Cell News

Newsletter of the German Society for Cell Biology
Volume 37, 4/2011

Focus on Cell Biology in Dresden
Annual Meeting 2012

Deutsche Gesellschaft für Zellbiologie

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CONTENT

Mitteilungen der Deutschen Gesellschaft für Zellbiologie

Focus on Cell Biology in Dresden

In this issue we want to, inspired by our next annual meeting, introduce labs that are active in cell biology research in Dresden. In the last issue, Ewa Paluch from the *Max Planck Institute for Cell Biology and Genetics* (MPI-CBG) already presented her field of interest and expertise. Here now, six groups introduce their work: Four from the MPI-CBG, and two from the Technical University of Dresden, in particular one from the *Center for Regenerative Therapies Dresden* (CRTD) and one from the *DFG–Research Center and Cluster of Excellence for Regenerative Therapies Dresden, Medical Faculty*. We hope that their reports will inspire attendees of the meeting to contact them for exchange of ideas and discussion.

In addition, we complete our series with articles from physicists that turn into biology with Claus Fütterer from Leipzig. Eventually, we offer two Research News articles, one on Dictyostelium by Annette Müller-Taubenberger (München) and one on the nuclear envelope by Sascha Neumann (Köln).

A New Board on the Block

Furthermore, in this issue, you will find the election forms to vote for – or against – the candidates for the *Executive Board* and the *Advisory Board*, and we hope that many of you will use their right to vote. Of course, we considered if we can start to vote via internet, but this will need some time as we will have to change our constitution, which says that the election has to take place in written form. In their October meeting, the executive and the advisory board of the DGZ have agreed on Eugen Kerkhoff (Regensburg), our former secretary, as candidate for the next President of the DGZ. Ralph Gräf (Potsdam) is now up for Vice-President. These two colleagues were already on the board as Secretary and Chief Operating Officer, respectively. The two new candidates are Oliver Gruss (Heidelberg), who runs for the Chief Operating Officer (Geschäftsführer), and Klemens Rottner (Bonn), who is the candidate for the Secretary of the society. The CVs of all four are found on p. 5 and 6. The DGZ office will stay in Heidelberg and will be operated by Sabine Reichel-Klingmann. She has provided a good deal of stability for the DGZ for more than ten years and we are glad that she will stay.

The alert observer will notice that we introduce a "boy band". However, please be insured that we have tried hard to have a female president but it did not work out. Our hope now is that in the next round, this will change. At least, on the new advisory board the gender issue is balanced as we have six women and six men. According to our DGZ constitution, three candidates of the *Advisory Board* have to be elected. As you will see in the forms, Volker Gerke, Eckhart Lammert and Doris Wedlich agreed to be set up as candidates again. This is indeed very good for the continuity of our work as they are experienced in the matters of the DGZ. Again, the "mix" is good as with the beginning of my presidency we called several scientists being relatively early in their career onto the board, in particular Sylvia Erhardt, Anne Spang and Zuzanna Storchova.

The Program of the Annual Meeting in Dresden 2012

On page 7 and 8 you will find the programme for the meeting as it stands now. One of the highlights will be the opening with Fiona Watt delivering the Carl Zeiss Lecture. After her talk, it will continue with the first Plenary Session on Nuclear organization. Interesting enough, and not by accident, three established researchers from Edinburgh join us here – chaired by Ivan Raska (Prague) and complemented by Karla Neugebauer (Dresden). Actually a sign how deeply the DGZ is collaborating with colleagues in Great Britain, and we will try to enhance this collaboration in future meetings.

Three New Lecture Series

In order to make the meeting even more attractive, especially but not exclusively for the young scientists, we have initiated – in addition to the Carl-Zeiss Lecture – three new lecture series within the annual meeting. The first one, "Frontiers in Cell Biology", will be given on the upcoming meeting by Kai Simons (Dresden). After a long successful research at the EMBL in Heidelberg, Kim became one of the founding Max Planck directors for the MPI-CBG in Dresden in 1998. Kai has performed outstanding work in the field of cellular membranes, and that is why he was entitled "membrane master" in a recent JCB portrait.

