (2008). Adult zebrafish as a model for successful central nervous system regeneration. *Restor Neurol Neurosci* **26**, 71-80.

Chapouton, P., Jagasia, R. and Bally-Cuif, L. (2007). Adult neurogenesis in non-mammalian vertebrates. *Bioessays* **29**, 745-57.

Chapouton, P., Skupien, P., Hesl, B., Coolen, M., Moore, J. C., Madelaine, R., Kremmer, E., Faus-Kessler, T., Blader, P., Lawson, N. D. et al. (2010). Notch activity levels control the balance between quiescence and recruitment of adult neural stem cells. *J Neurosci* **30**, 7961-74.

Fleisch, V. C., Fraser, B. and Allison, W. T. (2011). Investigating regeneration and functional integration of CNS neurons: Lessons from zebrafish genetics and other fish species. *Biochim Biophys Acta* **1812**, 364-80.

Font, E., Desfilis, E., Perez-Canellas, M. M. and Garcia-Verdugo, J. M. (2001). Neurogenesis and neuronal regeneration in the adult reptilian brain. *Brain Behav Evol* **58**, 276-95.

Ganz, J., Kaslin, J., Hochmann, S., Freudenreich, D. and Brand, M. (2010). Heterogeneity and Fgf dependence of adult neural progenitors in the zebrafish telencephalon. *Glia* **58**, 1345-1363.

Grandel, H., Kaslin, J., Ganz, J., Wenzel, I. and Brand, M. (2006). Neural stem cells and neurogenesis in the adult zebrafish brain: origin, proliferation dynamics, migration and cell fate. *Dev Biol* **295**, 263-77.

Hans, S., Freudenreich, D., Geffarth, M., Kaslin, J., Machate, A. and Brand, M. (2011). Generation of a non-leaky heat shock-inducible Cre line for conditional Cre/lox strategies in zebrafish. *Dev Dyn* **240**, 108-15.

Hans, S., Kaslin, J., Freudenreich, D. and Brand, M. (2009). Temporally-controlled site-specific recombination in zebrafish. *PLoS One* **4**, e4640.

Horner, P. J. and Gage, F. H. (2000). Regenerating the damaged central nervous system. *Nature* **407**, 963-70.

Kaslin, J., Ganz, J. and Brand, M. (2008). Proliferation, neurogenesis and regeneration in the non-mammalian vertebrate brain. *Philos Trans R Soc Lond B Biol Sci* **363**, 101-22.

Kaslin, J., Ganz, J., Geffarth, M., Grandel, H., Hans, S. and Brand, M. (2009). Stem cells in the adult zebrafish cerebellum: initiation and maintenance of a novel stem cell niche. *J Neurosci* **29**, 6142-53.

Kirsche, W. (1965). [Regenerative processes in the brain and spinal cord]. *Ergeb Anat Entwicklungsgesch* **38**, 143-94.

Kirsche, W. (1967). [On postembryonic matrix zones in the brain of various vertebrates and their relationship to the study of the brain structure]. *Z Mikrosk Anat Forsch* **77**, 313-406.

Kirsche, W. and Kirsche, K. (1961). [Experimental studies on the problem of regeneration and function of the tectum opticum of Carassium carassium L.]. *Z Mikrosk Anat Forsch* **67**, 140-82.

Kizil C. and Brand M. (2011). Cerebroventricular Microinjection (CVMI) into Adult Zebrafish Brain Is an Efficient Misexpression Method for Forebrain Ventricular Cells. *PLoS ONE* 6(11): e27395. doi:10.1371/journal.pone.0027395

Kizil, C., Kaslin, J., Kroehne, V. and Brand, M. (2012). Adult neurogenesis and brain regeneration in zebrafish. *Dev Neurobiol* **72**, 429-461.

Kroehne, V. (2011). Analysis of brain regeneration in adult zebrafish after traumatic injury. Dissertation. Fakultät für Mathematik und Naturwissenschaften, Technische Universität Dresden.

Kroehne, V., Freudenreich, D., Hans, S., Kaslin, J. and Brand, M. (2011). Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors. *Development* **138**, 4831-41.

Rahmann, H. (1968). [Autoradiographic studies on the DNA metabolism (mitosis frequency) in the CNS of Brachydanio rerio Ham. Buch. (Cyprinidae, Pisces)]. *J Hirnforsch* **10**, 279-84.

Ramón y Cajal, S. and May, R. M. (1928). Degeneration and regeneration of the nervous system. Oxford; London: Oxford University Press; Humphrey Milford.

Tanaka, E. M. and Ferretti, P. (2009). Considering the evolution of regeneration in the central nervous system. *Nat Rev Neurosci* **10**, 713-23.

Tong, S. K., Mouriec, K., Kuo, M. W., Pellegrini, E., Gueguen, M. M., Brion, F., Kah, O. and Chung, B. C. (2009). A cyp19a1b-gfp (aromatase B) transgenic zebrafish line that expresses GFP in radial glial cells. *Genesis* **47**, 67-73.

Zhao, C., Deng, W. and Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell* **132**, 645-60.

Zupanc, G. K., Hinsch, K. and Gage, F. H. (2005). Proliferation, migration, neuronal differentiation, and long-term survival of new cells in the adult zebrafish brain. *J Comp Neurol* **488**, 290-319.

Volker Kroehne

DFG-Research Center for Regenerative Therapies Dresden/Cluster of Excellence and Biotechnology Center, Technische Universität Dresden, Fetscherstraße 105, 01307 Dresden, Germany

1999 – 2004: Diploma studies in "Molecular Biotechnology", Bielefeld University, Germany; 2005 – 2006: PhD student in the group of Michael Brand at the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Dresden; 2005 – 2011: Fellow of the "International Max Planck Research School for Molecular Cell Biology and Bioengineering"; 2007 – 2011: PhD student in the group of Michael Brand at the Biotechnology Center & DFG-Center for Regenerative Therapies (CRTD), TU Dresden; 2011 – 2012: Post-doctoral fellow in the group of Michael Brand at the CRTD & BIOTEC, TU Dresden

Research interests:

- Engineered reconstruction of skeletal muscle
- Adult neural stem cells and regeneration of the CNS
- Engineering of neural tissues from pluripotent stem cells

Michael Brand

DFG-Research Center for Regenerative Therapies Dresden/Cluster of Excellence and Biotechnology Center, Technische Universität Dresden, Fetscherstraße 105, 01307 Dresden, Germany
E-Mail: michael.brand@biotec.tu-dresden.de

1986: M.A., Harvard University; Diploma, University of Cologne; 1989: PhD, University of Cologne; 1990: Post-doctoral work at University of California San Francisco; 1992: Helmholtz Fellow, MPI for Developmental Biology, Tübingen; 1995-1999: Group Leader at the University of Heidelberg; 2000-2002: Group Leader at MPI-CBG, Dresden; since 2003: Professor of Developmental Genetics, TU Dresden; since 2005: Director, Biotechnology Center TU Dresden (BIOTEC), and Director, DFG-Research Center for Regenerative Therapies Dresden-Cluster of Excellence (CRTD); Vice-Speaker, DFG SFB 655 - Cells into Tissues

Research interests:

- Adult neural stem cells and regeneration of the vertebrate brain
- Regeneration of the adult retina
- Human neurodegenerative disease models in zebrafish
- Patterning of the neural plate

Neuronal Regeneration – Some Insight

Mike O. Karl

Introduction

Neuronal injury and diseases are still too often life threatening and very devastating for us, because we know that neurons lost are gone forever. Studies in lower, cold-blooded vertebrates are mesmerizing, because some fish and types of salamanders may completely regenerate structurally and functionally various parts of their nervous system [1-5]. Interestingly, some species are more restricted than others in its regenerative capacity by organ, tissue region, number and type of cells, as well as functional recovery. Further, some brain areas, many in fish and few in mice, even remain the capacity of physiological adult neurogenesis providing live-long ongoing addition of new neurons independent of, but often affected by, injury and disease [6]. The mechanisms that decide about the persistence of adult neural stem cells as sources of adult neurogenesis are unknown as well as those that limit regeneration in some but not others. It will be exciting to find out to what extent adult physiological and damaged induced-regenerative neurogenesis are related. In both cases parts of the underlying programs will be similar to embryogenesis and the differences need to be taken apart. Many fundamental questions remain, some of which we are addressing in our currently ongoing studies in the retina as a model area of neuronal regeneration in the brain. To name a few questions: which parts of regenerative mechanisms are species and tissue specific, how does damage induce the process, what defines the plasticity of a cell as a source of regeneration and are regenerative mechanisms in mammals just restricted or are some parts not existing. Thus, studies from many scientist around the world will be necessary using varies animal models and systems to reveal the cell biology of regeneration and its limitations. Ultimately, one major question will be whether or not in neurodegenerative diseases the surviving cells have any capacity that may be utilized to therapeutic benefit.

Our working model – Roadblocks of retinal regeneration

The retina is part of the central nervous system (CNS) and has a long-standing history as an approachable part of the brain [7]. In brief, vertebrates retinas major cell type are photoreceptor cells responding to light and relaying the information through interneurons (bipolar, amacrine and horizontals) to retinal ganglion cells, which project with their axons to the higher visual brain centers. The major type of retinal glia cells – Müller glia, often compared to radial glia in the developing brain – are located across the retina and radially span its full width. Various retina diseases or injuries may lead to loss of vision in humans due to neuronal cell loss and deficiency in regenerative cell replacement. Interestingly, in adult zebrafish retina Müller glia generate photoreceptors

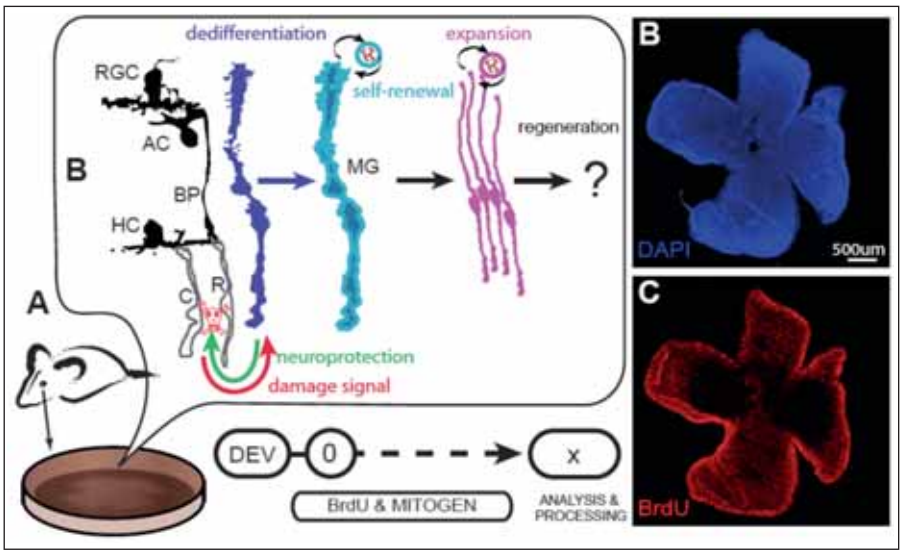


Figure: NEURONAL REGENERATION OF THE RETINA: A) Working model: We are currently using in vivo and ex vivo approaches using mouse retina as a model species to understand the molecular and cellular mechanisms of retinal regeneration. We hypothesize that upon neuronal damage Müller glia respond by de-differentiation, which includes but is not limited to changes in their cellular and molecular phenotype (referred to as regulated reprogramming [5]). Latter process may lead to cell cycle re-entry, so that Müller glia divide, self-renew and generate progenitor progeny, which might expand to provide sufficient cell numbers differentiating into regenerated neurons for functional repair. **B-C) Ex vivo approach:** Using mouse retina explant culture others and we observed induction of Müller glia proliferation (DAPI stains all cell nuclei; BrdU labels any cell that underwent DNA synthesis indicating cell proliferation; picture B & C are kindly provided by Kati Löffler in my lab). For in vivo approach see Ref. [12].

throughout live [8]. Upon damage of retinal neurons Müller glia are reactivated and regenerate all types of neurons until vision is restored. First evidence that Müller glia are a cell source for neuronal regeneration has been provided by studies in posthatch chicken [9]. Retinal regeneration is much more limited in chicken compared to fish – with lower cell numbers being replaced including all cell types but not photoreceptors.

During my postdoctoral work, we hypothesized that proper stimulation may induce parts of a regenerative program in adult mouse retina in vivo. In rats and mice developmental retinal progenitor proliferation (embryonic retinogenesis) peaks around the day of birth and declines until about the end of the first postnatal week [10]. After this time, there is little evidence for renewed proliferation of either progenitors or Müller glia in the mammalian retina [11]. Even in cases of severe degeneration Müller glia show in most cases only a low level of proliferation. In mice, various labs showed that few, if any, Müller glia proliferate after damage in vivo, which we confirmed for neurotoxic damage of ganglion and amacrine cells as well as light damage of photoreceptors [12-14].

Strikingly, a few groups have reported in recent years that upon stimulation with growth factors or transcription factors a very limited amount of neurons can be regenerated in the mouse and rat retina in vivo or in vitro (reviewed in Karl & Reh 2010 Trends Mol Med [2]). Application of various mitogenic factors stimulates a small number of Müller glia to proliferate. Using different types of damage we observed in adult mice that mitogens EGF and FGF were superior to other to induce cell cycle re-entry of Müller glia. Initially, after one intraocular shot of mitogen up to 10% Müller glia were labeled by BrdU suggesting proliferation [13]. Daily injection of up to four subsequent days with a combination of FGF and insulin maintained the highest number of Müller glia progeny amounting to 1% of the initially stimulated population. At this time BrdU+ Sox2+ positive cells are either Müller glia or potentially Müller glia derived progenitor cells. Generation of progenitor like cells is supported by our gene expression studies indicated by specific changes in cell cycle and progenitor related genes. Proliferation ceases below detection level with the last round of mitogen application. When we investigated de-novo neurogenesis we observed BrdU+ cells colabelled for neuronal markers NeuN+ and Calretinin not before 8 days after injury. Interestingly, using a transgenic reporter mouse labeling all GABAergic neurons with GFP under the GAD67 promotor, I found the first GAD67-GFP+ BrdU+ double-positive cells on day 8 after injury, which still slightly increased until day 30. Using confocal microscopy [12] we fully analyzed complete intact retina explants and counted on average 7+- 0.5 GAD67-GFP+ BrdU+ cells. Based on a detailed analysis current data suggest that of all Sox2+ BrdU+ cells less than 5% differentiate into NeuN+, 0.1% Calretinin+ and 0.05% GAD67-GFP expressing cells. In sum, we observed that only a minor population of Müller glia participate, its progeny does not significantly expand, an even smaller part survives and still less differentiate into neurons. In sum, findings so far provide first evidence, but the endogenous capacity for regenerative neurogenesis in mammal's remains latent and without functional consequence.

Conclusions

To understand and overcome the roadblocks of mammalian regeneration the mechanisms that enable, control and limit regeneration need to be discovered, which we currently pursue in the lab. Combining ex vivo and in vivo approaches (see Figure) we increased the efficiency to reach our ultimate goals: to focus particularly on regenerative mechanisms and roadblocks that limit the number of Müller cells undergoing regulated reprogramming (de-differentiation) to re-enter cell cycle, that restrict progeny expansion as well as those which prevent regeneration of photoreceptors and retinal ganglion cells in the adult animal in vivo. By doing so we might not only learn how to utilize the surviving cells capacity to provide therapeutic benefit, but also whether degenerative processes hinder endogenous self-repair and neuroprotective mechanisms or even induce secondary neuronal cell loss. In detail, most pathological conditions in the retina lead to changes in Müller glia, analogous to the response of astrocytes to injury in other regions of the CNS – often called reactive gliosis [2, 11]. It is a non-stereotypical response of glia associated with a pathological state that serves as an umbrella term describing various phenotypic changes. In humans, Müller glial cell activation and changes in phenotype – gliosis, are associated with virtually every major retinal disease. Gliosis is correlated with many changes, but whether it is always an impediment to tissue recovery (scarring and proliferative gliosis), protection to secondary lesion or both is still a crucial question. Here our major hypothesis is whether gliosis is an aberrant regenerative response or an independent entity.

Acknowledgment

I wish to thank all members of my lab for being curious, eager and brave to investigate and to seek ways to overcome the limits of neuronal regeneration. Further, without the long-standing and kind support of K.E., O.S., M.C., T.R., my family and friends none of this might have been possible and surely much less enjoyable. Most certainly, my start and current progress in Dresden would not have been possible without the kind help of many more at CRTD, DZNE, TUD and beyond. Research funding and support to our lab and lab members has been kindly provided by DFG, DZNE, Herbert-Funke Stiftung, DAAD and ProRetina.

References

1. Lamba, D., M. Karl, and T. Reh, Neural regeneration and cell replacement: a view from the eye. *Cell Stem Cell*, 2008. **2**(6): p. 538-49.
2. Karl, M.O. and T.A. Reh, Regenerative medicine for retinal diseases: activating endogenous repair mechanisms. *Trends Mol Med*, 2010.
3. Kizil, C., et al., Adult neurogenesis and brain regeneration in zebrafish. *Developmental neurobiology*, 2011.
4. Tanaka, E.M. and P. Ferretti, Considering the evolution of regeneration in the central nervous system. Nature reviews. *Neuroscience*, 2009. **10**(10): p. 713-23.
5. Bermingham-McDonogh, O. and T.A. Reh, Regulated reprogramming in the regeneration of sensory receptor cells. *Neuron*, 2011. **71**(3): p. 389-405.
6. Kempermann, G., Adult Neurogenesis 22010: Oxford University Press.
7. Dowling, J.E., The Retina: An Approachable Part of the Brain1987: Belknap Press.
8. Bernardos, R.L., et al., Late-stage neuronal progenitors in the retina are radial Muller glia that function as retinal stem cells. *J Neurosci*, 2007. **27**(26): p. 7028-40.
9. Fischer, A.J. and T.A. Reh, Muller glia are a potential source of neural regeneration in the postnatal chicken retina. *Nat Neurosci*, 2001. **4**(3): p. 247-52.
10. Close, J.L., B. Gumuscu, and T.A. Reh, Retinal neurons regulate proliferation of postnatal progenitors and Muller glia in the rat retina via TGF beta signaling. *Development*, 2005. **132**(13): p. 3015-26.
11. Bringmann, A., et al., Cellular signaling and factors involved in Muller cell gliosis: neuroprotective and detrimental effects. *Prog Retin Eye Res*, 2009. **28**(6): p. 423-51.
12. Karl, M.O., Reh, T.A., 15. Studying the Generation of Regenerated Retinal Neuron from Müller glia in the Mouse Eye, in Retinal Development, Methods and Protocols, Series: Methods in Molecular Biology, Vol. 884, S.-Z. Wang, Editor 2012, Humana Press.
13. Karl, M.O., et al., Stimulation of neural regeneration in the mouse retina. *Proc Natl Acad Sci U S A*, 2008. **105**(49): p. 19508-13.
14. Lamba, D.A., et al., Baf60c is a component of the neural progenitor-specific BAF complex in developing retina. *Dev Dyn*, 2008. **237**(10): p. 3016-23.

Mike O. Karl

Technische Universität Dresden
DFG-Center for Regenerative Therapies Dresden (CRTD) & Cluster of Excellence, and joint affiliation with German Center for Neurodegenerative Diseases (DZNE), Germany
http://www.mokalab.org

Mike Karl was born in 1976 in Germany. He received his medical degree and completed his scientific degree at the University of Hamburg (1996-2002). As a junior fellow he gained further research training at the University of Pennsylvania in Philadelphia (2001-2002). Two years ago, after performing his post-doctoral work at the University of Washington in Seattle (2005-2009) he started his research group as an independent research associate at the Center for Regenerative Therapies Dresden (CRTD). Since 2011 he has a joint group leader affiliation with the German Center for Degenerative Diseases (DZNE; Helmholtz Association). His laboratory studies embryonic and regenerative neurogenesis as well as develops human retina model systems.