The second series to be started is that of the "Distinguished Lecturer", for which we invited Kim Nasmyth (Oxford) this year. Kim has been a leading figure in the establishment of the Institute of Molecular Pathology (IMP) in Vienna, where he stayed from 1988 until 2005. His research on sister chromatid cohesion was indeed ground breaking. Reason enough for the University of Oxford to eventually recruit him as Professor for Biochemistry.

Moreover, in order to remember and to honour the achievements of one of the pioneers in German cell biology and cell biology in general, we decided to open a third series, the "Matthias Schleiden Lecture". It will be started by Günter Blobel (New York), who himself is a pioneer in the way how we look

PREFACE

at cells and who is a founder of the concept how biological activities are coordinated in a cell. For the very young colleagues, it may be appropriate to mention that Günter Blobel was awarded the Nobel price for Physiology or Medicine in 1999 (for his Nobel lecture see http://www.nobelprize.org/nobel_prizes/medicine/laureates/1999/blobel-lecture.html). His work on protein sorting within the cell, or more generally on how "cells organize themselves into distinct membranes and compartments", as well as on one of the largest "molecular machines" of the cell, i.e. the nuclear pore complex, represents a milestone of modern cell biology.

With the establishment of these three series, we want to highlight the achievements of colleagues who proved over time through their scientific discoveries that cell biology is an adventure far ahead of just simple methodological issues in preparation for translational research or as a basis for biotech companies. Thereby we wish to provide our younger colleagues with role models in research that they may try to follow in good sense.

With the best wishes for the year 2012
Harald Herrmann

Obituary

Lynn Margulis, outspoken proponent of the endosymbiont hypothesis of cell evolution

One thing is clear: the endosymbiont hypothesis is a hypothesis no longer. It is so well grounded in experimental evidence that it can now be considered a fact, just as evolution is a certainty and not a theory any more. But that wasn't always so. In 1966, a fledgling 28 year old biologist wrote a paper, The Origin of Mitosing Cells, that was rejected by an estimated 15 journals before it eventually got published in the Journal of Theoretical Biology, "that waste bin of loony ideas", as a colleague once remarked. This paper, and not the widely known 1970 book "Origin of Eukaryotic Cells", first expounded the idea that eukaryotic cells evolved as a result of multiple endocytic and symbiotic interactions among bacteria (archaea were not known at the time). It is less well known because it was published under the name Lynn Sagan (she married the world-famous astronomer Carl Sagan at age 19). Despite ferocious opposition Lynn Margulis stuck to the idea and accumulated piece after piece of supporting evidence. Today, as every student knows, the endosymbiont hypothesis is a cornerstone of our current thinking of how life evolved on this planet and in fact is almost considered old hat. The course of events that accompanied her idea is a paradigm for the old quip: When it was first proposed, they said: it isn't true; when the truth was finally revealed, they said: it's not important; and when the importance could no longer be denied, they said: anyway, it isn't new. Today, the hypothesis-turned-fact is supported by countless lines of cell and molecular biological evidence as well as present-day cases of cellular biocoenosis that represent various stages of symbiotic events "caught in the act".

Once her idea was accepted, Lynn Margulis was elected to several Science Academies and received numerous awards, including the National Medal of Science presented to her by Bill Clinton. In 2008, she was one of 13 recipients of the Darwin-Wallace Medal of the Linnean Society, awarded only every 50 years. She died on November 22, 2011 from the consequences of a hemorrhagic stroke.

Manfred Schliwa

DGZ ELECTION 2012-2014

The Executive Board and three members of the Advisory Board, two auditors and three members of the prize jury shall be elected or the term of office 2012 – 2014.

The candidates are:

Executive Board:
Eugen Kerkhoff (Regensburg) as president, Ralph Gräf (Potsdam) as vice president, Oliver Gruss (Heidelberg) as chief operating officer, Klemens Rottner (Bonn) as secretary.