Mechanical tension, cell sorting and tissue organization

Jens-Christian Röper and Christian Dahmann

During the development of multicellular organisms cells collectively organize to form structured tissues and organs. Tissue organization requires chemical signals and mechanical processes like cell division and cell rearrangements that are driven by physical forces. The past decades have revealed signalling pathways important for structuring tissues, however, the contributions of mechanical processes to tissue organization are less well understood. In this review, we discuss some recent advances in our understanding of the mechanical processes underlying the sorting of cells.

Cell sorting

The sorting of cells with different identities and fates during development is an important principle to organize functional tissues and other adult structures. In classic experiments, Holtfreter and colleagues demonstrated that *in vitro* mixtures of different vertebrate cell types would sort out and spontaneously form aggregates in which the relative positions of the different cell types resembled their prior arrangement in the embryo (Townes and Holtfreter, 1955). The physical mechanisms that drive these cell sorting processes, however, are still debated. In his Differential Adhesion Hypothesis, Steinberg proposed that the sorting behaviour of cells follows directly from quantitative differences in the strength of adhesion between cells (Steinberg, 1963). Indeed, quantitative differences in the expression of cell adhesion molecules of the cadherin superfamily can drive cell sorting in tissue culture and during animal development (Godt and Tepass, 1998; Gonzalez-Reyes and St Johnston, 1998; Nose et al., 1988; Steinberg and Takeichi, 1994). More recent hypotheses explain cell sorting by differences in interfacial tension (Brodland, 2002; Harris, 1976). Interfacial tension arises at the bonds between cells from contractile forces exerted by the cell's cytoskeleton and opposing forces generated by cell-cell adhesion.

Compartment boundaries

We study the mechanisms guiding cell sorting at compartment boundaries in *Drosophila*. Compartment boundaries are lineage restrictions that partition tissues into adjacent non-mixing cell populations, ter-

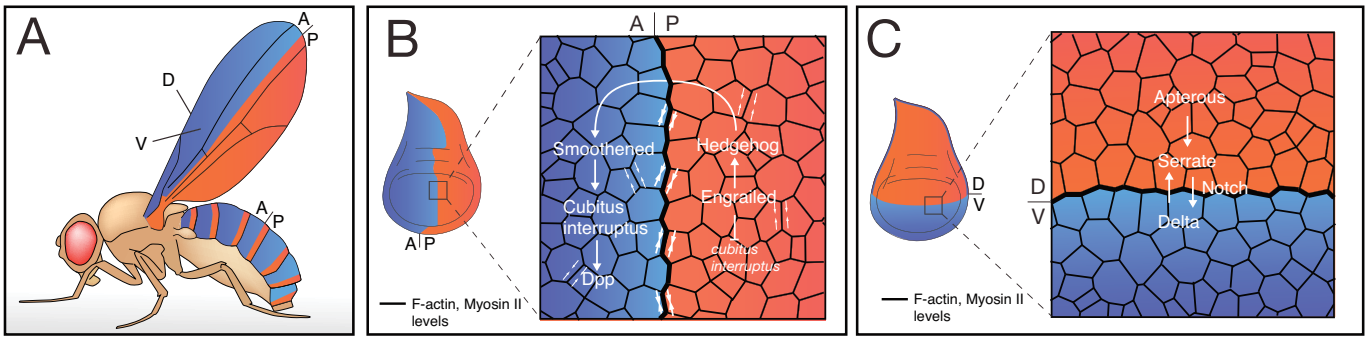


Figure 1:* Compartment boundaries in *Drosophila
(A) *Drosophila* adult fly. Anterior (A, blue) and posterior (P, red) compartments in the wing and abdomen are shown. Dorsal (D) and ventral (V) compartments correspond to the upper and lower surface layers of the wing blade, respectively. **(B,C)** Selector genes and signalling pathways in the wing imaginal disc. **(B)** In cells of the posterior compartment, the selector gene *engrailed* induces the expression of the signalling molecule Hedgehog and at the same time represses transcription of *cubitus interruptus*, a gene encoding for a transcription factor transducing the Hedgehog signal. Secreted Hedgehog spreads a few rows of cells into the anterior compartment. Transduction of the Hedgehog signal in these cells requires Smoothened, a seven-span transmembrane protein and Cubitus interruptus. In response to Hedgehog signalling several target genes are activated, including *dpp*. Dpp acts as a long-range signalling molecule directing growth and patterning along the A/P axis of the wing imaginal disc. F-actin and Myosin II accumulate along the compartment boundary. Mechanical cell bond tension (arrows) is locally increased. **(C)** Cells of the dorsal compartment express the selector gene *apterous* that induces expression of the Notch ligand Serrate. Ventral cells express the Notch ligand Delta. Notch signalling at the D/V compartment boundary leads to increased F-actin and Myosin II levels and is required to maintain this boundary.

med compartments (Dahmann et al., 2011; Monier et al., 2011). They are characterized by a straight and sharp morphology. Lineage tracing first identified compartments in insects (Garcia-Bellido et al., 1973; Lawrence, 1973) and then later also in vertebrate embryos. The embryonic central nervous system and the limb buds are two examples of vertebrate structures that are subdivided into compartments (Altabef et al., 1997; Arques et al., 2007; Fraser et al., 1990; Langenberg and Brand, 2005).

In *Drosophila*, the wing imaginal disc, a single cell layered epithelium that is the precursor of the adult wing, is consecutively subdivided into four compartments (**Figure 1**) (Garcia-Bellido et al., 1973). An early-arising compartment boundary subdivides the wing imaginal disc from embryonic stages onwards into anterior (A) and posterior (P) compartments. Subsequently, during larval stages a second compartment boundary orthogonal to the first one partitions the tissue further into dorsal (D) and ventral (V) compartments.

Compartments are units of developmental control and are instrumental in structuring insect and vertebrate tissues. Local chemical signalling between adjacent compartments sets up organizing centres along compartment boundaries. These organizing centres produce long-range signalling molecules that spread through the tissue and control in a concentration-dependent manner the expression of target genes. These target genes, in turn, direct growth and patterning of the tissue (Lawrence and Struhl, 1996).

Maintaining straight and sharp compartment boundaries during development is a challenging task, because cell proliferation and tissue movements cause cells to rearrange and hence to mix. The activity of 'selector genes' and local signalling between compartments is required to maintain compartment boundaries. In *Drosophila*, maintenance of the A/P compartment boundary requires the selector gene *engrailed* (Morata and Lawrence, 1975) and signalling by Hedgehog and Decapentaplegic (Dpp), a member of the family of Bone morphogenetic proteins (BMPs) (**Figure 1B**) (Blair and Ralston, 1997; Dahmann and Basler, 2000; Rodriguez and Basler, 1997; Shen and Dahmann, 2005). The selector gene *apterous* (Blair et al., 1994) and Notch signalling are important to maintain the D/V boundary in the developing fly wing (**Figure 1C**) (Micchelli and Blair, 1999; Rauskolb et al., 1999).

Mechanical tension

What are the physical mechanisms that maintain compartment boundaries? It has long been assumed that differences in the affinity (or adhesiveness) of cells from neighbouring compartments drive cell sorting at compartment boundaries (Garcia-Bellido et al., 1973). Recent work in *Drosophila*, however, has instead revealed an important role of local increases in mechanical tension at adherens junctions along compartment boundaries.

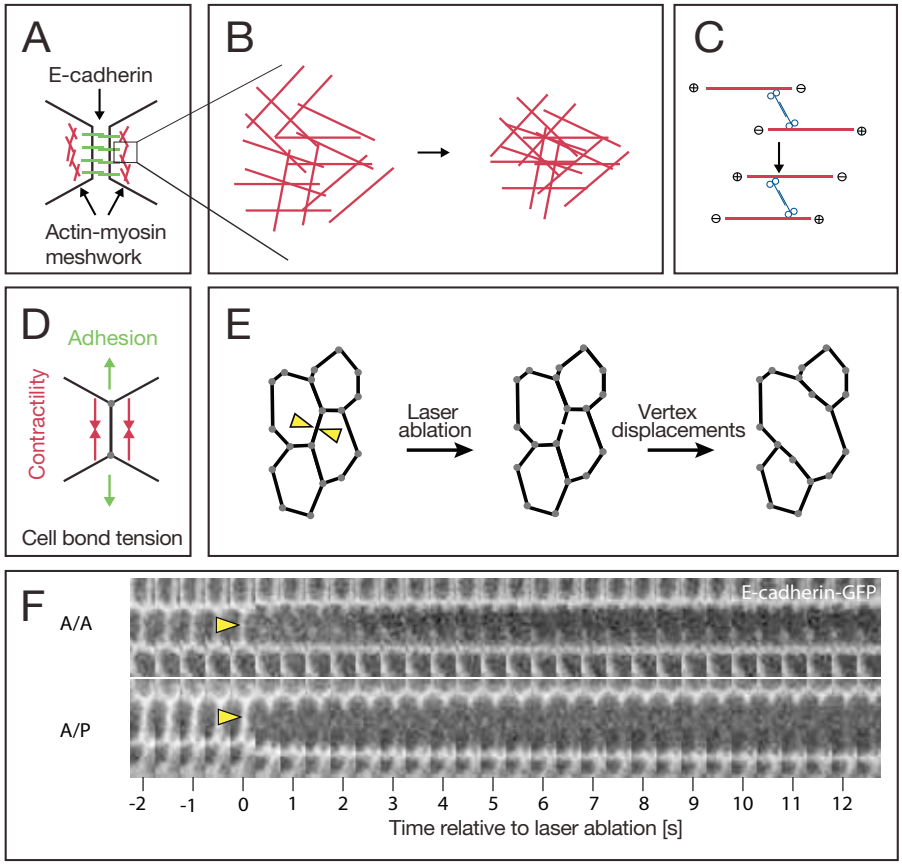


***Figure 2:* Processes generating cell bond tension**
(A) E-Cadherin molecules (green) form homophilic interactions at the level of adherens junctions linking two cells. Underlying this is a meshwork of actin filaments and Myosin II motors (red). **(B)** This actin-myosin meshwork shows contractile behaviour. **(C)** Bipolar myosin dimers can slide anti-parallel actin-filaments past each other, which leads on a larger scale to a contractile behaviour of the meshwork. **(D)** Cell bond tension is influenced by both E-cadherin mediated adhesion and actin-myosin mediated contractility. **(E)** Cell bond tension can be measured by laser ablation. Arrowheads indicate the point of ablation. Upon laser ablation the two vertices at the ends of the ablated bond (grey) are displaced. **(F)** Kymograms of A/A and A/P cell bonds in wing imaginal discs visualized by E-cadherin-GFP before and after laser ablation.

In epithelia, adherens junctions form an adhesive circumferential belt mechanically linking neighbouring cells to each other by the homophilic binding of E-cadherin molecules (**Figure 2A**). The cytoplasmic tail of E-cadherin dynamically interacts via catenins with a meshwork of filamentous (F-) actin and myosin motor proteins underlying the plasma membrane (**Figure 2B**) (Yonemura, 2011). Bipolar assemblies of non-muscle Myosin II (Myosin II) slide antiparallel actin filaments past each other enabling this cortical meshwork to contract (**Figure 2C**) (Quintin et al., 2008). The ensuing contractile forces are balanced by opposing forces resulting from cell-cell adhesion (**Figure 2D**). The combination of cortical contractility and cell-cell adhesion thus generates mechanical tension at the adherens junctions linking two cells (hereafter referred to as cell bond tension). It was therefore intriguing to find that F-actin and Myosin II are enriched at adherens junctions along compartment boundaries in flies (Landsberg et al., 2009; Major and Irvine, 2005; Major and Irvine, 2006; Monier et al., 2010).

We have used laser ablation to directly quantify the mechanical tension at individual cell bonds within the wing imaginal disc epithelium (Landsberg et al., 2009). Ablation of single cell bonds using an UV laser beam causes an imbalance of forces in the adherens junctional network leading to the displacement of cell corners (vertices) (Farhadifar et al., 2007) (**Figure 2E**). The initial speed and magnitude of this vertex displacement is a relative measure of the tension that acted along the cell bond before ablation. We found that cell bond tension is similar in the anterior and the posterior compartment, indicating that the mechanical properties of anterior and posterior cells are alike. Cell bond tension along the A/P compartment boundary, however, is approximately 2.5 fold higher compared to cell bond tension within the compartments **(Figure 2F)**. Cell bond tension is reduced in the presence of the drug Y-27632, an inhibitor of Rho-kinase (Uehata et al., 1997), which is a major activator of Myosin II. These results demonstrate that actomyosin-based cell bond tension is locally increased along the A/P compartment boundary.

Is Myosin II activity required to maintain straight and sharp compartment boundaries? Decreasing Myosin II activity using mutant alleles of *zipper*, the gene encoding myosin heavy chain, results in irregular compartment boundaries in the wing imaginal disc and embryonic epidermis of the fly (Landsberg et al., 2009; Monier et al., 2010). Performing live imaging in the embryonic epidermis showed that cells dividing along the A/P compartment boundary transiently push into the neighbouring compartment and thereby locally deform the compartment boundary (Monier et al., 2010). However, these cells are subsequently pushed back and a straight compartment boundary is re-established. To directly address the role of Myosin II at the compartment boundary, Monier et al. locally inactivated Myosin II using chromophore assisted laser inactivation (CALI) (Monier et al., 2010). Using this technique, Monier et al. were able to show that when Myosin II was specifically inactivated at the compartment boundary, dividing cells were no longer pushed back resulting in a rough and irregular compartment boundary. These experiments demonstrate a crucial role of actin-myosin based contractility in the separation of different cell populations at compartment boundaries.

Are local increases in cell bond tension sufficient to maintain sharp and straight compartment boundaries? We used a vertex model to simulate tissue growth to address this question (**Figure 3A**). In this model (Farhadifar et al., 2007; Landsberg et al., 2009), the network of adherens junctions is described by the position of vertices and the connections between them. Stable and stationary network configurations arise when the forces acting on each vertex balance each other. These configurations correspond to local minima of a work function, which describes the elasticity of cells and junctional forces arising from cell-cell adhesion and actomyosin contractility (**Figure 3A**). Cell proliferation in these networks is simulated by randomly selecting a cell, increasing its area two-fold, and inserting a new cell bond through the centre of the cell. The network is subsequently allowed to relax to a new local minimum. Compartment boundaries

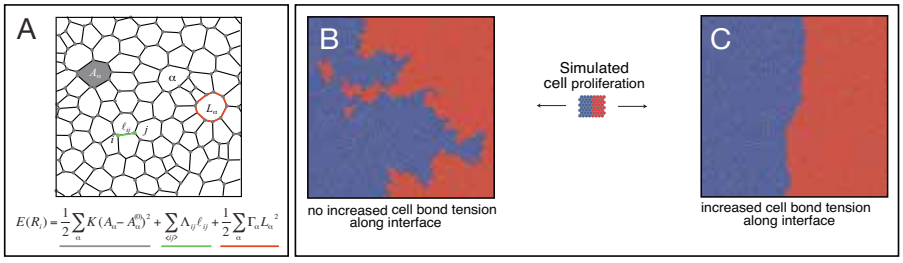


Figure 3: Simulation of growth processes
(A) The network of adherens junctions is defined in the vertex model by the position *Ri* of vertices *i* and the connection between them *ij*. Stable configurations are local minima of a work function (bottom of panel). The first term describes an area elasticity of cells indexed by α, with an actual area A_α, and a preferred area $A^{(0)}$, and an elastic coefficient K (grey). The effects of tension Λ_{ij} along a cell bond of length l_{ij} connecting the vertices i and j are described by the second term (green). The third term describes the elasticity of the cell perimeter L_α with coefficient Γ (red). **(B,C)** Different outcomes of growth simulations using the vertex model for two adjacent cell populations. **(B)** All cell bonds have the same tension. **(C)** Cell bond tension is locally increased 2.5 fold along the compartment boundary.

are introduced by starting with a network configuration in which two adjacent groups of cells are separated by a straight interface. We showed by using these simulations that the initially straight interface between the two cell populations became rough and irregular during cell proliferation and cells started to mix (**Figure 3B**) (Landsberg et al., 2009). These findings support the idea that cell division leads to cell rearrangements promoting cell mixing. Importantly, however, when cell bond tension along the interface was locally increased in the simulations, cells did not mix and the interface between the two compartments remained straight and sharp (**Figure 3C**) (Landsberg et al., 2009). Therefore, these simulations indicate that a local increase in cell bond tension is sufficient to maintain straight and sharp compartment boundaries between two proliferating cell populations.

Taken together, the results of these experimental and theoretical approaches suggest a model in which chemical signalling between compartments result in local increases in actomyosin-based cell bond tension. Increases in cell bond tension guide junctional rearrangements after cell division to prevent cell mixing and to maintain compartment boundaries.

Differences in actomyosin-dependent cell cortex tension have recently been implicated in sorting cells from different germlayers during zebrafish gastrulation (Krieg et al., 2008). Modulations of mechanical tension might therefore be a more general mechanism underlying cell sorting.

Local modulations in mechanical tension at cell junctions are not limited to cell sorting processes, but seem to be common during animal development. In gastrulating *Drosophila* embryos, for example, tissue elongation involves concerted shrinkage and extension of cell junctions. Shrinking junctions are enriched in F-actin and Myosin II, and display higher mechanical tension (Bertet et al., 2004; Fernandez-Gonzalez and Zallen, 2009; Rauzi et al., 2008).

Many open questions remain. How is mechanical tension at cell junctions along compartment boundaries locally increased? How is this increase in tension linked to the chemical signalling pathways that control compartment boundary maintenance? And, how do cell adhesion molecules contribute to cell sorting at compartment boundaries? One way that cell adhesion molecules contribute to cell sorting is by providing physical linkage between cells enabling the contractile actomyosin network to generate tension. More intriguingly, however, previous results suggest that differences in the expression levels of cell adhesion molecules trigger local accumulations of F-actin and Myosin II at cell junctions. In *Drosophila* epithelia, for example, borders between cells expressing the homophilic adhesion molecule Echinoid and cells that do not express Echinoid are enriched in F-actin and Myosin II (Wei et al., 2005). Differences in the expression of cell adhesion molecules between compartments could thus result in local accumulations of F-actin and Myosin II, and hence lead to increased cell bond tension along the compartment boundary.

In conclusion, live imaging and sophisticated approaches to perturb protein and cell function *in vivo* combined with computational modelling has advanced our understanding of how compartment boundaries are formed and maintained. Local increases of mechanical tension have emerged as a crucial physical mechanism driving cell sorting. Further analysis of mechanical processes promises to shed new light on questions in cell and developmental biology.

Acknowledgements

We thank Daiki Umetsu and Maryam Aliee for help in preparing figures and members of our group for critical comments on the manuscript. Work in the laboratory is supported by the Max Planck Society and by grants from Deutsche Forschungsgemeinschaft and the Human Frontier Science Program.

References

Altabef, M., J.D. Clarke, and C. Tickle. 1997. Dorso-ventral ectodermal compartments and origin of apical ectodermal ridge in developing chick limb. *Development*. **124**:4547-4556.

Arques, C.G., R. Doohan, J. Sharpe, and M. Torres. 2007. Cell tracing reveals a dorsoventral lineage restriction plane in the mouse limb bud mesenchyme. *Development*. **134**:3713-3722.

Bertet, C., L. Sulak, and T. Lecuit. 2004. Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature*. **429**:667-671.