Advisory Board:
Volker Gerke (Münster), Eckhard Lammert (Düsseldorf), Doris Wedlich (Karlsruhe).

Auditors:
Marie-Christine Dabauvalle (Würzburg), Hans-Georg Mannherz (Bochum).

Prize Jury:
Frank Schnorrer (Martinsried), Sabine Werner (Zürich), Walter Witke (Bonn)

For the election, please use the enclosed ballot paper. You can propose additional candidates. Ballot papers with additional notes are not valid.

Please insert the ballot paper into the blue envelope. Do not mark the blue envelope. Insert the blue envelope into the white envelope and send it by

January 31, 2012

to the DGZ office:

Deutsche Gesellschaft für Zellbiologie (DGZ)
Sekretariat, z.H. Frau Sabine Reichel-Klingmann
c/o Deutsches Krebsforschungszentrum
Im Neuenheimer Feld 280
69120 Heidelberg

If you have any question, please do not hesitate to contact the DGZ office at dgz@dkfz.de.

DGZ ELECTION 2012-2014

Prof. Dr. rer. nat. Eugen Kerkhoff as president

Date of birth: 13.06.1964
University Hospital Regensburg, Department of Neurology

Education: Chemistry studies at the University of Cologne (1983-1989). Diploma work at the Institute of Physical Chemistry in the laboratory of Prof. Dr. Georg Ilgenfritz; degree: Diplom Chemiker (1989). Dissertation at the Institute of Biochemistry, University of Cologne Medical School, in the laboratory of Prof. Dr. Klaus Bister (1989-1992); degree: Dr. rer. nat. (1992).

Positions: Postdoctoral fellow at the New York University Medical Center, Howard Hughes Medical Institute, New York, USA, in the laboratory of Prof. Dr. Edward B. Ziff (1992-1994). Postdoctoral fellow at the Imperial Cancer Research Fund (ICRF), London, UK, in the laboratory of Dr. Hartmut Land (1995). Postdoctoral fellow at the Institut für Medizinische Strahlenkunde und Zellforschung, University of Würzburg Medical School, in the laboratory of Prof. Dr. Ulf R. Rapp (1995-1998). Research group leader at the Institut für Medizinische Strahlenkunde und Zellforschung, University of Würzburg Medical School, (1998-2008). Junior Groupleader of the Bavarian Genome Research Network (BayGene) (since 2006), W2 Professorship at the Institute of Functional Genomics, University of Regensburg (2008-2010). W2 Professorship at the Department of Neurology, University Hospital Regensburg (since 2010).

Research focus: Actin dynamics at vesicle membranes, neuronal differentiation and signalling, signal transduction in the regulation of cell polarity, migration, adhesion and cell/cell contacts.

Prof. Dr. rer. nat. Ralph Gräf as vice president

Born March 22nd, 1965
Institute for Biochemistry and Biology, Dept. of Cell Biology,
University of Potsdam

Education and career: Study of biology at the Technische Universität München until 1990. 1991-1994 Ph.D. in the lab of Prof. Helmut Wieczorek at the Institute for Zoology at the Ludwig-Maximilians-Universität München (LMU). Topic: "Molecular Analysis Of The Insect V-ATPase Catalytical Complex". 1994-1996 Postdoc with Prof. W. R. Harvey at the Temple University, Philadelphia, USA in an NIH joint project with Prof. Wieczorek. 1996-2005 group leader at the Institute for Cell Biology of the LMU headed by Prof. Manfred Schliwa. 2002 habilitation in "cell biology" at the medical faculty of the LMU with the topic "Molecular And Functional Analysis Of The Dictyostelium Centrosome". 2005 - 2006 employment as an imaging specialist for laser scanning microscopy at the Carl Zeiss MicroImaging GmbH, Jena. Since October 2006 professor for cell biology at the University of Potsdam.