Blair, S.S., D.L. Brower, J.B. Thomas, and M. Zavortink. 1994. The role of apterous in the control of dorsoventral compartmentalization and PS integrin gene expression in the developing wing of *Drosophila*. *Development*. **120**:1805-1815.

Blair, S.S., and A. Ralston. 1997. Smoothened-mediated Hedgehog signalling is required for the maintenance of the anterior-posterior lineage restriction in the developing wing of *Drosophila*. *Development*. **124**:4053-4063.

Brodland, G.W. 2002. The Differential Interfacial Tension Hypothesis (DITH): a comprehensive theory for the self-rearrangement of embryonic cells and tissues. *J Biomech Eng*. **124**:188-197.

Dahmann, C., and K. Basler. 2000. Opposing transcriptional outputs of Hedgehog signaling and engrailed control compartmental cell sorting at the *Drosophila* A/P boundary. *Cell*. **100**:411-422.

Dahmann, C., A.C. Oates, and M. Brand. 2011. Boundary formation and maintenance in tissue development. *Nature reviews. Genetics*. **12**:43-55.

Farhadifar, R., J.C. Röper, B. Aigouy, S. Eaton, and F. Jülicher. 2007. The influence of cell mechanics, cell-cell interactions, and proliferation on epithelial packing. *Current biology : CB*. **17**:2095-2104.

Fernandez-Gonzalez, R., and J.A. Zallen. 2009. Cell mechanics and feedback regulation of actomyosin networks. *Sci Signal*. **2**:78.

Fraser, S., R. Keynes, and A. Lumsden. 1990. Segmentation in the chick embryo hindbrain is defined by cell lineage restrictions. *Nature*. **344**:431-435.

Garcia-Bellido, A., P. Ripoll, and G. Morata. 1973. Developmental compartmentalisation of the wing disk of *Drosophila*. *Nat New Biol*. **245**:251-253.

Godt, D., and U. Tepass. 1998. *Drosophila* oocyte localization is mediated by differential cadherin-based adhesion. *Nature*. **395**:387-391.

Gonzalez-Reyes, A., and D. St Johnston. 1998. The *Drosophila* AP axis is polarised by the cadherin-mediated positioning of the oocyte. *Development*. **125**:3635-3644.

Harris, A.K. 1976. Is cell sorting caused by differences in the work of intercellular adhesion? A critique of the steinberg hypothesis. *Journal of Theoretical Biology*. **61**:267-285.

Krieg, M., Y. Arboleda-Estudillo, P.H. Puech, J. Kafer, F. Graner, D.J. Muller, and C.P. Heisenberg. 2008. Tensile forces govern germ-layer organization in zebrafish. *Nat Cell Biol*. **10**:429-436.

Landsberg, K.P., R. Farhadifar, J. Ranft, D. Umetsu, T.J. Widmann, T. Bittig, A. Said, F. Jülicher, and C. Dahmann. 2009. Increased cell bond tension governs cell sorting at the *Drosophila* anteroposterior compartment boundary. *Curr Biol*. **19**:1950-1955.

Langenberg, T., and M. Brand. 2005. Lineage restriction maintains a stable organizer cell population at the zebrafish midbrain-hindbrain boundary. *Development*. **132**:3209-3216.

Lawrence, P.A. 1973. A clonal analysis of segment development in Oncopeltus (Hemiptera). *J Embryol Exp Morphol*. **30**:681-699.

Lawrence, P.A., and G. Struhl. 1996. Morphogens, compartments, and pattern: lessons from *Drosophila*? *Cell*. **85**:951-961.

Major, R.J., and K.D. Irvine. 2005. Influence of Notch on dorsoventral compartmentalization and actin organization in the *Drosophila* wing. *Development*. **132**:3823-3833.

Major, R.J., and K.D. Irvine. 2006. Localization and requirement for Myosin II at the dorsal-ventral compartment boundary of the Drosophila wing. *Dev Dyn*. **235**:3051-3058.

Micchelli, C.A., and S.S. Blair. 1999. Dorsoventral lineage restriction in wing imaginal discs requires Notch. *Nature*. **401**:473-476.

Monier, B., A. Pelissier-Monier, A.H. Brand, and B. Sanson. 2010. An actomyosin-based barrier inhibits cell mixing at compartmental boundaries in *Drosophila* embryos. *Nature cell biology*. **12**:60-65.

Monier, B., A. Pelissier-Monier, and B. Sanson. 2011. Establishment and maintenance of compartmental boundaries: role of contractile actomyosin barriers. *Cellular and molecular life sciences : CMLS*. **68**:1897-1910.

Morata, G., and P.A. Lawrence. 1975. Control of compartment development by the *engrailed* gene in *Drosophila*. *Nature*. **255**:614-617.

Nose, A., A. Nagafuchi, and M. Takeichi. 1988. Expressed recombinant cadherins mediate cell sorting in model systems. *Cell*. **54**:993-1001.

Quintin, S., C. Gally, and M. Labouesse. 2008. Epithelial morphogenesis in embryos: asymmetries, motors and brakes. *Trends Genet*. **24**:221-230.

Rauskolb, C., T. Correia, and K.D. Irvine. 1999. Fringe-dependent separation of dorsal and ventral cells in the *Drosophila* wing. *Nature*. **401**:476-480.

Rauzi, M., P. Verant, T. Lecuit, and P.F. Lenne. 2008. Nature and anisotropy of cortical forces orienting *Drosophila* tissue morphogenesis. *Nat Cell Biol*. **10**:1401-1410.

Rodriguez, I., and K. Basler. 1997. Control of compartmental affinity boundaries by hedgehog. *Nature*. **389**:614-618.

Shen, J., and C. Dahmann. 2005. The role of Dpp signaling in maintaining the *Drosophila* anteroposterior compartment boundary. *Dev Biol*. **279**:31-43.

Steinberg, M.S. 1963. Reconstruction of tissues by dissociated cells. Some morphogenetic tissue movements and the sorting out of embryonic cells may have a common explanation. *Science*. **141**:401-408.

Steinberg, M.S., and M. Takeichi. 1994. Experimental specification of cell sorting, tissue spreading, and specific spatial patterning by quantitative differences in cadherin expression. *Proc Natl Acad Sci U S A*. **91**:206-209.

Townes, P.L., and J. Holtfreter. 1955. Directed movements and selective adhesion of embryonic amphibian cells. *Journal of Experimental Zoology*. **128**:53-120.

Uehata, M., T. Ishizaki, H. Satoh, T. Ono, T. Kawahara, T. Morishita, H. Tamakawa, K. Yamagami, J. Inui, M. Maekawa, and S. Narumiya. 1997. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature*. **389**:990-994.

Wei, S.Y., L.M. Escudero, F. Yu, L.H. Chang, L.Y. Chen, Y.H. Ho, C.M. Lin, C.S. Chou, W. Chia, J. Modolell, and J.C. Hsu. 2005. Echinoid is a component of adherens junctions that cooperates with DE-Cadherin to mediate cell adhesion. *Developmental cell*. **8**:493-504.

Yonemura, S. 2011. Cadherin-actin interactions at adherens junctions. *Current opinion in cell biology*. **23**:515-522.

Jens-Christian Röper

Technische Universität Dresden, Institut für Genetik
Helmholtzstr. 10, D-01069 Dresden, Germany
Tel.: +49 (351) 463-39537, Fax: +49 (351) 463-39579
e-mail Jens-Christian.Roeper@mailbox.tu-dresden.de

Jens-Christian Röper studied Biochemistry at the Free University of Berlin. For his PhD he moved to the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden to work on the biophysical mechanisms underlying tissue organization under the supervision of Suzanne Eaton. In 2010, he joined the laboratory of Christian Dahmann at the Institute of Genetics of the Dresden University of Technology to investigate the mechanisms involved in cell sorting.

Christian Dahmann

Technische Universität Dresden, Institut für Genetik
Helmholtzstr. 10, D-01069 Dresden, Germany
Tel.: +49 (351) 463-39537, Fax: +49 (351) 463-39579
e-mail Christian.Dahmann@mailbox.tu-dresden.de

Christian Dahmann studied Biology at the University of Tübingen. He received his graduate training at the Research Institute of Molecular Pathology (IMP) in Vienna under the supervision of Kim Nasmyth. For his postdoctoral studies he joined the laboratory of Konrad Basler at the University of Zürich, where he began to work on signaling pathways directing cell sorting in *Drosophila*. In 2001, he started his own research group at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden. Since 2010, he is a fellow of the DFG Heisenberg Programme at the Institute of Genetics. His lab explores the molecular and biomechanical mechanisms of cell sorting and tissue organization using a variety of tools ranging from genetics to biophysical approaches.

"Macrophages on the move: how podosomes contribute to immune cell invasion"

Stefan Linder

Podosomes and invadopodia: organelles of invasive cells

For successful migration, invasive cells have to overcome many barriers, in particular the dense meshwork of interconnected fibres that makes up the extracellular matrix (ECM). Two different modes of migration present themselves: amoeboid migration, where cells squeeze through the holes between ECM fibres, and mesenchymal migration, with cells proteolytically degrading local ECM obstructions (1). In consequence, cells using the mescenchymal mode have to develop methods to fine-tune the local degradation of matrix material.

Podosomes and invadopodia, collectively called "invadosomes", are cell-matrix contacts with an inherent ability to lyse extracellular matrix material (2-5). This is achieved by localized release of matrix-lytic factors, especially proteases of the matrix metalloproteinase (MMP) family (6). Podosomes are mostly formed in a physiological context and have been described for monocytic cells such as macrophages, dendritic cells and osteoclasts, but also in endothelial cells and smooth muscle cells. Invadopodia are formed by several types of cancer cells and thus seem to contribute to a more pathological scenario (2-6). Both types of structures have attracted widespread attention during the last few years, and the invadosome field is progressing significantly, as evidenced by the ever-growing amount of publications and of labs joining the field (www.invadosomes.org).

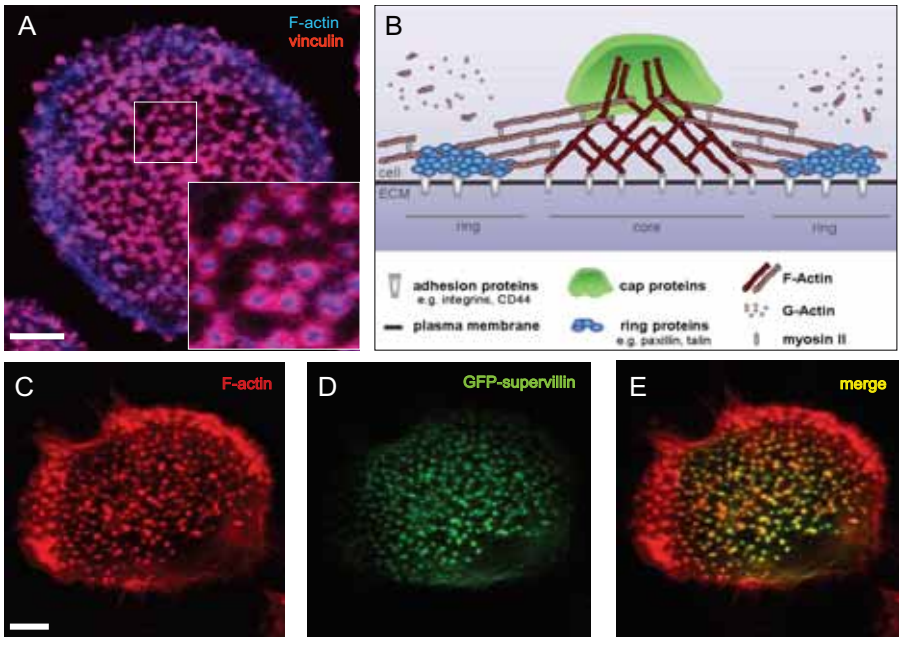


***Figure 1:* Podosome structure.** (A) Confocal micrograph of a primary human macrophage stained for F-actin (blue) and vinculin (red). Note that each actin-rich podosome core is surrounded by a vinculin-containing ring structure. Image by Petra Kopp. (B) Podosome model: a core of branched f-actin (dark red), surrounded by possibly unbranched actin filaments (light red) bundled by myosin II (dark grey), a cap structure on top of the actin core (green), and the surrounding ring structure (blue). Contact to the matrix is established by integrins and CD44 (light grey). A surrounding cloud of G- and F-actin is indicated above the podosome. Reprinted, with permission, from (5). (C-E) Podosome subpopulations show different molecular composition. Confocal micrograph of primary macrophage stained for F-actin (C), and expressing GFP-supervillin (D), with merge in (E). Note that large precursor podosomes in the cell periphery are virtually devoid of GFP-supervillin. Images by Susanne Cornfine. Bars in (A,C): 10 µm.

Podosomes in macrophages: multiple uses for the molecular toolbox

Podosomes in primary macrophages are a relevant and accessible system to study invadosome regulation for several reasons: i) primary macrophages show constitutive formation of podosomes, ii) they display high numbers of often up to 500 podosomes per cell, which allows statistical analysis, and iii) primary macrophages are unaltered, i.e. not immortalized, allowing the study of signal cascades in their practically pristine configuration.

Podosomes show a typical architecture: a core structure consisting of F-actin and actin-associated proteins such as WASP, Arp2/3, gelsolin or cortactin, and a ring structure of plaque proteins such as talin, vinculin, paxillin or zyxin (2). Recent findings also demonstrate the presence of a cap structure on top of the actin core, which contains the formin FMNL-1 (7) or the membrane-associated protein supervillin (8), and

several other proteins (P.Cervero and S. Linder, unpublished). This structure may regulate podosome growth or could function as a hub for incoming vesicels (Figure 1).

The ultrastructure of podosomes is currently under intensive investigation, and recent evidence hints at the existence of a layer of unbranched actin filaments that surround the branched network of the core. This would also be in line with the detection of myosin around the core (9,10), and the involvement of actomyosin-dependent contractility in both mechanosensing by podosomes (see below) and turnover of the podosome structure itself (11,12). Actomyosin cables also run between podosomes and thus connect individual structures into a higher-ordered group. This actomyosin-generated tug-of-war may also explain the striking regularity of the podosome pattern in cells and help to coordinate net movement of a field of podosomes.

Podosomes are multifunctional organelles that combine several key features. First, they most probably function as adhesive hotspots of cells, as they are enriched in adhesion-promoting proteins such as integrins (4,13,14) or CD44 (Chabadel et al., 2007). TIRF microscopy also revealed close contact of podosomes to the underlying substratum (15). A second key feature is the ability to locally degrade the extracellular matrix (see below). A third intriguing feature is the ability for mechanosensing. Podosomes can detect traction forces, and both spacing and lifetime of podosomes is modulated by matrix rigidity (16,17). Again, the actomyosin system seems to be critical for this ability. A fourth, and mostly speculative, function concerns the possible role of podosomes in providing adhesion points within newly established protrusions, thus supporting the directional migration of cells. Further experiments will be needed to determine whether this ability can be added to the growing repertoire of these multipurpose organelles.

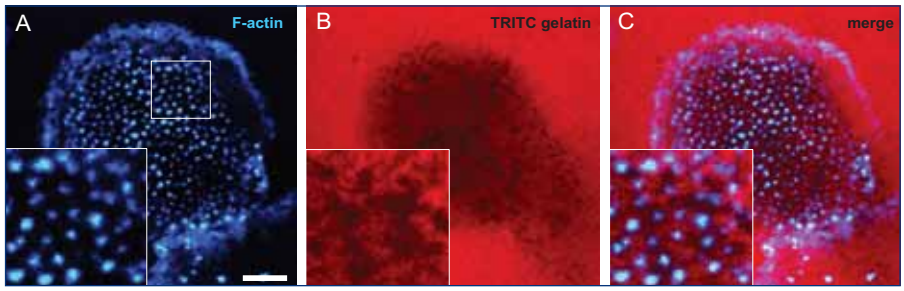


Figure 2: **Podosomes are sites of extracellular matrix degradation.** Confocal micrographs of a primary human macrophage, stained for F-actin (A; blue), seeded on fluorescently labeled gelatin matrix (B; red). Sites of matrix degradation are visible as black defects and mostly coincide with podosomes (C). White box in (A) indicates detail images shown as insets. White bar: 10 µm. Images by Christiane Wiesner.

All podosomes are equal – but some are more equal than others

In several systems, podosomal structures can be induced by overexpression of active RhoGTPases such as $CDC42^{V12}$ (18), stimulating PKC pathways by adding phorbol esters (19), or transformation with oncogenes such as Src (20). While these are highly effective treatments, more upstream regulators that induce maturation or diversification of the structure may thus be bypassed. In fact, one of the most intriguing features of cells that show constitutive podosome formation is the existence of podosome subpopulations. In primary human macrophages, at least two subpopulations exist that show distinct characteristics such as size, lifetime, dynamics and subcellular localization. Larger podosomes at the cell periphery or the leading edge of migrating cells, called precursors, have

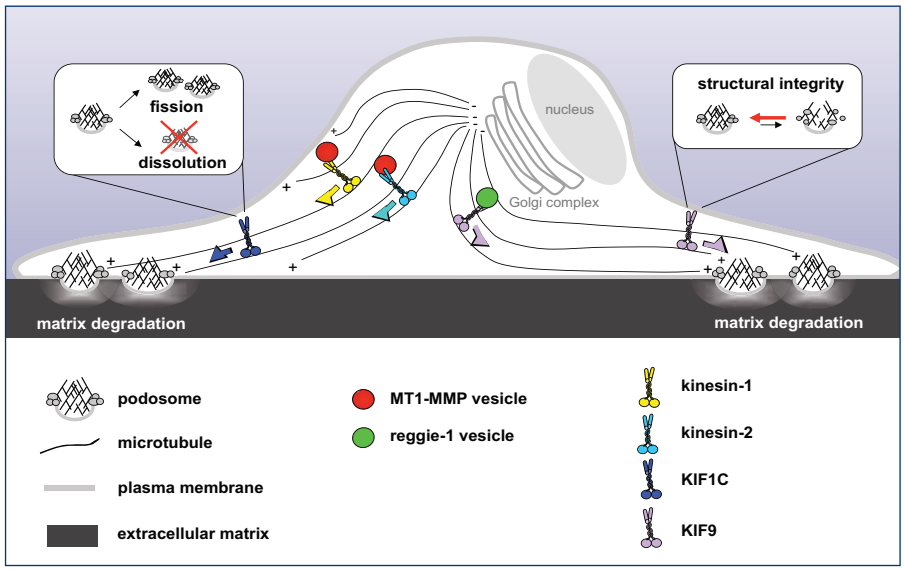


Figure 3: **Regulation of podosomes by microtubule-dependent transport.** Microtubules contact podosomes with their plus ends. Plus end–directed kinesin motors transport cargo vesicles to podosomes, which influence podosome structure and function. Kinesin-1 and kinesin-2 transport MT1-MMP-positive vesicles that probably influence matrix degradation. KIF1C carries as yet unidentified cargo and regulates the dynamics of precursor podosomes. KIF9 transports vesicles positive for reggie-1/flotillin-2 and influences matrix degradation, while another, as yet unidentified cargo of KIF9 supports the structural integrity of podosomes. Modified from (5), and reprinted with permission.

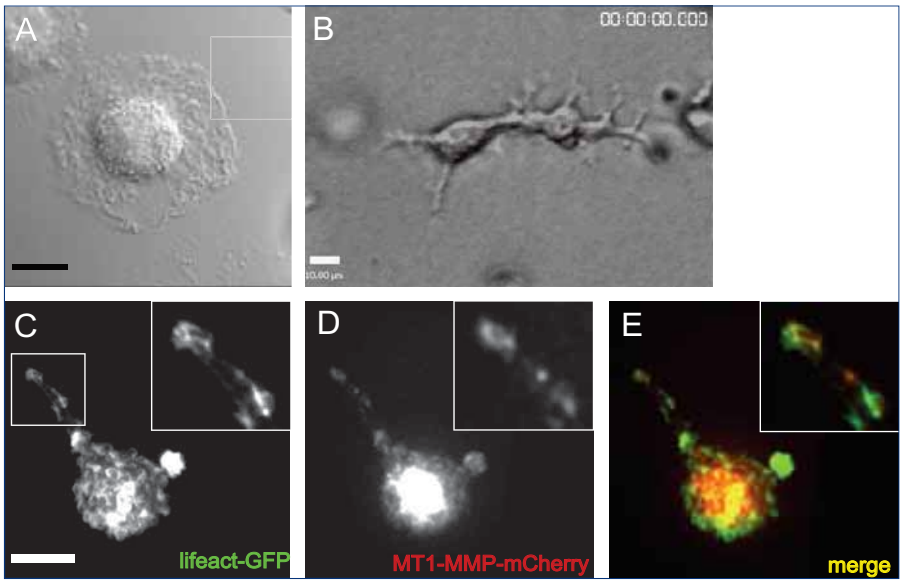


***Figure 4:* Macrophages in in 2D and 3D.** (A, B) Macrophages change their morphology in 3D. (A) Brightfield micrograph of macrophage seeded on glass coverslip. Note central dome-shaped part containing the nucleus and most of the cytoplasm, and surrounding thin layer forming multiple ruffles. Image by Mirko Himmel. (B) Still images from time lapse movie of macrophages embedded in gelled collagen I. Note numerous finger-like protrusions. (C-E) Confocal micrographs of a primary human macrophage embedded in gelled collagen I, and overexpressing lifeact-GFP, detecting F-actin (C) and MT1-MMP-mCherry (D), with merge shown in (E). Note the absence of typical podosomes, but the formation of F-actin rich clusters at cell protrusions, that also contain MT1-MMP-mCherry. White box indicates area of detail images. Bars: 10 µm. Images by Christiane Wiesner.

been shown to turn over quickly and also split off daughter podosomes that move into the more inner regions of the cell (21). This process is influenced by contact of microtubule plus ends (9). The population of smaller podosomes, called successors, do not show fission processes and are interconnected by a meshwork of contractile actomyosin cables. Local myosin contractility at podosomes is controlled by supervillin, a member of the villin family, that binds both contractile myosin and myosin light chain, which leads to further stimulation of myosin activity, and thus induces a feed-forward cycle of increasing actomyosin contraction resulting in podosome dissolution (8). Interestingly, also precursor podosomes acquire supervillin and myosin prior to their dissolution. Collectively, these findings indicate that podosome subpopulations in macrophages differ in their molecular makeup, and that their composition alters during their lifecycle. Further molecular differences between podosome subpopulations that fine-tune podosome architecture and function are to be expected.

Breaching the matrix: proteolytic degradation at podosomes

Degradation of extracellular matrix material is one of the hallmarks and defining features of podosomes (6). It also helps to discriminate between podosomes and other actin-rich structures of cells. Classically, matrix degradation is demonstrated by seeding cells on fluorescently labeled matrix. Sites of local ECM degradation then appear underneath podosomes, as the matrix is degraded and the label is lost (Figure 2). Podosomes are cellular all-purpose weapons that can attack a large variety of ECM materials such as fibronectin, collagen or gelatin. They do this by locally concentrating and releasing ECM-lytic factors, most notably proteins of the matrix metalloproteinase family (MMP) such as MT1-MMP, but also other metalloproteinases such as ADAMs (6,18,20). Accumulation of proteinases at podosomes is probably achieved through microtubule-dependent trafficking (see below). For example, MT1-MMP has been demonstrated to travel along microtubules towards podosomes in vesicles that are powered by kinesin-1 and kinesin-2 (22). However, the fine-tuning of this process, the regulatory molecules and the podosome substructures that are involved, are still to be determined. An intriguing question is also how onset and cessation of matrix degradation at podosomes is timed, and whether all podosomes are able to degrade ECM in equal measure and at all times.

Podosome traffic: a busy hub for intracellular transport

Due to their composition, podosomes depend on actin-regulatory processes. However, it is increasingly apparent that podosomes are also influenced by other parts of the cytoskeleton, and particularly by microtubules and microtubule-dependent trafficking. For example, intact microtubules are necessary for the formation of podosomes (23), and live cell imaging has demonstrated the dynamic contact of microtubule plus ends with podosomes. This contact also influences the dynamics of podosome precursors, suggesting the delivery of regulatory factors along microtubules to podosomes (9). Consistently, a variety of motor proteins have been identified that regulate different aspects of podosome dynamics and function: kinesin-1 and kinesin-2 are important for the delivery of the key metalloproteinase MT1-MMP to podosomes (22), the kinesin KIF1C regulates the fission rates of podosome precursors (9), and the kinesin KIF9 has emerged as an important regulator of both podosome stability and matrix degadration (24). It seems that podosomes act as subcellular hubs that coordinate an intricate and highly dynamic cargo delivery system (Figure 3). It will be highly interesting to determine how this system

is fine tuned to ensure correct and timely delivery of both podosome components and regulatory factors.

More than a special effect: podosomes in 3D

So far, podosomes and invadopodia have been studied mostly on 2 dimensional, artificial surfaces. This may be relevant, as also in some physiological situations flat, 2 dimensional interfaces are likely to exist between the matrix and podosome forming cells (5). These include contact of monocytic cells with vessel walls, of endothelial cells with the basement membrane and of osteoclasts with the bone surface. In these situations, cells most likely form podosome structures that are similar to their *in vitro* cousins.

However, cells embedded in 3 dimensional environments show drastic alterations of their morphology and behaviour (Figure 4). Consequently, it is to be expected that also podosomes and related structures, if they indeed exist within tissues, have altered appearances. Initial experiments with macrophages embedded in 3D gelled collagen show that cells lose their typical "fried egg" appearance and display a central body that forms multiple and highly dynamic extensions. Intriguingly, these extensions often end in dot-like, actin-rich accumulations that also contain typical podosomal proteins such as cortactin or vinculin. Moreover, they also accumulate proteases such as MT1-MMP (Figure 4), a prerequisite for local matrix degradation. Further studies are necessary to determine if these structure are indeed degrading matrix material and can thus be identified as 3D equivalents of 2D podosomes.

Conclusions

Podosomes are a, if not *the*, major feature of the actin cytoskeleton of macrophages. These cells invest considerable resources in the formation, upkeep and highly fine-tuned turnover of podosomes. Not unwisely so, as podosomes are multipurpose organelles that combine several key abilities of macrophages, including adhesion, localized matrix degradation and mechanosensing. They should thus be instrumental for macrophages in gathering information about the environment, adhering to suitable surface features, and paving the way during invasive migration. Current research activities point to the existence of equivalent structures also in 3D situations, underlining the likely in vivo relevance of podosomes. Looking back, podosomes have come a long way from their discovery in the 1980s, when they were mostly regarded as "funny curiosities". It is to the credit of pioneers like Pier Carlo Marchisio that we are now able to see and study these structures as relevant, multifunctional and integral parts of cells.

Acknowledgments

I would like to thank all present and past members of the lab, as well as my colleagues at LMU (Munich) and UKE (Hamburg). Work in my lab is currently funded by the DFG (LI 925/3-1), the Wilhelm Sander-Stiftung (2007.20.02) and the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° FP7-237946 (T3Net).

References

1. Friedl P and Wolf K. 2003. Tumour-cell invasion and migration: diversity and escape mechanisms. *Nature Reviews Cancer.* **3**:362-74
2. Linder S. and Aepfelbacher M. (2003). Podosomes: adhesion hot-spots of invasive cells. *Trends Cell Biol* **13**:376-85.
3. Weaver AM. (2006). Invadopodia: specialized cell structures for cancer invasion. *Clin. Exp. Metastasis* **23**:97-105
4. Gimona M, Buccione R, Courtneidge SA, Linder S. (2008). Assembly and biological role of podosomes and invadopodia. *Curr. Opin. Cell Biol.* **20**(2):235-41
5. Linder S., Wiesner C., Himmel M. (2011). Degrading devices: invadosomes in proteolytic cell invasion. *Annu. Rev. Mol. Cell Biol.* **27**:185-211.
6. Linder S. (2007). The matrix corroded: podosomes and invadopodia in extracellular matrix degradation. *Trends Cell Biol* **17**:107-17.
7. Mersich AT, Miller MR, Chkourko H, Blystone SD. (2010). The formin FRL1 (FMNL1) is an essential component of macrophage podosomes. *Cytoskeleton* (Hoboken). **67**(9):573-85
8. Bhuwania R., Cornfine S., Fang Z., Krüger M., Luna E.J., Linder S. (2012). Supervillin couples myosin-dependent contractility to podosomes and enables their turnover, *J. Cell Sci.*, in press
9. Kopp P, Lammers R, Aepfelbacher M, Woehlke G, Rudel T, Machuy N., Steffen W., Linder, S. (2006). The kinesin KIF1C and microtubule plus ends regulate podosome dynamics in macrophages. *Mol. Biol. Cell.* **17**(6):2811-23
10. van Helden SF, Oud MM, Joosten B, Peterse N, Figdor CG, van Leeuwen FN. (2008). PGE2-mediated podosome loss in dendritic cells is dependent on actomyosin contraction downstream of the RhoA-Rho-kinase axis. *J. Cell Sci.* **121**(7):1096-106
11. Luxenburg C, Geblinger D, Klein E, Anderson K, Hanein D, Geiger B, Addadi L. (2007). The architecture of the adhesive apparatus of cultured osteoclasts: from podosome formation to sealing zone assembly. *PLoS One.* **2**(1):e179
12. Akisaka T, Yoshida H, Suzuki R, Takama K. (2008). Adhesion structures and their cytoskeleton-membrane interactions at podosomes of osteoclasts in culture. *Cell Tissue Res.* **331**(3):625-41
13. Zambonin-Zallone A, Teti A, Grano M, Rubinacci A, Abbadini M, Garboli M, Marchisio PC. (1989). Immunocyto-chemical distribution of extracellular matrix receptors in human osteoclasts: a beta 3 integrin is colocalized with vinculin and talin in the podosomes of osteoclastoma giant cells. *Exp. Cell Res.* **182**(2):645-52
14. Chabadel A, Banon-Rodriguez I, Cluet D, Rudkin BB, Wehrle-Haller B, Genot E., Jurdic P, Anton IM, Saltel F. (2007). CD44 and beta3 integrin organize two functionally distinct actin-based domains in osteoclasts. *Mol. Biol. Cell.* **18**(12):4899-910
15. Linder S and Kopp P. (2005). Podosomes at a glance. *J. Cell Sci.* **118**(P10):2079-82
16. Collin O, Tracqui P, Stephanou A, Usson Y, Clément-Lacroix J, Planus E. (2006). Spatiotemporal dynamics of actin-rich adhesion microdomains: influence of substrate flexibility. *J. Cell Sci.* **119**(9):1914-25
17. Collin O, Na S, Chowdhury F, Hong M, Shin ME, Wang F, Wang N. (2008). Self-organized podosomes are dynamic mechanosensors. *Curr. Biol.* **18**(17):1288-94.
18. Tatin F, Varon C, Genot E, Moreau V. (2006). A signalling cascade involving PKC, Src and Cdc42 regulates podosome assembly in cultured endothelial cells in response to phorbol ester. *J. Cell Sci.* **119**:769-81
19. Hai CM, Hahne P, Harrington EO, Gimona M (2002) Conventional protein kinase C mediates phorbol-dibutyrate-induced cytoskeletal remodeling in a7r5 smooth muscle cells. *Exp.Cell Res.* **280**: 64-74
20. Abram CL, Seals DF, Pass I, Salinsky D, Maurer L, Roth TM, Courtneidge SM. (2003). The adaptor protein fish associates with members of the ADAMs family and localizes to podosomes of Src-transformed cells. *J. Biol. Chem.* **278**(19):16844-51
21. Evans J, Correia I, Krasavina O, Watson N, Matsudaira P. (2003). Macrophage podosomes assemble at the leading lamella by growth and fragmentation. *J. Cell Biol* **161**:697–705
22. Wiesner C, Faix J, Himmel M, Bentzien F, Linder S. (2010). KIF5B and KIF3A/KIF3B kinesins drive MT1-MMP surface exposure, CD44 shedding and extracellular matrix degradation in primary macrophages. *Blood* **116**:1559-69
23. Linder S, Hufner K, Wintergerst U, Aepfelbacher M. (2000). Microtubule-dependent formation of podosomal adhesion structures in primary human macrophages. *J Cell Sci* **113**:4165-76.
24. Cornfine S, Himmel M, Kopp P, el Azzouzi K, Wiesner C, Krüger M, Rudel T, Linder, S. (2011). The kinesin KIF9 and reggie/flotillin proteins regulate matrix degradation by macrophage podosomes. *Mol. Biol. Cell.* **22**(2):202-15

Stefan Linder

Institute for Medical Microbiology, Virology and Hygiene
University Medical Center Eppendorf
Martinistr. 52
20246 Hamburg
Germany

Email: s.linder@uke.de
Web: http://www.uke.de/institute/infektionsmedizin/index_56522.php?id=-1

Stefan Linder studied Biology at the Ludwig Maximilians University in Munich. He gained his PhD in the lab of Manfred Schliwa (Institute for Cell Biology, LMU), where he became fatally interested in the cytoskeleton. For a postdoc, he moved to the lab of Martin Aepfelbacher (Institute for Cardiovascular Diseases, LMU), where he stumbled upon podosomes and got hooked ever since. He is now professor for Cellular Microbiology at the University Medical Center Eppendorf (UKE). He holds positions as an editor of *European Journal of Cell Biology*, co-president of the *Invadosome Consortium* (www.invadosomes.org), and coordinator of the EU-FP7 programme *Tissue Transmigration Training Network* (T3Net; www.t3net-itn.org). His lab studies cytoskeletal regulation in primary human cells, especially macrophages and endothelial cells, in the context of adhesion, migration, invasion and phagocytosis.

Chromothripsis in childhood brain tumors – an unexpected link to cancer predisposition

Stefan M. Pfister, Peter Lichter and Jan O. Korbel

Chromosothripsis - an alternative mechanism of cancer development

In early 2011, researchers from the Welcome Trust Sanger Center (Cambridge, UK) have proposed a novel, alternative concept of tumor initiation in a small proportion of cancers through a single catastrophic cellular event that leads to dozens to hundreds of clustered genomic rearrangements in a progenitor cell, with the rearrangements being restricted to one or few chromosomes [1], Figure 1. This genetic phenomenon, which the authors referred to as chromothripsis (from Greek: chromo for chromosome and thripsis for shattering), cannot be explained by the text-book model initially proposed by Fearon and Vogelstein in colon cancer according to which cancer is caused by a cumulous acquisition of multiple genetic hits over time, which ultimately leads to the growth advantage of a cancer-initiating cell clone [2]. Convincing evidence was provided that these two mechanisms are fundamentally different by the facts that in case of chromothripsis (i) affected chromosomes often display only two alternating copy-number states, (ii) genomic breakpoints are highly significantly spatially clustered, (iii) heterozygosity is frequently retained in regions with two copies or more (which would not be expected for early acquired genomic deletions), and (iv) the identical genetic rearrangements are frequently present in relapse tumors from the same patients after cytotoxic treatment and or radiotherapy, and yet no obvious additional acquired genetic hits are necessary to render the tumor therapy-resistant. Screening thousands of published SNP profiling datasets from primary tumors, Stephens et al. identified this phenomenon overall in 2-3% of cancers with some entities (e.g., bone cancers) being much more frequently affected by chromothripsis than others [1].

Furthermore, it is indeed fundamentally important to distinguish chromothripsis from general 'genomic instability', which would under no circumstances be expected to occur in such a spatially restricted (yet massive)

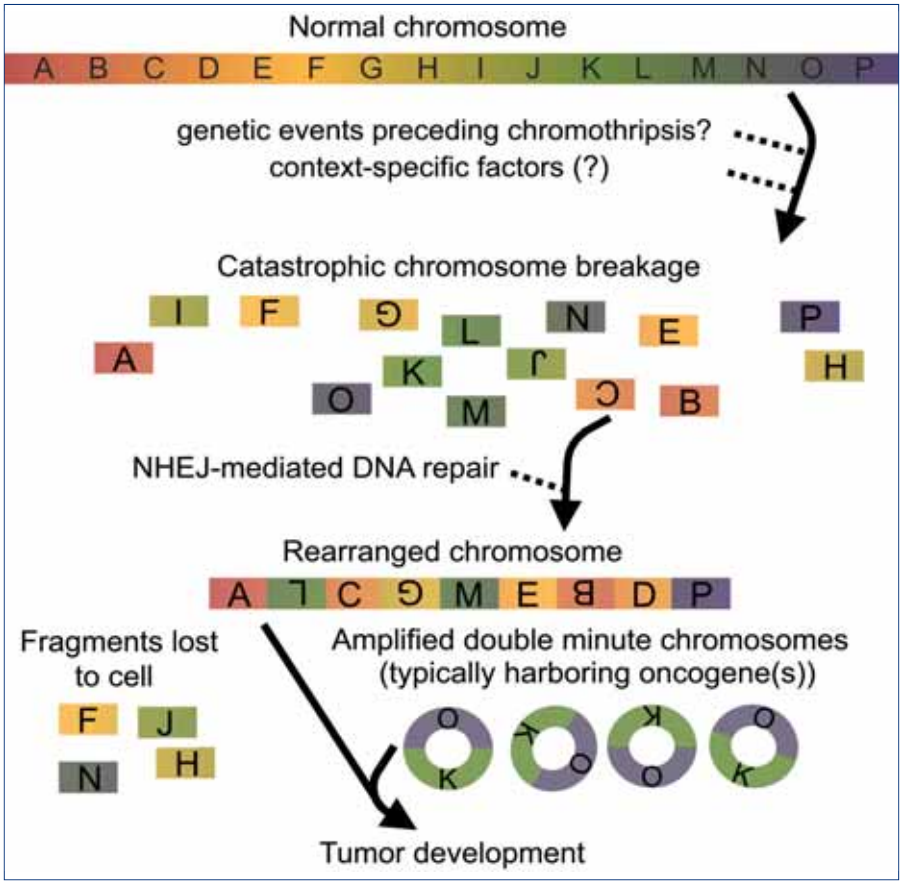


Figure 1: Schematic illustration of chromothripsis: one or few chromosomes (or chromosome arms) are shattered into small pieces in a single event and are then re-joined in a random fashion by non-homologous end-joining. Genetic events preceding tumor initiation in tumors with chromothripsis have been unknown so far, as were context-specific factors, which could explain why some cell types (in defined developmental stages) are more prone to undergo chromothripsis than others. Frequently, during the process of re-joining, certain fragments are lost, whereas others lead to the formation of separate mini-chromosomes and get amplified because they contain a driver oncogene.

manner (i.e., only affecting a single or maximally few chromosomes) and would typically involve many different copy-number states per chromosome.

The most plausible mechanism of re-joining the shattered pieces is presumed to be non-homologous end joining (NHEJ), which on the one hand is error-prone, and which on the other hand frequently leads to the complete loss of genetic material, as is often observed in chromothripsis (Figure 1).

Stephens et al. described two different patterns of chromothripsis in their seminal paper, one of them being characterized by countless re-joined pieces alternating between only two defined copy-number states (we refer to it as 'shattering phenotype', Figure 3), and another one distinguished by few pieces from one or several chromosomes joining together and forming a separate mini-chromosome ('double minute chromosome'), which is then amplified under the strong selective advantage of a driver oncogene (e.g., MYC a family oncogene) that is contained in the mini-chromosome (we termed this 'amplifier phenotype', Figure 2). Although this first study on chromothripsis convincingly established an alternative mechanism of cancer initiation (which certainly is a revolutionary finding by itself), the underlying molecular mechanism, and factor(s) making a cell susceptible for chromothripsis remained unknown. Hence, it has remained unclear (i) whether there are genetic events preceding chromothripsis, and (ii) how context-specific factors influence chromothripsis in different cell types (Figure 1)?