Memberships: ASCB, DGZ (secretary from 2001-2005, chief operating officer from 2010-2012)

Current research: Functional analyses of centrosome and microtubule-associated proteins, interactions of centrosomes with nuclear structures using Dictyostelium amoebae as a model system. These issues are invesigated with molecular biologal, biochemical and microscopical methods.

DGZ ELECTION 2012-2014

Prof. Dr. rer. nat. Oliver Gruss as Chief Operating Officer

Born 13.10.1967
Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH)
DKFZ-ZMBH Alliance

Education and career: Study of biochemistry in Regensburg 1989-1994. PhD thesis work at the ZMBH in Heidelberg 1994-1998 on translocation of proteins across the ER membrane with Prof. Dr. Bernhard Dobberstein. Postdoctoral training at EMBL with Prof. Dr. Iain Mattaj: work on the function of the Ran GTPase in spindle formation. Since 2003: independent group leader at the ZMBH working on nuclear proteins in open mitosis. Since 2010: Professor for molecular biology of cell division at the ZMBH.

Memberships: DGZ, GBM, ASCB

Research areas: Regulation of spindle formation: biochemical analysis of spindle function in cell free extracts of Xenopus. Preotemic approaches for the identification of novel spindle assembly proteins in Xenopus extracts and human cells. Splicing protein in open mitosis. snRNP biogenesis, phospho-regulation of the SMN complex.

Surveys of our research can be found in the following reviews:
Bärenz F, Mayilo D, Gruss OJ. Centriolar Satellites: Busy orbits around the centrosome. *Eur J Cell Biol* (2011), **90**, 938-9
Hofmann JC, Husedzinovic A, Gruss OJ. The functions of spliceosome components in open mitosis. *Nucleus* (2010), **1**, 447-59

Prof. Dr. rer. nat. Klemens Rottner as Secretary

born: 08.04.1970
Rheinische Friedrich-Wilhelms-Universitaet Bonn

Education: study of Biology in Salzburg/Austria 1988-1994. PhD thesis at the Institute for Molecular Biology, Austrian Academy of Sciences, Salzburg/Austria with Prof. Dr. J. Victor Small
1995-1999: Regulation of the actin cytoskeleton in migration. 2000-2003: EMBO-longterm fellow and postdoctoral training with Prof. Dr. Juergen Wehland at Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany, work on Actin Dynamics in Motility and Host-pathogen Interaction. 2004-2010: Independent Group Leader "Cytoskeleton Dynamics" at HZI Braunschweig. Since 2010: Professor and Head of Actin Dynamics and Motility Unit at the Institute of Genetics, Rheinische Friedrich-Wilhelms-Universitaet Bonn

Membership: Germany Society for Cell Biology

Research areas: actin turnover in motility processes, molecular regulation of cell surface protrusions, imaging actin dynamics in cells (using TIRF and/or photomanipulation approaches), signalling to and from Rho-GTPases, regulation of actin nucleation in vivo, exploitation of the actin cytoskeleton by pathogens

More detailed information on our research can be found in:
Hänisch, J., Stradal, T.E.B., and Rottner, K. (2011) A novel contractility pathway operating in Salmonella invasion. *Virulence*, in press.
Rottner, K., Stradal, T.E.B. (2011) Actin Dynamics and Turnover in Cell Motility. *Curr Opin Cell Biol*, **23**(5), 569-78.
Rottner, K., Hänisch, J., Campellone, K. (2010) WASH, WHAMM and JMY: Regulation of Arp2/3 complex and beyond. *Trends Cell Biol*, **20**(11), 650-61.