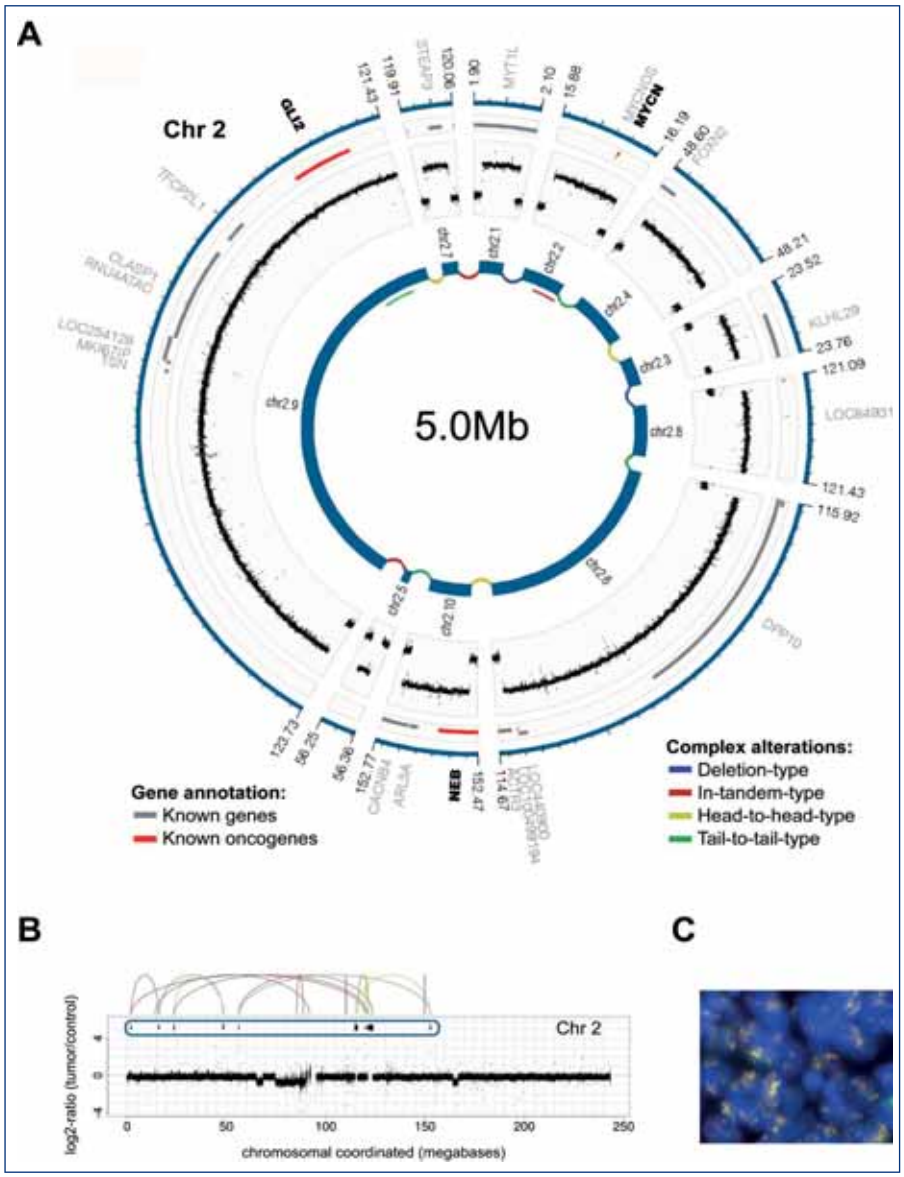


Figure 2: Medulloblastoma showing chromothripsis of the 'amplifier phenotype' including the formation of a circular mini-chromosome (A) which is getting amplified (B) under the selective advantage of carrying two known medulloblastoma oncogenes (MYCN and GLI2). Double-minute chromosomes were verified by FISH (C).

Chromothripsis in childhood brain tumors and its link to p53 and cancer predisposition

A recent study that we carried out in the context of the International Cancer Genome Consortium (ICGC) project on pediatric brain tumors (ICGC PedBrain Tumor; www.pedbraintumor.org) which was published in Cell in January 2012 yielded new insights in this regard [3]. Analyzing the genomes of childhood medulloblastoma brain tumor patients we found that chromothripsis is tightly linked with predisposing germline mutations in the *TP53* gene encoding the p53 protein, a condition known as Li-Fraumeni Syndrome. Li-Fraumeni Syndrome, which was initially described by Li and Fraumeni in 1969 [4], is associated with a very high lifetime risk of developing malignancies, in woman close to 100% and in men approx. 80% until the age of 50 years. It was previously known, that medulloblastoma, a highly aggressive embryonal WHO grade IV malignancy arising in the cerebellum, was within the spectrum of Li-Fraumeni associated tumors [5, 6]. However, the current study shed new light on this connection as detailed below.

We performed whole-genome sequencing of a Sonic Hedgehog-subtype medulloblastoma (SHH-MB) from a patient with a germline *TP53* mutation. Analysis of the genome revealed a striking pattern of massive, highly localized DNA rearrangements indicative of chromothripsis [3], with the dramatic chromosome alterations displaying a striking similarity to the chromothripsis 'amplifier phenotype'. This involved the formation of an oncogene-carrying double-minute chromosome, which appeared highly amplified in the medulloblastoma cells (Figure 2A, B). By using fluorescence *in-situ* hybridization (FISH), we verified the presence of such double minute chromosomes in basically all cells of medulloblastomas with chromothripsis (Figure 2C), which suggested that chromothripsis-associated double minute chromosome formation occurred as an early (possibly initiating) event during development of the tumor. Computational simulations we performed on the basis of the genomic rearrangements detected in the pateint were in strong support of a key assumption of the chromothripsis model, *i.e.* the formation of catastrophic chromosome rearrangements in a one-step dramatic event – leading us to reject the textbook-model model of progressive rearrangements as a possible mechanism of tumor formation [3].

Hypothesizing that the predisposing *TP53* mutation in the patient was linked with the massive chromosome rearrangements, we went on integrating *TP53* status with SNP array based copy-number alteration data in additional medulloblastomas from all four medulloblastoma subtypes [7]. We observed both rearrangements of the chromothripsis 'shattering phenotype' (Figure 3) as well as the 'amplifier phenotype' (Figure 2), with the latter appearing to be particularly frequent in SHH-MB compared to tumors studied by Campbell and Co-workers [1, 3]. Furthermore, our analyses revealed a strong statistical association between mutant *TP53* and chromothripsis: namely, 10/10 *TP53*-mutated

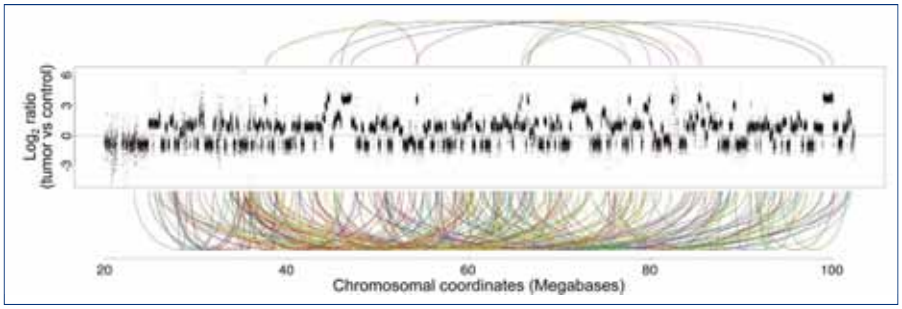


Figure 3: Another medulloblastoma tumor sample showing a striking example of the 'shattering phenotype' with more than 100 breakpoints in one chromosome.

SHH-MBs showed chromothripsis, whereas none (0/22) of the wild-type *TP53* SHH-MBs harboured rearrangements resulting from chromothripsis ($P=1.6x10^{-8}$, Fisher's exact test). We additionally found that 5/6 suspected sporadic SHH-MB patients with chromothripsis, for which constitutional DNA samples were available (enabling the inference of the gene's "germline" status), harboured *TP53* germline mutations. This was an important observation, since it implied that by detecting chromothripsis in a patient with SHH-MB, previously undiagnosed TP53 germline mutations – i.e. a cancer predisposition syndrome with a very high penetrance – may be encountered. In the sixth case, an acquired *TP53* mutation appeared to be a very early somatic event – *i.e.*, we were unable to detect residual levels of wild-type *TP53* in the tumor cells of the respective patient. Hence, our results causally implicate predisposing TP53 mutations with the catastrophic genome alterations observed in SHH-MB patients, indicating a role of p53 in predisposing cells to chromothripsis, or in facilitating cell survival in the context of massive DNA damage. Interestingly, chromothripsis was very rarely observed in the other medulloblastoma subtypes (i.e., Non-SHH MBs) indicating that activated SHH signaling might serve as a context-specific factor (Figure 4).

Further analyses of SNP array data showed a strong association between *TP53* mutations and chromothripsis also in another malignancy, *i.e.*, acute myeloid leukemia with complex karyotype, in this case involving somatically acquired *TP53* mutations. Moreover, in the presence of *TP53* germline mutations, malignancies other than medulloblastoma displayed a markedly increased frequency of chromothripsis (30-40%) compared to the 2-3% observed in Campbell and Co-workers [1]. We did, however, not observe a link between chromothripsis and somatically acquired *TP53* mutations in tumors of the WNT-medulloblastoma subtype, in which TP53 mutations were never seen as an early somatic event as extrapolated by allele frequency of the mutant TP53 allele. Collectively, these results revealed a context-specific role for p53 in the initiation of chromothripsis, or in the cell's reaction to the massive rearrangements [3].

How do these findings affect clinical decision making?

This close association of germline (or very early somatic) TP53 mutations and chromothripsis and its restriction to medulloblastomas of the SHH subgroup may be quite easily translated into a clinical setting. Each patient with a SHH-driven medulloblastoma, which harbors a somatic TP53 mutation (or a medulloblastoma patient whose tumor shows clear evidence of chromothripsis) could be tested for a TP53 germline mutation and, in case of a positive result, the entire family could be subsequently subjected to genetic counseling. But how would

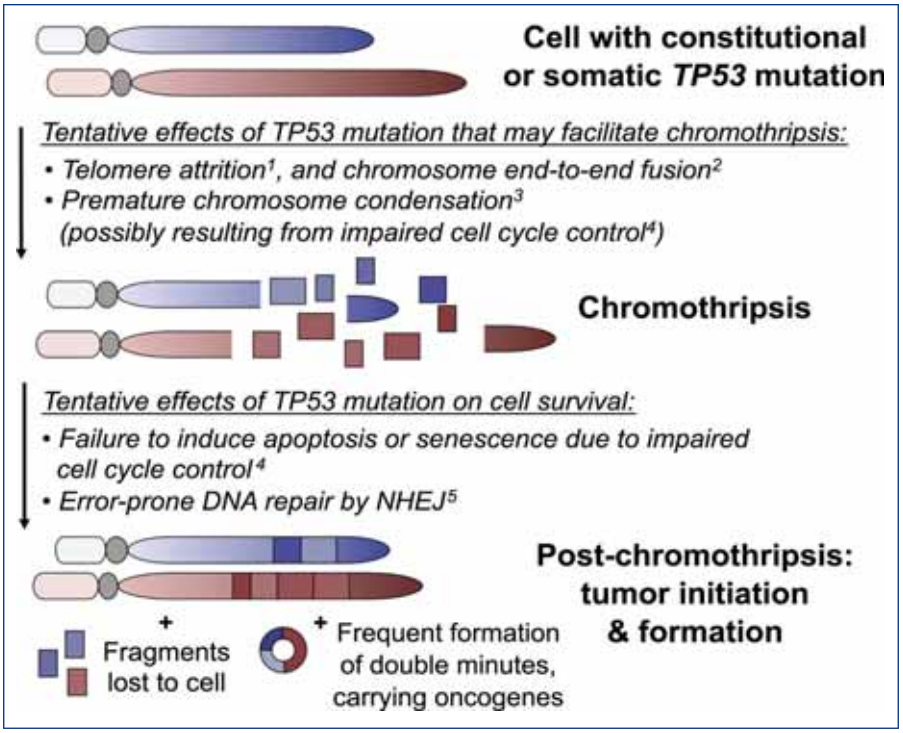


Figure 4: Model of the tumorigenesis of a SHH-subtype medulloblastoma in a patient with Li-Fraumeni Syndrome. In this work, we for the first time identified a genetic event preceding chromothripsis, and we identified active SHH signaling as a context-specific factor that obviously facilitates or contributes to chromothripsis in external granule cells, the cells of origin for SHH-driven medulloblastomas.

this help the patient? Would patients and their family members really want to know that they have a very high risk to develop a malignant tumor some time during their lifespan, most of them before 50 years of age? This certainly is a personal decision that we should ultimately leave up to the patient. However, we should be prepared to give a recommendation how to deal with this issue and a growing body of evidence suggests that for two reasons it is useful to have this information being a Li-Fraumeni patient: (i) It has been convincingly demonstrated by Villani et al. [8], that putting such patients on a surveillance program that includes regular whole-body MRI and blood tests will help to diagnose their tumors at relatively early stages and thus to dramatically increase the chances of cure. (ii) Furthermore, in patients with known Li-Fraumeni Syndrome, administration of high-dose radiotherapy or DNA-damaging chemotherapy has to be thoroughly weighed against the potential of these modalities of readily inducing severe side effects, and secondary malignancies, in these patients.

In summary, we have identified a genetic factor preceding chromothripsis in malignant cells, namely germline (or early somatic) TP53 mutations. Furthermore, we have established that context-specific factors (in our case SHH-dependence) seem to predispose certain types of precursor cells for chromothripsis (or serve as an additional selection factor for cells that underwent chromothripsis). As outlined above, these findings not only help dissecting the molecular mechanisms underlying this intriguing genetic phenomenon, but are also of immediate clinical relevance in terms of recommendations for genetic counseling, surveillance programs, and therapeutic decisions.

Acknowledgements

We thank Andrey Korshunov, Thomas Zichner, Tobias Rausch, and David TW Jones for assistance in preparing the Figures. S.P., P.L., and J.K. are supported by grants from the German Cancer Aid (109252) and the BMBF in the context of the ICGC PedBrain Tumor project (www.pedbraintumor.org).

Literature

[1.] Stephens PJ, Greenman CD, Fu B, Yang F, Bignell GR, Mudie LJ, Pleasance ED, Lau KW, Beare D, Stebbings LA, et al: **Massive Genomic Rearrangement Acquired in a Single Catastrophic Event during Cancer Development**. *Cell* 2011, **144**:27-40.

[2.] Fearon ER, Vogelstein B: **A genetic model for colorectal tumorigenesis**. *Cell* 1990, **61**:759-767.

[3.] Rausch T, Jones D, Zapatka M, Stütz A, Zichner T, Weischenfeldt J, Jäger N, Remke M, Shih D, Northcott P, et al: **Sequencing of the childhood brain tumor medulloblastoma links catastrophic DNA rearrangements with TP53 mutations**. *Cell* 2012, **January 19, 2012, epub ahead of print**.

[4.] Li FP, Fraumeni JF, Jr.: **Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome?** *Ann Intern Med* 1969, **71**:747-752.

[5.] Petitjean A, Mathe E, Kato S, Ishioka C, Tavtigian SV, Hainaut P, Olivier M: **Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database**. *Hum Mutat* 2007, **28**:622-629.

[6.] Malkin D, Li FP, Strong LC, Fraumeni JF, Jr., Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, et a: **Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms**. *Science* 1990, **250**:1233-1238.

[7.] Taylor M, Northcott P, Korshunov A, Remke M, Cho Y-J, Clifford S, Eberhart C, Parsons D, Rutkowski S, Gajjar A, et al: **Molecular subgroups of medulloblastoma: the current consensus**. *Acta Neuropathologica* 2011, **Dec 2. [Epub ahead of print]**.

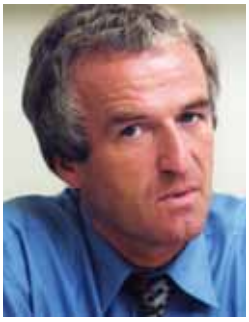
[8.] Villani A, Tabori U, Schiffman J, Shlien A, Beyene J, Druker H, Novokmet A, Finlay J, Malkin D: **Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: a prospective observational study**. *The Lancet Oncology* 2011, **12**:559-567.

Stefan M. Pfister, PD Dr. med.

Head, Division of Pediatric Neurooncology (B062)
German Cancer Research Center (DKFZ) Heidelberg
Im Neuenheimer Feld 280, D-69120 Heidelberg

Stefan Pfister was appointed head of the Division Pediatric Neurooncology at the German Cancer Research Center (DKFZ) in 2012. Being a pediatrician by training, Pfister received his MD from Tübingen University, and his clinical education at Mannheim and Heidelberg University Hospitals. As a physician-scientist, he completed postdoctoral fellowships with Christopher Rudd at the Dana-Faber Cancer Institute/Harvard Medical School, and with Peter Lichter at the German Cancer Research Center, Division of Molecular Genetics. Pfister´s research focuses on the genetic characterization of childhood brain tumors by applying next-generation profiling methods and subsequently translating novel findings into a clinical context. This might be achieved by establishing prognostic biomarkers, by identifying new drug targets and genetic cancer predispositions, or by providing models for preclinical drug testing. For his translational neurooncology projects, Pfister received several prestigious awards, including the Kind-Philipp Award for Pediatric Onclology 2009, and the Alfred-Müller Award for Neurooncology in 2011. Together with Peter Lichter he is currently conducting the whole-genome sequencing part of the PedBrainTumor project, the first German contribution to the International Cancer Genome Consortium (ICGC), in which whole genome sequencing is being performed on 600 tumor and 600 normal samples. Furthermore, Pfister is coordinating the BMBF-funded project "Molecular diagnostics in medulloblastoma", which aims to prospectively validate a number of highly promising molecular biomarkers in this aggressive childhood brain tumor for future clinical application.

Peter Lichter, Prof. Dr. rer. nat.



Head, Division of Molecular Genetics (B060)
German Cancer Research Center (DKFZ) Heidelberg
Im Neuenheimer Feld 280, D-69120 Heidelberg

Peter Lichter is heading the Division of Molecular Genetics at the German Cancer Research Center (DKFZ) since 1992. After receiving his PhD at Heidelberg University, Lichter joined the group of D.C. Ward at Yale University as a postdoctoral scientist. Currently, his main research interests include the identification and validation of prognostic and predictive biomarkers from genome-wide screening datasets in various cancer entities including CLL and brain tumors, the functional characterization of tumor-specific genetic events in vitro and in vivo, the establishment of novel molecular targets for therapeutic interference, and the development of molecular genetic methods. To this end, Lichter was pioneering the development of fluroescence in-situ hybridization (FISH) techniques and array-based comparative genomic hybridization (array-CGH), two methods nowadays routinely used in genetics laboratories around the globe. Furthermore, Lichter was inventor of novel models of a functional nuclear architecture. For his groundbreaking developments in the field of molecular genetics Lichter was awarded the German Cancer Award (Deutscher Krebspreis) in 2002, and the Award "Deutsche Krebshilfe" in 2004 (among others). Lichter is currently coordinating the large-scale sequencing project "PedBrainTumor", the first German contribution to the International Cancer Genome Consortium (ICGC), and has been coordinating numerous other national genomics projects including projects in the framework of the NGFN (national genome research network).