Dresden, March 21 - 24, 2012

Scientific Programme

Wednesday, March 21

14:00 – 14:15 **Opening Ceremony**

14:15 – 17:00 **Plenary Session 1: Nuclear organization**
Chair: Ivan Raska (Prag)

Invited speakers: Wendy Bickmore (Edinburgh), William Earnshaw (Edinburgh), Karla Neugebauer (Dresden), Eric Schirmer (Edinburgh)

17:00 – 17:15 Break

17:15 – 18:15 **DGZ Award Ceremony**

18:15 – 19:00 **Carl Zeiss Lecture: Fiona Watt (Cambridge)**

19:00 **Poster Session and Welcome Reception**

Thursday, March 22

09:00 – 12:00 **Symposium 1: Modelling in cell biology**
Chair: Ewa Paluch (Dresden)

Invited speakers: Martin Howard (Norwich), Achim Kramer (Berlin)

and speakers selected from the abstracts

09:00 – 12:00 **Symposium 2: miRNA and cancer**
Chair: Marcus Peter (Chicago)

Invited speakers: Reuven Agami (Amsterdam), Thomas Brabletz (Freiburg)

and speakers selected from the abstracts

09:00 – 12:00 **Symposium 3: Cell metabolism and cell homeostasis**

Chair: Mike Hall (Basel)

Invited speakers: Johan Auwerx (Lausanne), Tobias Huber (Freiburg)

and speakers selected from the abstracts

09:00 – 12:00 **Symposium 4: Meiosis**
Chair: Wolfgang Zachariae (München)

Invited speakers: Scott Keeney (New York), Marie-Helene Verlhac (Paris),
and speakers selected from the abstracts

09:00 – 12:00 **Symposium 5: Microtubules and Motors**
Chair: Zeynep Ökten (München)

Invited speakers: Joe Howard (Dresden), Carsten Janke (Paris)

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**

14:15 – 15:15 **Lunch Symposium: ibidi GmbH**
Cell Culture Assays

15:30 – 18:30 **Plenary Session 2: Cell and tissue morphogenesis**
Chair: Elisabeth Knust (Dresden)

Invited speakers: Darren Gilmour (Heidelberg), Thomas Lecuit (Marseille), James Nelson (Stanford), Benjamin Podbilewicz (Haifa)

15:30 – 18:30 **Plenary Session 3: Frontiers in microscopy**
Chair: Petra Schwille (Dresden/München)

Invited speakers: Jan Huisken (Dresden), Holger Stark (Göttingen), Philip Tinnefeld (Braunschweig), Andreas Zumbusch (Konstanz)

19:00 - 20:00 **Distinguished Lecturer: Kim Nasmyth (Oxford)**

Friday, March, 23

09:00 – 12:00 **Symposium 6: Asymmetric division – Mechanics of cell division**
Chair: Daniel Gerlich (Zürich)

Invited speakers: Matthieu Piel (Paris), Melina Schuh (Cambridge)

and speakers selected from the abstracts

09:00 – 12:00 **Symposium 7: Autophagy and cross-talk between organelles**
Chair: Zvulun Elazar (Rehovot)

Invited speakers: Christian Behl (Mainz), Jon Lane (Bristol)

and speakers selected from the abstracts

09:00 – 12:00 **Symposium 8: Cell biology of the immune response**
Chair: Jack Neefjes (Amsterdam)

Invited speakers: Ariel Savina (Paris), Tim Lämmermann (Bethesda)

and speakers selected from the abstracts

09:00 – 12:00 **Symposium 9: Control of cell and organ size**
Chair: Aurelio Teleman (Heidelberg)

Invited speakers: Ernst Hafen (Zürich), Alison Lloyd (London)

and speakers selected from the abstracts

09:00 – 12:00 **Symposium 10: Cytoskeleton mechanics**
Chair: Andreas Bausch (München)

Invited speakers: Stephan Grill (Dresden), Xavier Trepat (Barcelona)

and speakers selected from the abstracts

12:00 – 15:30 **Poster Session / Lunch**

12:15 – 13:15 **Lunch Symposium**

14:15 – 15:15 **Lunch Symposium**

15:30 – 18:30 **Plenary Session 4: Cilia**
Chair: Lotte Pedersen (Kopenhagen)

Invited speakers: Karl Lechtreck (Athens, USA), Heymut Omran (Münster), Nathalie Spassky (Paris), Peter Swoboda (Stockholm)