Jan Korbel, Dr. rer. nat.

Group Leader
European Molecular Biology Laboratory (EMBL) Heidelberg
Meyerhofstraße 1, 69117 Heidelberg

Jan Korbel leads a research group at EMBL Heidelberg since 2008. He was awarded his PhD from EMBL Heidelberg/Humboldt University, Berlin in 2005 and undertook postdoctoral research at Yale University, USA from 2005-2007. The research in Jan's group combines experimental and computational approaches for studying the extent, functional impact and mutational origins of genetic variation, with a focus on genomic structural variation in the germline and in cancer. Structural variants, including copy-number variants, inversions, and translocations, are responsible for most of the genetic variation in the human genome. Recent advances in massively parallel DNA sequencing enable dissecting the impact of this form of variation on disease mechanisms, including on tumorigenesis. The Korbel group has adopted a systems biology rationale in which computational biology research feeds into the experimental laboratory, and vice versa. Jan Korbel is involved in three cancer genome projects participating in the International Cancer Genome Consortium (ICGC). As a co-chair of the Structural Variation Analysis group of the 1000 Genomes Project he is further in charge of the analysis of structural variants identified in the germline of several thousands of individuals, sequenced by that international project.

13th **Young Scientist Meeting** of the German Society for Cell Biology (DGZ)

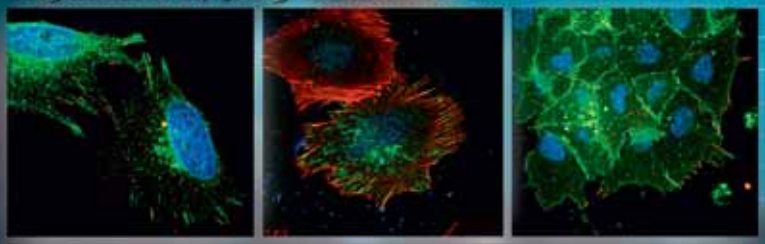
Cell Biology shapes the Embryo

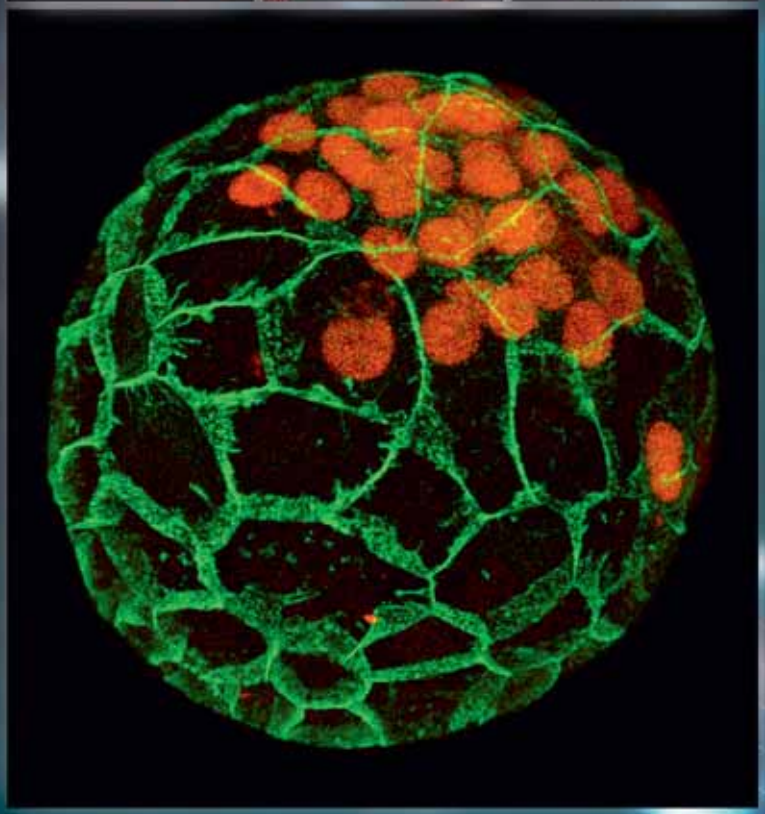
Jena, September 20-22, 2012

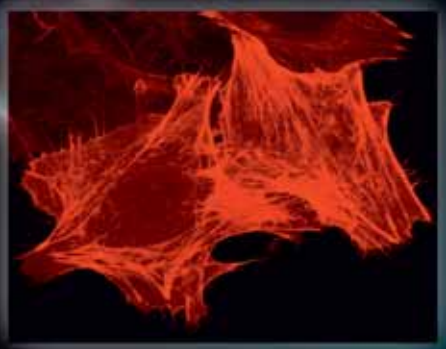
Speakers

Butz Baum
Yohanns Bellaiche
Antonio Jacinto
Ray Keller
Rolf Kemler
Michel Labouesse
Heiko Lickert
Jeremy Nance
James Nelson
Mark Peifer
Erez Raz
Pernille Rorth
Eyal Schejter
Giorgio Scita
Benedicte Sanson

*Organized by **Jörg Großhans** and **Doris Wedlich***







Registration 120€, Students 90€, **free for DGZ members**.
Registration **includes accommodation and meals**. The number of participants **is limited**.

Master- or PhD-students, postdocs and young group leaders are invited to apply for participation with an abstract. Participants wil present their work as a poster or a short talk.
For more information and registration visit **www.zellbiologie.de**
Please send your application and questions by email to the DGZ office at **dgz@dkfz.de**

APPLICATION DEADLINE
August 10th, 2012

DGZ

ZEISS

Poster by Thomas Stoy, ZEBio, KIT | Thomas.Stoy@kit.edu

From Genes to Functions - implications of DNA-repair dysfunction in the development and treatment of breast cancer

Andreea Julia Stahl and Lisa Wiesmüller

DNA double-strand break repair defects and susceptibility to breast cancer

Breast cancer is the most common malignancy affecting women worldwide. Besides sporadic forms, hereditary predisposition is responsible for its development in 5–10% of all breast cancer cases (Ralhan et al., 2007) (Figure 1). One of the biggest discoveries in the field of breast cancer research was the identification of the breast cancer 1 and 2 (*BRCA1* and *BRCA2*) genes already eighteen years ago (Miki et al., 1994; Wooster et al., 1995). Both BRCA genes play fundamental roles in DNA double-strand break repair (DSBR) (Narod and Foulkes, 1994; Ralhan et al., 2007). However, *BRCA1* and *BRCA2* germline mutations are responsible for only about 20% of familial breast cancer. Additional predisposing genes with high and moderate penetrance have been described to play a role in disease development. Thus, until today eleven breast cancer predisposing genes (*BRCA1*, *BRCA2*, *TP53*, *PTEN*, *RAD51C*, *ATM*, *CHEK2*, *RAD50*, *NBN*, *BRIP1*, *PALB2*) have been discovered that are known to play a role in DSBR (Walsh and King, 2007; Meindl et al., 2010). Breast cancer susceptibility genes, which are responsible for other cellular functions, like *CDH1* (encoding a calcium dependent glycoprotein, which functions in cell-to-cell adhesion; Keller et al., 1999) and *STK1/LKB1* (a serine/threonine kinase, which negatively regulates the mTOR pathway; Hearle et al., 2006) have more rarely been reported. All until now identified susceptibility genes together explain about 30% of familial cases meaning that 70% are still of unknown origin (Ralhan et al., 2007). Therefore, besides already defined mutations in high and moderate penetrance genes, cumulative effects of subtle aberrations in DSBR due to single nucleotide polymorphisms (SNPs) and epigenetic alterations are also thought to be responsible for breast carcinogenesis (Ralhan et al., 2007). Consistently, the individual risk for developing breast cancer in *BRCA1* and *BRCA2* mutation carriers varies between 40-87%. Recent studies have identified SNPs in modifier genes that modulate the risk in these individuals, supporting the hypothesis of cumulative effects (Wang et al., 2010).

Breast cancer risk assessment in developed countries relies on pedigree analysis and genetic testing of *BRCA1* and *BRCA2*. Recent progress in drug development revealed that poly (ADP-ribose) polymerase (PARP) inhibitors specifically induce cell death in *BRCA1* and *BRCA2* deficient cells impacting on potential use for targeted therapy (Bryant et al., 2005; Farmer et al., 2005). Since *BRCA1* and *BRCA2* and other susceptibility genes are tightly linked to DSBR, another possible approach for defining susceptibility might be cell-based functional testing to directly identify DSBR defects. This principle would

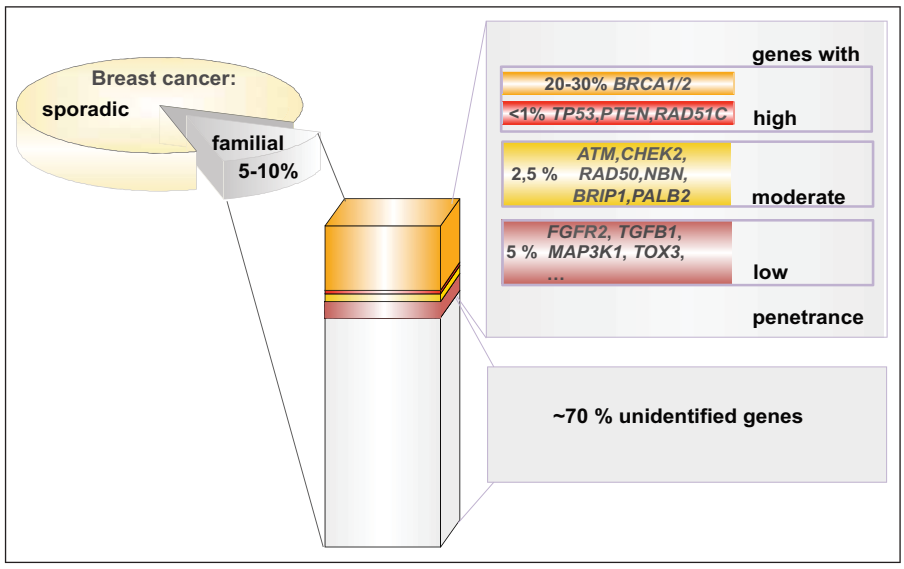


Figure 1: Schematic overview of susceptibility genes for familial breast cancer.
5-10% of breast cancer cases are of familial origin. So far eleven genes that play a role in DNA double strand break repair (DSBR) have been identified. They are divided into high and moderate penetrance groups, and modifier genes with low penetrance. However, around 70% of the hereditary breast cancer cases cannot be explained by defined gene alterations.

be applicable on risk assessment for persons with or without familial background and as predictor for targeted therapies. In this article, we summarize our lab´s recent advances in establishing a functional test for using DSBR defects as a marker for determining both breast cancer predisposition and targeted therapies.

DSBR pathways and the role of breast cancer susceptibility gene products

A diversity of exogenous genotoxic hazards like ionizing radiation (IR), cancer chemotherapeutic agents and endogenous normal cellular processes lead to DNA double strand breaks (DSB). DSBs are among the most severe types of DNA damage, causing cell death if left unrepaired. On the other hand, DNA damage needs to be repaired correctly in order to avoid gene mutations, chromosomal translocations, aneuploidies, and ultimately malignant transformation. Therefore the cells are capable of managing several different injuries to DNA using diverse repair pathways. For DSBR two major evolutionarily conserved repair pathways namely homologous recombination (HR) and non-homologous end joining (NHEJ) exist. These DSBR pathways enable distinct reactions and therefore differ in the fidelity of the repair process (Valerie and Povirk, 2003).

DNA damage signaling activates recruitment of specialized complexes for repair to the affected site and inhibits cell cycle progression until the damage is removed. Disastrous damage, which is beyond repair, leads to p53-induced cell death. Ataxia-telangiectasia mutated (ATM), a serine protein kinase and master regulator of cellular DSB responses, orchestrates repair initiation and cell cycle progression by interaction and modification of several proteins (Shiloh 2003). Additional important regulators of the cell cycle are p53 and BRCA1. BRCA1 is recruited to DSBs where it interacts with a number of proteins. BRCA1 facilitates p53 phosphorylation by ATM, which in turn leads to G1/S arrest after IR-induced damage through p53-mediated p21 transcription (Fabbro et al., 2004). Furthermore BRCA1 is also implicated in S and G2/M arrest after DNA damage by synergizing with ATM, Chk1, and Chk2 in the signaling cascade (Xu et al., 2001; Yarden et al., 2002). Another important function of BRCA1 at DNA damage sites is its interaction with PALB2 and BRCA2 leading to stabilization of these proteins (Sy et al., 2009; Zhang et al., 2009). Additionally BRCA1 interacts with BACH1, a DNA dependent ATPase and 5'-3' DNA helicase (encoded by *BRIP1*), that supports error-free HR (Cantor et al., 2001). HR occurring during S/G2-phase is the most precise pathway, since a particular DNA damage is repaired using its homologous sequence. The initial protein complex engaged in HR is the MRE11–RAD50–Nibrin (MRN) complex. Moreover, upon DNA damage, ATM phosphorylates histone H2AX on Ser139, which is then named γ–H2AX. γ–H2AX accumulates in the nucleus in characteristic focal structures (foci) and is one of the most important damage markers (Paull et al., 2000). In the absence of DNA breaks H2AX is constitutively phosphorylated at Tyr142. After dephosphorylation at Tyr142 γ–H2AX is recognized by its sensor, mediator of DNA damage checkpoint protein 1 (MDC1) (Cook et al., 2009). Nibrin (formerly called NBS1) binds MDC1 and ATM, which ensures that ATM stays at the damage site spreading H2AX phosphorylation to neighbouring nucleosomes (Horejsi et al., 2004; Lee et al., 2005). Nibrin is a component of the heterotrimeric MRN complex, which is a crucial ele-

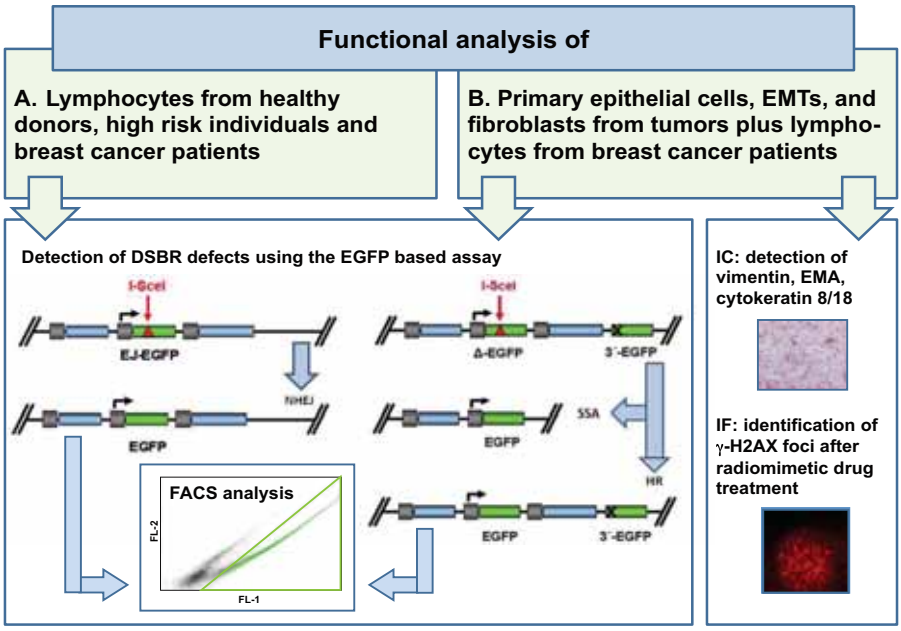


Figure 2: Detection of specific DSBR defects in primary cells.
(A) Peripheral blood lymphocytes from different cohorts (cases: high risk individuals, breast cancer patients; controls: healthy individuals) were comparatively analysed using our EGFP-based DSBR assay system. **(B)** Various types of primary cells derived from breast cancer patients (epithelial cells, EMTs, fibroblasts originating from tumor specimens and lymphocytes from blood samples) are being classified by imunocytochemical (IC) detection of marker proteins (vimentin, EMA, cytokeratin 8/18). Classified cell populations are subjected to the EGPF-based DSBR assay and immunofluorescence microscopy (IF) for γ–H2AX foci quantification. The schematic drawing outlines representative DNA substrates for measurements of specific DSBR activities, here NHEJ and homology-directed DSBR (SSA plus HR). These substrates comprise an I-*Sce*I recognition sequence within a mutated EGFP gene, enabling targeted introduction of a DSB. After NHEJ, SSA, and HR, respectively, the wild-type *EGFP* gene is reconstituted, and the fraction of EGFP-positive cells quantified flow cytometrically by FACS analysis.

ment for DNA end-processing and alignment (Carney et al., 1998; Paull and Gellert, 1998; Williams et al., 2007). The resulting 3`single-stranded DNA (ssDNA) is covered with Replication Protein A (RPA). The assembly of the recombinase RAD51 on ssDNA is mediated by BRCA2, which is able to interact with both RAD51 and ssDNA (Moynahan et al., 2001; Davies and Pellegrini, 2007; Thorslund et al., 2010). RAD51 catalyses central processes in HR, namely homology search, DNA strand invasion, homologous pairing, and DNA strand exchange (Sung, 1994; Baumann et al., 1996). PTEN, which is well-known as inhibitory component of the phosphatidyl inositol 3-kinase (PI3K)/Akt pathway, has been connected with HR through regulation of *RAD51* transcription (Shen et al., 2007).

During NHEJ DSBs are repaired either by direct ligation of the two broken DNA ends or processing of the ends by nucleases and polymerases and subsequent religation. In the canonical NHEJ pathway, the ends are bound by Ku70 and Ku80. Afterwards the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is recruited to DSB ends. The complex formed by Ku70, Ku80, and DNA-PKcs, named DNA-PK, has been described to protect the ends from nucleases as well as to facilitate, if necessary, the end processing reactions. Finally, religation is catalysed by the XRCC4/DNA-Ligase IV complex. Since canonical NHEJ often involves end processing, it can lead to mutations and is therefore a potentially error-prone DSBR pathway (Valerie and Povirk, 2003; Lieber, 2008). Alternative NHEJ reactions have been described *in vitro* and *in vivo*, which are always error-prone, but the molecular make-up of these reactions has not yet been fully resolved (Nussenzweig and Nussenzweig, 2007).

Another error prone pathway is single strand annealing (SSA). Like HR SSA relies on homologies. Different from RAD51-dependent HR, SSA anneals ssDNA stretches RAD52-dependently, whereby pairing results in unpaired, non-homologous sequences that are removed before ligation (Valerie and Povirk, 2003). Hence SSA is always associated with the deletion of DNA sequences. SSA plays a major role in repairing spontaneous and induced DSBs between repeated sequences.

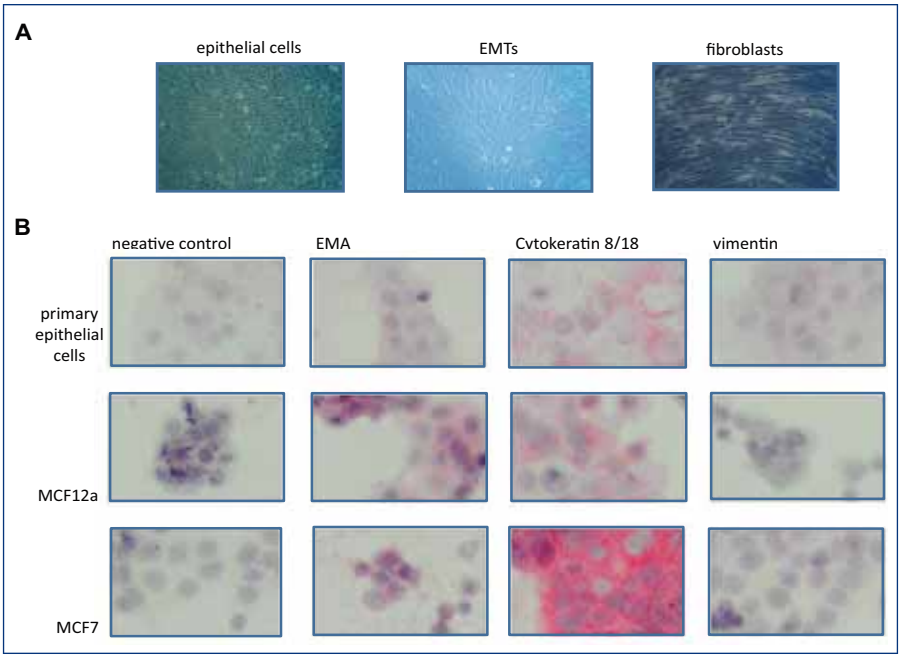


Figure 3: Characterization of primary cell cultures derived from breast cancer specimens. For classification of primary cells we scrutinized the cellular morphology via bright field microscopy **(A)** and performed immunocytochemistry (IC) using antibodies directed against EMA, cytokeratin 8 plus 18, vimentin, and no primary antibody (negative control), respectively (B). Red signals indicate immunostaining, blue signals mark nuclei.

Development of a sensitive cell-based method for detection of breast cancer predisposing DSBR defects

In our laboratory a sensitive test for the detection of specific DNA repair defects has been developed more than ten years ago (Akyüz et al., 2002). The assay system requires at least two plasmids, which are serially or concomitantly transferred into the cells of interest either by viral infection or DNA transfection. One plasmid carries the information for expression of the rare-cutting I-*Sce*I restriction enzyme targeting an 18bp recognition site. The second plasmid includes two homologous sequences, a so-called donor and recipient version of the *EGFP* gene. Both sequences have been mutated such that only after successful DNA recombination green fluorescing EGFP protein is expressed. The recipient carries the I-*Sce*I recognition sequence in place of the chromophore encoding region and is driven by a CMV promoter. The donor lacks a promoter. Specific I-*Sce*I-mediated cleavage within the acceptor sequence creates an artificial DSB. Using the donor sequence as a template the break is repaired by the particular mechanisms of the cell, thus, leading to EGFP reactivation. Different *EGFP* sequences have been generated which allow the specific detection of HR between long and short homologies, SSA, NHEJ, and combinations thereof. In DSBR experiments, transfection efficiencies are determined through co-transfection of wild-type EGFP expression plasmid in split samples, enabling DSBR data normalization. Taking advantage of this technique after establishment in immortalized cell lines, a pilot study called BRENDA (Die Qualität der

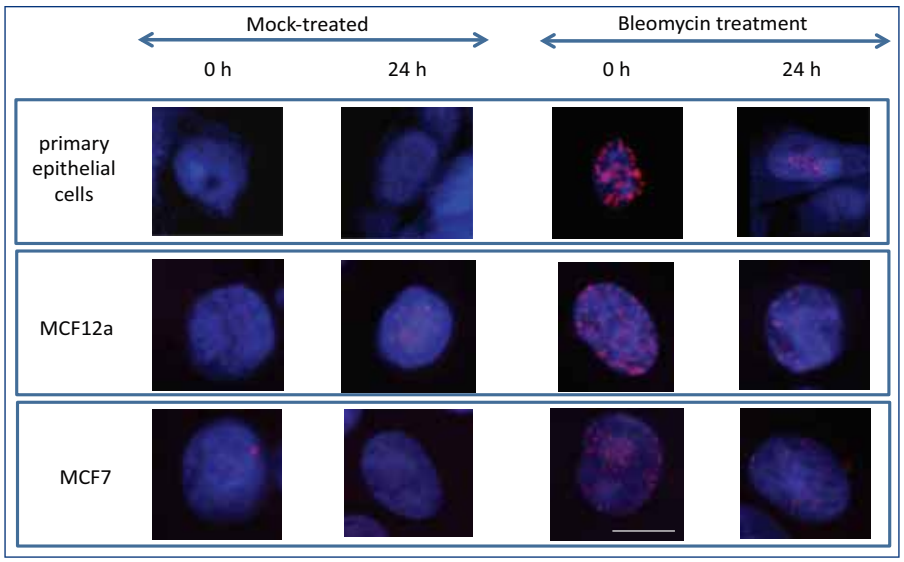


Figure 4. γ–H2AX foci analysis of primary and immortalized mammary epithelial cells.
The cells were incubated either with the radiomimetic drug bleomycin (20 mU) for 24 h or mock-treated in controls. The cells were fixed with 3.7 % formaldehyde immediately after treatment (0 h) or after recultivation for 24 h in fresh medium. Subsequently the cells were stained with an antibody directed against γ–H2AX and a secondary Alexa Fluor 555-conjugated antibody. Representative immunofluorescence images are shown (bar: 20 µM). Fluorescence micrographs were collected on an Olympus BX51 epifluorescence microscope equipped with UPLAN FI objective lenses fitted with a thermoelectronically cooled, charge-coupled device camera. AnalySIS software including the mFIP module (Soft Imaging System) was used for image acquisition, overlays, and analysis. For the automated identification and visualization of nuclear foci with high fluorescence intensity versus diffuse staining, a colour intensity treshold is set and maintained for all images.

Brustkrebsversorgung unter evidence-basierten Leitlinien) was started in order to investigate potential differences in DNA damage repair between primary lymphocytes from healthy donors (245), individuals from high breast cancer risk families (35) and breast cancer patients (175) (Figure 2). Preceding experiments with lymphoblastoid cell lines had shown that there are indeed specific patterns of DSBR changes depending on the defective gene (Keimling et al., 2011). The pilot case-control study followed the rationale that by identification of these particular repair patterns, it might be possible to detect defects in the BRCA1 and BRCA2 pathways without knowing the affected gene, mutation, or polymorphism. Primary lymphocytes from blood samples were isolated by Ficoll-gradient and stimulated for three days with phytohemagglutinin. Subsequently, the cells were transfected according to amaxa protocols using pathway-specific DSBR substrates and I-*Sce*I expression plasmid followed by flow cytometric quantification of EGFP-positive cells after 24 h. Our results from the analysis of lymphocytes in the case-control format showed that error-prone DSBR activities (NHEJ, SSA) were increased in women with familial risk and in breast cancer patients particularly of younger age (<50), whereas non-hereditary factors had no influence (Keimling et al., 2012). The predictive power of pathway-specific DSBR frequencies (odds ratios, AUC values) was strikingly similar for allocation to the groups of high risk individuals and young breast cancer patients, which is compatible with early-onset disease being one of the hallmarks of hereditary breast cancer. These data suggest that the detection of error-prone DSBR activities in cells derived from blood samples may serve as a powerful tool for breast cancer risk assessment.

DSBR analysis in primary cells from breast cancer specimens

With the advent of drugs like PARP inhibitors targeting HR dysfunctional tumors (Bryant et al., 2005; Farmer et al., 2005), markers are needed to select those patients who will benefit from treatment. The mutational status of *BRCA1* or *BRCA2* was found to predict therapeutic responsiveness to PARP inhibitor treatment (Fong et al., 2009). Our functional approach utilizing lymphocytes is limited by the fact that reversion mutations and deregulated expression of DSBR proteins in the tumor itself may modulate the sensitivity to PARP inhibitor treatment. Therefore, we also established protocols for isolation of different primary cells, from breast tumors, namely epithelial cells, EMTs (epithelial mesenchymal transitions cells), and fibroblasts for use in DSBR analysis by our EGFP-based assay system. The aim of this ongoing project is to evaluate DSBR testing of tumor- versus blood-derived cells as a marker for therapeutic responsiveness to conventional genotoxic and novel targeted therapies.

Preceding experiments engaging primary cells from five breast cancer specimens revealed that pathway-specific DSBR testing in these cell types can identify *BRCA1/2*-like DSBR defectiveness (Keimling et al., 2008). Interestingly, this *BRCA1/2*-like DSBR pattern, characterized by elevated levels of the error-prone activities NHEJ and SSA, was particularly obvious with primary cell cultures from a patient with familial breast cancer tumor. Because *BRCA1* and *BRCA2* gene sequences were wild-type in this patient according to genomic DNA sequence analysis, this observation suggested that this approach will pick up DSBR aberrations in the BRCA1- and BRCA2-pathways independently of a mutation in the corresponding genes. These promising results encouraged the investigation of tumor derived primary cells on a larger scale.

In order to detect DSBR defects by our method in primary tumor-derived epithelial

cells, EMTs, and fibroblasts, a multistep isolation protocol has been developed in our lab. Tumor samples are cut into small pieces and incubated in special growth media for epithelial cells and fibroblasts. Outgrowing cells are first classified in epithelial and non-epithelial cells according to their morphology (Figure 3A). Since epithelial cells are attached more strongly to the culture flask than EMTs and fibroblasts, differential typsinization is used next for purification of epithelial cell cultures. Finally, positive immuno-selection separates EMTs from fibroblasts. Epithelial-mesenchymal transition (EMT), the conversion of epithelial cells to a mesenchymal phenotype, is important in several processes during embryonic development e.g. for migration of neural crest cells out of the neuroectoderm. This transformation includes several steps like loss of epithelial cell polarity and cell to cell junctions, downregulation of epithelial-specific and expression of mesenchymal-specific genes. EMTs are migratory cells and therefore play a role in cancer progression and metastasis (Kang and Massague, 2004).

At the time point of functional analysis the cell cultures are characterized by specific cell surface markers using immunocytochemical (IC) staining. Vimentin expression serves as marker to identify EMTs and fibroblasts, Human Epithelial Membrane Antigen (EMA) and Cytokeratin 8 plus 18 serve for epithelial cells and EMTs (Figure 3B) (Vincent-Salomon and Thiery, 2003; Kang and Massague, 2004; Rodriguez-Pinilla et al., 2007). Cultures of early passage, i.e. passage two to three, are applied to DSBR experiments using our EGFP-based assay system. As internal references breast epithelial cell lines (MCF12a, MCF7, and/or MDA-MB-157) are analysed in parallel on each experimental day. We intend to compare the results from EGFP-based DSBR testing with quantification of an established DSB marker, which enables monitoring DSB removal and thus overall DSBR capacity (independently of the pathway and quality). Therefore, we analyse nuclear γ–H2AX foci by immunofluorescence microscopy (IF) in split samples of the cultures subjected to DSBR measurements. For γ–H2AX foci analysis, the cultured primary cells were treated with the radiomimetic drug bleomycin and subsequently immunostained specifically for γ–H2AX (Figure 4). Preliminary evaluation of the data from >100 tumors suggests that indeed there are significant differences between the DNA repair capacity and fidelity of the primary cells derived from these tumors, and it will be interesting to see whether the EGFP- and γ–H2AX-based marker systems are coupled. The final evaluation will include subgroup analysis of the DSBR data regarding clinical characteristics of tumor specimens and patients such as age, grading, hormone receptor status, or response to treatment.

In summary, we provide evidence suggesting that cell-based

analysis of specific DSBR mechanisms in primary human cells might be a promising and powerful tool for determination of breast cancer predisposition. In view of the fact that functional analysis can identify the consequences of combinations of known and unknown mutations, SNPs, and epigenetic aberrations, this approach will be particularly interesting also for the development of individually tailored therapeutic regimens targeting DSBR-defective tumors.

Literature

Akyüz N, Boehden GS, Süsse S, Rimek A, Preuss U, Scheidtmann KH, Wiesmüller L. DNA substrate dependence of the p53-mediated regulation of double-strand break repair. *Mol Cell Biol* (2002) **22**, 6306–6317

Baumann P, Benson FE, West SC. Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. *Cell* (1996) **87**, 757–766

Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* (2005) **434**, 913–917

Cantor SB, Bell DW, Ganesan S, Kass EM, Drapkin R, Grossman S, Wahrer DC, Sgroi DC, Lane WS, Haber DA, Livingston DM. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell* (2001) **105**, 149–160

Carney JP, Maser RS, Olivares H, Davis EM, Le Beau M, Yates JR 3rd, Hays L, Morgan WF, Petrini JH. The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell* (1998) **93**, 477–486

Chapman JR, Jackson SP. Phospho-dependent interactions between NBS1 and MDC1 mediate chromatin retention of the MRN complex at sites of DNA damage *EMBO reports* (2008) **9**, 795-801

Cook PJ, Ju BG, Telese F, Wang X, Glass CK, Rosenfeld MG. Tyrosine dephosphorylation of H2AX modulates apoptosis and survival decisions. *Nature* (2009) **458**, 591-596

Davies OR, Pellegrini L. Interaction with the BRCA2 C terminus protects RAD51-DNA filaments from disassembly by BRC repeats. *Nat Struct Mol Biol* (2007) **14**, 475-383

Fabbro M, Savage K, Hobson K, Deans AJ, Powell SN, McArthur GA, Khanna KK. BRCA1-BARD1 complexes are required for p53Ser-15 phosphorylation and a G1/S arrest following ionizing radiation-induced DNA damage. *J Biol Chem* (2004) **279**, 31251–31258

Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, Martin NM, Jackson SP, Smith GC, Ashworth A. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* (2005) **434**, 917–921

Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, Ashworth A, Carmichael J, Kaye SB, Schellens JH, De Bono JS Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med* (2009) **361**, 123-134

Hearle N, Schumacher V, Menko FH, Olschwang S, Boardman LA, Gille JJ, Keller JJ, Westerman AM, Scott RJ, Lim W, Trimbath JD, Giardiello FM, Gruber SB, Offerhaus GJ, De Rooij FW, Wilson JH, Hansmann A, Moslein G, Royer-Pokora B, Vogel T, Phillips RK, Spigelman AD, Houlston RS. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. *Clin Cancer Res* (2006) **12**, 3209–3215

Horejsi Z, Falck J, Bakkenist CJ, Kastan MB, Lukas J, Bartek J. Distinct functional domains of Nbs1 modulate the timing and magnitude of ATM activation after low doses of ionizing radiation. *Oncogene* (2004) **23**, 3122–3127

Kang Y, Massague J. Epithelial-mesenchymal transitions: twist in development and metastasis. *Cell* (2004) **118**, 277-279

Keimling M, Kaur J, Bagadi SA, Kreienberg R, Wiesmüller L, Ralhan R. A sensitive test for the detection of specific DSB repair defects in primary cells from breast cancer specimens. (2008) *Int J Cancer* **123**, 730-736

Keimling M, Volcic M, Csernok A, Wieland B, Dörk T, Wiesmüller L. Functional characterization connects individual patient mutations in ataxia telangiectasia mutated (ATM) with dysfunction of specific DNA double-strand break-repair signaling pathways. *FASEB J* (2011) 3849-3860

Keimling M, Deniz M, Varga D, Stahl A, Schrezenmeier H, Kreienberg R, Hoffmann I, König J, Wiesmüller L. The Power of DNA Double-Strand Break (DSB) Repair Testing to Predict Breast Cancer Susceptibility. *FASEB J* (2012) in press

Keller G, Vogelsang H, Becker I, Hutter J, Ott K, Candidus S, Grundei T, Becker KF, Mueller J, Siewert JR, Hofler H. Diffuse type gastric and lobular breast carcinoma in a familial gastric cancer patient with an E-cadherin germline mutation. *Am J Pathol* (1999) **155**, 337–342

Lee JH, Paull TT. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. *Science* (2005) **308**, 551–554.

Lieber MR. The mechanism of human nonhomologous DNA end joining. *J Biol Chem* (2008) **283**, 1–5

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* (1994) **266**, 66–71

Moynahan ME, Pierce AJ, Jasin M. BRCA2 is required for homology directed repair of chromosomal breaks. *Mol Cell* (2001) **7**, 263-272

Narod SA, William D. Foulkes. BRCA1 and BRCA2: 1994 and beyond. *Nat Rev Cancer* (2004) **4**, 665-676

Nussenzweig A, Nussenzweig MC. A backup DNA repair pathway moves to the forefront. *Cell* (2007) **131**, 223-225

Paull TT, Gellert M. The 3 to 5 exonuclease activity of Mre11 facilitates repair of DNA double-strand breaks. *Mol Cell* (1998) **1**, 969–979.

Paull TT, Rogakou EP, Yamazaki V, Kirchgessner CU, Gellert M, Bonner WM. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr Biol* (2000) **10**, 886–895

Ralhan R, Kaur J, Kreienberg R, Wiesmüller L. Links between DNA double strand break repair and breast cancer: Accumulating evidence from both familia and nonfamilial cases *Cancer Lett* (2007) **248**, 1–17

Rodriguez-Pinilla SM, Sarrio D, Honrado E, Moreno-Bueno G, Hardisson D, Calero F, Benitez J, Palacios J. Vimentin and laminin expression is associated with basal-like phenotype in both sporadic and BRCA1-associated breast carcinomas. *J Clin Pathol* (2007) **60**, 1006-1012

Shen WH, Balajee AS, Wang J, Wu H, Eng C, Pandolfi PP, Yin Y. Essential role for nuclear PTEN in maintaining chromosomal integrity. *Cell* (2007) **128**, 157-170

Shiloh, Y. ATM and related protein kinases: safeguarding genome integrity. *Nat Rev Cancer* (2003) **3**, 155–168

Sung P. Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. *Science* (1994) **265**, 1241–1243

Sy SM, Huen MS, Chen J. PALB2 is an integral component of the BRCA complex required for homologous recombination repair. *Proc Natl Acad Sci USA* (2009) **106**, 7155–7160

Thorslund T, McIlwraith MJ, Compton SA, Lekomtsev S, Petronczki M, Griffith JD, West SC. The breast cancer tumor suppressor BRCA2 promotes the specific targeting of RAD51 to single-stranded DNA. *Nat Struct Mol Biol* (2010) **17**, 1263-1265

Valerie K, Povirk LF. Regulation and mechanisms of mammalian double-strand break repair. *Oncogene* (2003) **22**, 5792-5812

Van Gent DC, Hoeijmakers JH, Kanaar R. Chromosomal stability and the DNA double-stranded break connection. *Nat Rev Genet* (2001) **3**, 196-206

Vincent-Salomon A, Thiery JP. Host microenvironment in breast cancer development: epithelial-mesenchymal transition in breast cancer development. *Breast Cancer Res* (2003) **5**, 101-106

Walsh T, King MC. Ten genes for inherited breast cancer. *Cancer Cell* (2007) **11**, 103-5

Wang X, Pankratz VS, Fredericksen Z, Tarrell R, Karaus M, McGuffog L, Pharaoh PD, Ponder BA, Dunning AM, Peock S, Cook M, Oliver C, Frost D; EMBRACE, Sinilnikova OM, Stoppa-Lyonnet D, Mazoyer S, Houdayer C; GEMO, Hogervorst FB, Hooning MJ, Ligtenberg MJ; HEBON, Spurdle A, Chenevix-Trench G; kConFab, Schmutzler RK, Wappenschmidt B, Engel C, Meindl A, Domchek SM, Nathanson KL, Rebbeck TR, Singer CF, Gschwantler-Kaulich D, Dressler C, Fink A, Szabo CI, Zikan M, Foretova L, Claes K, Thomas G, Hoover RN, Hunter DJ, Chanock SJ, Easton DF, Antoniou AC, Couch FJ. Common variants associated with breast cancer in genome-wide association studies are modifiers of breast cancer risk in BRCA1 and BRCA2 mutation carriers. *Hum Mol Genet* (2010) **19**, 2886-2897

Williams RS, Williams JS, Tainer JA. Mre11-Rad50-Nbs1 is a keystone complex connecting DNA repair machinery, double-strand break signaling, and the chromatin template. *Biochem Cell Biol* (2007) **85**, 509–520

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G. Identification of the breast cancer susceptibility gene BRCA2. *Nature* (1995) **378**, 789-792

Xu B, Kim S, Kastan MB. Involvement of Brca1 in S-phase and G(2)-phase checkpoints after ionizing irradiation. *Mol Cell Biol* (2001) **21**, 3445–3450

Yarden RI, Pardo-Reoyo S, Sgagias M, Cowan KH, Brody LC. BRCA1 regulates the G2/M checkpoint by activating Chk1 kinase upon DNA damage. *Nat Genet* (2002) **30**, 285–289

Zhang F, Fan Q, Ren K, Andreassen PR. PALB2 functionally connects the breast cancer susceptibility proteins BRCA1 and BRCA2. *Mol Cancer Res* (2009) **7**, 1110–1118

Acknowledgements

This work was supported by the Ministry for Education and Research (Bundesministerium für Bildung und Forschung, BMBF), Germany ("BRENDA", #012P0505), and the German Cancer Foundation (Deutsche Krebshilfe, #107744). We cordially thank Miriam Deniz and Dominic Varga, Ulm, for support during patient recruitment, Marina Wager and Julia Kaufmann, Ulm, for expert technical assistance, and Jochem König and Isabell Hoffmann, IMBEI Mainz, for statistical evaluation of the patient data.

Elisabeth (Lisa) Maria Wiesmüller, Prof. Dr. rer. nat.

Gynaecological Oncology, Head
Dept. of Obstetrics and Gynaecology of the University of Ulm
Prittwitzstrasse 43, D-89075 Ulm

Lisa Wiesmüller, Head of the Gynaecological Oncology Division, Ulm University, since 2001, finished her doctorate at the Max Planck Institute of Biochemistry, Munich, in 1990, followed by fellowships at the Institut Pasteur, Paris, and the Max Planck Institute of Medical Research in Heidelberg. In the 1990s her group started working on the role of p53 in DNA double-strand break repair at the Heinrich Pette Institute in Hamburg. During the course of her mechanistic studies on p53 and meanwhile also on many other DNA repair factors, she developed assays with promising applications. Thus, one of the procedures was awarded the first Ursula M. Händel Prize in 2004 for its potential in carcinogenicity testing. Most interestingly, she established new functional marker systems to detect increased breast cancer risk and to predict therapeutic responsiveness of breast cancer patients, for which she received the Innovation Award 2009 in Germany.

Dr. Andreea Julia Stahl (Csernok)

Department of Obstetrics and Gynaecology of the University of Ulm, Gynaecological Oncology
Prittwitzstrasse 43, D-89075 Ulm

Andreea J. Stahl (Csernok) studied biology at the Christian-Albrechts-University Kiel (prediploma) and Ruprecht-Karls-University Heidelberg (diploma), where she also received her PhD in 2008. During her PhD thesis at the German Cancer Research Center (DKFZ) Heidelberg, Division of Viral Transformation, she was analysing epigenetic mechanisms regulating the activation of silenced HPV-16 genomes. Afterwards she was working in the University of Ulm, Institute of Immunology. 2008 she joined the lab of Prof. L. Wiesmüller, University of Ulm, Gynaecological Oncology as a postdoc in breast cancer and DNA repair research.

DFG Deutsche Forschungsgemeinschaft

GRK1459
Sorting and Interactions between Proteins of Subcellular Compartments

Research Training Group 1459

The interdisciplinary Research Training Group (GRK) 1459 "Sorting and Interactions between Proteins of Subcellular Compartments"(www.grk1459.de) is constituted by scientists from the University Medical Center Hamburg-Eppendorf, the Institute of Biochemistry at the University of Kiel and the Bernhard-Nocht-Institute for Tropical Medicine in Hamburg. Ten PhD students and six MD students are funded by the Deutsche Forschungsgemeinschaft (DFG) since 2008. The general topic of the Research Training Group is the sorting and transport of selected proteins along the biosynthetic and endocytotic pathway and their interactions with cytosolic or luminal proteins. Missorted proteins may lead to loss of function in their target organelle, that may affect the well being of the cell and the organism as a whole. By focussing on disease-related proteins we are investigating basic mechanisms of the biogenesis of subcellular compartments as well as the balance of membrane transport between organelles. The majority of projects adresses sorting and transport processes under pathological conditions in cells derived from patients or mouse models of human diseases or cells infected by bacteria or in parasite cells.

The PhD and MD students go through an interdisciplinary curriculum of academic as well as non-academic courses which include i) monthly seminars with leading international guest scientists (among others J. Rothman, F. Wieland, J. Bonifacino, K. Simons, A. Helenius, T. Rapoport, R. Jahn, G. Warren, S. Pfeffer and S. Grinstein), ii) project-specific practical courses, iii) lectures on "Molecular Cell Biology of Subcellular Compartments", iv) annual retreats with external reviewers, v) three month scientific cooperative stay abroad and iv) the organization of an international symposium.

Thus, in 2010, the graduates and PIs of the GRK 1459 prepared the 1st International Symposium on "Protein Trafficking in Health and Disease" and the international Meeting on "Lysosomes" in 2011 in Hamburg. In this year we are now organizing the 2nd International Symposium on Protein Trafficking (www.trafficking-symposium2012.de). The meeting will take place 26th – 28th September 2012.

The graduates of the GRK 1459 invite PhD students and young scientists from all over Europe to come to Hamburg, meet the experts and get the hottest findings in this topic.

Annual Retreat of GRK1459, Sylt, October 2011

DFG - Research Training Group 1459

GRK 1459

2nd International Symposium

Protein Trafficking In Health And Disease

September 26th-28th, 2012
Hamburg, Germany

Keynote Speaker

Reinhard Jahn
(Max-Planck-Institute, Göttingen, Germany)

Confirmed speakers

William Balch
(Scripps Research Institute, La Jolla, USA)

Francis Barr
(University of Liverpool, UK)

Peter J. Cullen
(University of Bristol, UK)

Volker Haucke
(Freie Universität Berlin, Germany)

Ludger Johannes
(Institut Curie, Paris, France)

Tomas Kirchhausen
(Harvard Medical School, Boston, USA)

Harvey McMahon
(University of Cambridge, UK)

Mark Marsh
(University College London, UK)

Susan Ferro-Novick
(University of California, San Diego, USA)

Suzanne Pfeffer
(Stanford University, USA)

Mikael Pittet
(Harvard Medical School, Boston, USA)

Christian Ungermann
(University of Osnabrück, Germany)

Mark von Zastrow
(University of California, San Francisco, USA)

Topics

Golgi, Endosomes, Lysosomes, Membrane Fusion, Endocytosis, Vesicular Transport, Infections

www.trafficking-symposium2012.de

Register now!
A number of abstracts will be selected for oral presentations!

Abstract submission deadline:
May 15th, 2012
We are limited to 150 participants
1st come, 1st served!

Universität Hamburg

Universitätsklinikum Hamburg-Eppendorf

CAU Christian-Albrechts-Universität Kiel

Bernhard Nocht Institute for Tropical Medicine

ANNOUNCEMENT

International Meeting of the German Society for Cell Biology
Leipzig November 7-10, 2012

Molecular concepts in epithelial differentiation, pathogenesis and repair

Local organizers: Thomas Magin, Leipzig and Mechthild Hatzfeld, Halle

Wednesday, 7.11., 12:00h, welcome and lunch
14-16:30h – Epithelial morphogenesis and regeneration
E. Fuchs (New York), P. Martin (Bristol), C. Brakebusch (Kopenhagen), S. Werner (Zürich), Y. Barrandon (Lausanne), V. Botchkarev (Bradford)
Poster session and buffet dinner

Thursday, 8.11., 8.30-13h – Cell biology and biophysics of the cytoskeleton
J. Käs (Leipzig), P. Coulombe (Baltimore), T. Magin (Leipzig), R. Leube (Aachen), J.P. Spatz (Stuttgart)
Lunch and posters
14-17h – Visit to Porsche Factory

Thursday, 8.11., 18.30-21h – Skin barrier, inflammation and immunity
S. Tsukita (Osaka), M. Pasparakis (Köln), M. Amagai (Tokio), S. Rose-John (Kiel), A. Hovnanian (Paris)
Buffet dinner and poster session

Friday, 9.11., 8.30-13h – Adhesion receptors, cell contact and growth control
K. Green (Chicago), C. Niessen (Köln), M. Hatzfeld (Halle), W. Nelson (Stanford), A. Yap (Brisbane)
Lunch and posters
Visit to German National Library

Friday, 9.11., 18.30-21h – Matrix adhesion and migration
R. Fässler (Martinsried), J. Simon (Leipzig), A. Ridley (London), S. Hüttelmaier (Halle), M. Inagaki, (Nagoya), S. Iden (Köln)
Buffet dinner and poster session

Saturday, 10.11., 8.30-13h – Cell and molecule-based therapies and pespectives
M. DeLuca (Modena), L. Bruckner-Tuderman (Freiburg), S. Leachman (Salt Lake City), D. Roop (Denver), A. Balmain (San Francisco)
Lunch and departure

Impressum

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

Editorial Board:
Harald Herrmann-Lerdon
Ralph Gräf
Ludwig Eichinger
Oliver Gruss
Friedemann Kiefer
Thomas Magin

Every article stands in the responsibility of the author. For unsolicited sent manuscripts the society does not undertake liability. Reproduction, also in part, only with permission of the society and with reference.

Editorial Office
Manuscripts/Advertisements:
Sabine Reichel-Klingmann
Office of the German Society
for Cell Biology
c/o German Cancer Research Center
Im Neuenheimer Feld 280
69120 Heidelberg
Tel.: 06221/42-3451
Fax: 06221/42-3452
E-mail: dgz@dkfz.de
Internet: www.zellbiologie.de

Production/Press:
abcdruck GmbH
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:
2000 copies

Frequency of publication:
4 issues yearly

For DGZ members free of charge

If you are interested in advertising, please contact the DGZ office (dgz@dkfz.de)

The DGZ welcomes the following new members:

Kirstin ALBERS, Seyed Amir Hamze BEATI, Dr. Martin BECK, Martin BERGERT, Mandy BÖRMEL, Dipl.-Ing. (FH) Björn BOYSEN, Nicole BÜCHNER, Andrew CLARK, Miguel COELHO, Ivona DJURIC, Martin FISCHER, Dr. Claus FÜTTERER, Isabell GEHRING, Dr. Ulf GEUMANN, Catalina GUMHOLD, Dr. Christian HÄRING, Nadine HANNING, Peter HOBOTH, Jennifer HOFMANN, Prof. Dr. Rolf JESSBERGER, Sri Krishna Rajakiran KALEPU, Dr. Mike O. KARL, Maren KLINGER, MVDr. Drahomira KNITLOVA, Arne KNÖRCK, Sonja KROSCHWALD, Karen LINNEMANNSTÖNS, Ilde MANCINI LOMBARDI, Liliana MALINOVSKA, Msc. Sandra MARKMANN, Dr. Franz MEITINGER, Matthias MUNDER, Saravanan PALANI, Mgr. Filip PARDY, Dr. rer. nat. Markos PECHLIVANIS, Sandra PLEISER, Veena Nambiar POTHERA VEEDU, Dr. Veit RIECHMANN, Heike ROTH, Jun.-Prof. Dr. Dr. Karen ROTHER, Katrin RUDOLF, Annette SAMOL-WOLF, Alexandra SCHWARK, Dr. rer. nat. Jutta SHARBATI, Pieter STEENHUIS, Dr. Julia VON BLUME, Stefan WINHEIM, Carina ZÄPER

Missing members:

We have no valid address from the members listed below. If anybody can help us in this respect, please send a message to the DGZ office at dgz@dkfz.de.

Stephan Adelt
Koku Zikpi Adjogble
Marwan Al Falah
Dorit Arlt
Tanja Barendziak
Christian Barth
Manuel Bauer
Ulrike Bichelmeier
Jessica Blume
Peter Brandt
Theo Brigge
Julia Bubeck
Johann Bumann
Stacy Carl-McGrath
Rüdiger Cerff
Min Chen
Philip Dannhauser
Franziska Dietrich
Rozina Djamiarova
Cathrin Dressler
Hans-Georg Eckert
Stefanie Eichstaedt
Jana Frahm
Michael Fredrich
Eckhard Friedrich
Christiane Gerlach
Markus Grabenbauer
Julia Groß
Karl Reinhold Gruner
Horst Hameister
Jürgen Harf
Kristina Hartmann
Detlev Herbst
Joanna Hermainski
Michael Hilker
Anna-Lena Hillje
Giselbert Hinz
Christa Hochhuth
Jan Hönnemann
Jens Hoffmann
Thomas Jarchau
Simona Kafedziska
Günter Kahl
Antje Kettelhake
Karl-Hermann Korfsmeier
Martina Kralewski
Bernd Krüger
Ralf Kuchenbecker
Christian Kutzleb
Stefan Lakämper
Philipp Lange
Gilbert Lauter
Friederike Lehmann
Lasse Lehnert
Lothar Lucka
Elena Motrescu
Jens Müller
Sylvia Niebrügge
Philipp Niethammer
Thomas Noll
Tobias Ölschläger
Adaling Ogilvie
Andrea Pauli
Gerd Paulus
Matthias Peschke
Stephan Peter
Kirsten Peters
Winfried Peters
Alexander Petrovitch
Johannes Pohlner
Guofeng Qian
Ulrich Rausch
Filomena Ricciardi
Astrid Riehl
Kerstin Röser
Oliver Rößler
Josef Rüschoff
Klaus-Dieter Scharf
Hainfried Schenk
Katharina Schönrath
Daniela Schreiber
Gerd Schwarz
Klaus Seidl
Karsten Spring
Surayya Taranum
Lena Thiel
Nadime Ünver
Jürgen Voigt
Dietmar von der Ahe
Irene Wacker-Schröder
Wibke Wagner
Michaela Waibel
Christiane Walch-Solimena
Horst Waldvogel

MEMBERSHIP

Membership Application

I hereby declare my membership with immediate effect to the German Society for Cell Biology. The DGZ annual fee of EUR 52.00 for full members, EUR 35,00 for double members (combined membership in DGZ & GBM – Society for Biochemistry and Molecular Biology) and EUR 18,00 for student members is collected at the beginning of the year.

Name, title: .....

First name: .....

Adress (institution or private):

.....

.....

.....

Street, PO box: .....

Postal code, City: .....

E-mail: .....

Status: ☐ Full member ☐ Double member ☐ Student

Authorization

to collect the membership fee by direct debit

I agree that my DGZ-year review will be debited from my German bank account. When paying from abroad, please transfer or send a check.

Account number: .....

Bank code: .....

Bank institute: .....

Account holder: .....

Secretary of the DGZ:

Deutsche Gesellschaft für Zellbiologie e.V.
c/o Deutsches Krebsforschungszentrum
Im Neuenheimer Feld 280, D-69120 Heidelberg
Tel.: (06221) 42-3451, Fax: (06221) 42-3452
E-mail: dgz@dkfz.de
Internet: www.zellbiologie.de

.....
Date, Signature

Beitrittserklärung

Hiermit erkläre ich mit sofortiger Wirkung meinen Beitritt zur Deutschen Gesellschaft für Zellbiologie. Der DGZ-Jahresbeitrag in Höhe von EUR 52,00 für Vollmitglieder, EUR 35,00 für Doppelmitglieder in DGZ & GBM und EUR 18,00 für Studenten wird jeweils zu Jahresbeginn erhoben.

Name, Titel: .....

Vorname: .....

Anschrift (dienstlich oder privat):

.....

.....

.....

Straße, Postf.: .....

PLZ,Stadt:.....

E-mail: .....

Status: ☐ Vollmitglied ☐ Doppelmitglied ☐ Student

Ermächtigung

zum Einzug des Mitgliedsbeitrages per Lastschrift

Ich bin damit einverstanden, dass mein DGZ-Jahresbeitrag von meinem Konto abgebucht wird (Voraussetzung: deutsches Girokonto). Bei Zahlung vom Ausland aus bitte Überweisung oder Verrechnungsscheck.

☐ Jahresbeitrag Vollmitglied: EUR 52,00

☐ Jahresbeitrag Doppelmitglied: EUR 35,00

☐ Jahresbeitrag Student: EUR 18,00

Konto-Nr.: .....

BLZ: .....

Bankinstitut: .....

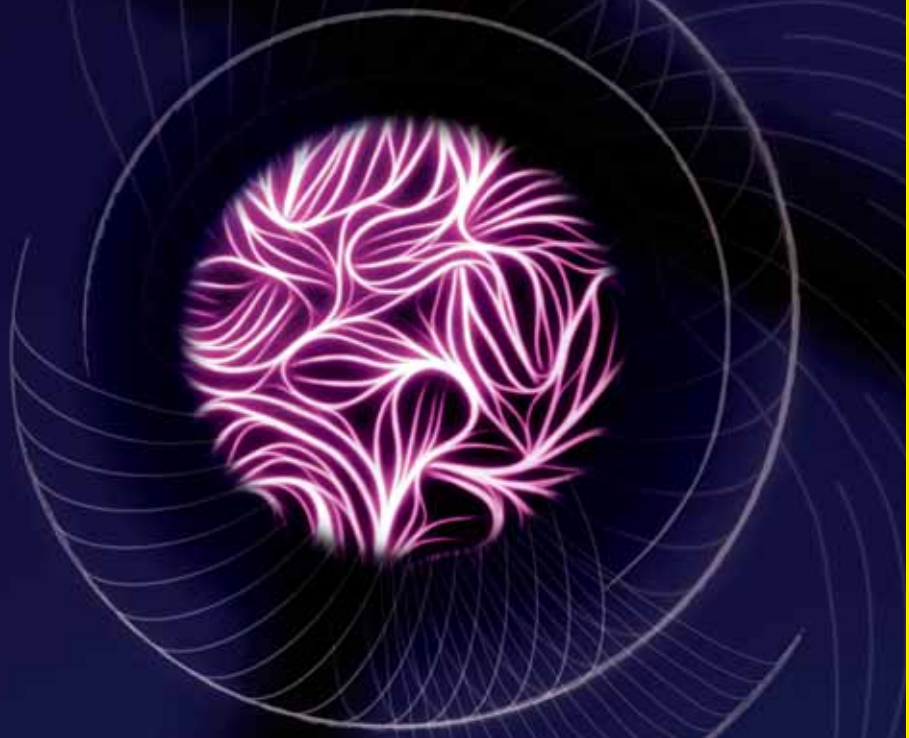
Kontoinhaber: .....

.....
Datum, Unterschrift

2nd International Meeting of the
German Society for Cell Biology (DGZ) on

Actin Dynamics

September 12th-15th, 2012
Regensburg, Germany



Organizers:

Eugen Kerkhoff (University of Regensburg, Germany)
Klemens Rottner (University of Bonn, Germany)
Theresia Stradal (University of Münster, Germany)

Further information and contact: www.actindynamics.org

DGZ DFG SPP 1464 BayGene Bayerisches Genomforschungsnetzwerk

Keynote Speakers:

Alan Hall, USA
Thomas D. Pollard, USA

Speakers:

William Brieher, USA
Nicholas Brown, UK
Richard E. Cheney, USA
Richard B. Dickinson, USA
David G. Drubin, USA
Elisabeth Ehler, UK
James Ervasti, USA
Dieter Fürst, Germany
John A. Hammer III, USA
Bernard Hoflack, Germany
Ohad Medalia, Switzerland
Alex Mogilner, USA
Keiichi Namba, Japan
Shuh Narumiya, Japan
David J. Odde, USA
Bob Robinson, Singapore
Michael Rosen, USA
Claudia Veigel, Germany
Niels Volkmann, USA
Matthew D. Welch, USA

The moment your data change
scientific minds.
This is the moment we work for.

www.zeiss.com/microscopy

ZEISS

We make it visible.