15:30 – 18:30 **Plenary Session 5: Cell adhesion and migration**
Chair: Maria Leptin (Heidelberg)

Invited speakers: Ralf Adams (Münster), John Condeelis (New York), Laura Machesky (Glasgow), Manuel Thery (Grenoble)

18:45 – 19:00 **Poster Awards**

19:00 – 20:00 **Frontiers in Science Lecture:**
Kai Simons (Dresden)

20:00 **Get Together**

Saturday, March 24

09:00 – 10:00 **Matthias Schleiden Lecture:**
Günter Blobel (New York)

10:00 – 13:00 **Symposium 11: Evolution of the cell**
Chair: Gaspar Jékély (Tübingen)

Invited speakers: Martin Embley (Newcastle), José Pereira Leal (Lissabon)

and speakers selected from the abstracts

10:00 – 13:00 **Symposium 12: Regeneration and stem cells**
Chair: Rüdiger Simon (Düsseldorf)

Invited speakers: Jochen Rink (Dresden), Elly Tanaka (Dresden)

and speakers selected from the abstracts

10:00 – 13:00 **Symposium 13: Cell biology of therapeutic delivery**
Chair: Leonard Rome (Los Angeles)

Invited speakers: Gert Storm (Utrecht), Ernst Wagner (München)

and speakers selected from the abstracts

10:00 – 13:00 **Symposium 14: Neuronal network**
Chair: Gaia Tavosanis (München)

Invited speakers: Caspar Hoogernaad (Rotterdam), Beatriz Rico (Alicante)

and speakers selected from the abstracts

10:00 – 13:00 **Symposium 15: Protein conformation diseases: cellular mechanisms and consequences**
Chair: Zoya Ignatova (Potsdam)

Invited speakers: Martin Vabulas (Frankfurt), Ina Vorberg (Bonn)

and speakers selected from the abstracts

13:00 **Closing Ceremony**

www.zellbiologie2012.de

General Information

Organizer
The German Society of Cell Biology
www.zellbiologie.de

Conference Organization
MCI Deutschland GmbH
Markgrafenstr. 56, 10117 Berlin, Germany
Phone: +49(0)30 / 204 59 0, Fax.: +49(0)30 / 204 59 50
E-mail: zellbiologie@mci-group.com

Conference Venue
MARITIM Hotel & Internationales Congress Center Dresden
Ostra-Ufer 2, 01067 Dresden, Germany

For travel information see **www.zellbiologie2012.de**

Conference Dates
Beginning of the conference: March 21, 2012
End of the conference: March 24, 2012

Social Events
Welcome Reception: Wednesday, March 21, 2012, 19:00
Get Together: Friday, March 23, 2012, 20:00

Important Dates and Deadlines
Online registration open: October 1, 2011
Abstract submission open: September 1, 2011
Abstract submission closing: January 31, 2012
Last day for early bird rate registration: February 15, 2012

Registration

Registration fee includes conference participation, welcome reception and the conference material.
Once your fax registration has been finished you will receive your confirmation of registration which is your invoice.

REGISTRATION fees Pre-Registration	until February 15, 2012	from February 16, 2012
Member DGZ	€ 150,00	€ 185,00
Non-Member	€ 210,00	€ 245,00
Students (Member DGZ)	€ 70,00	€ 90,00
Students	€ 120,00	€ 155,00
Invited speakers and Chairs	Free	Free
Day tickets (price per day)		
Regular	90,00	90,00
Students (Member DGZ)	30,00	30,00
Students (Non-Members)	40,00	40,00

For further details about registration, confirmation and cancellation: **www.zellbiologie2012.de**

Junior Scientists

We would like to encourage especially all junior scientists to join us in Dresden for this exciting meeting.
There is special emphasis to support the development of PhD students and PostDocs.

- A number of talks will be selected from abstracts
- Educational Session on Wednesday
- Poster awards will be given out
- Reasonable fees for registration

For any questions please contact:

MCI Deutschland GmbH
Markgrafenstr. 56, 10117 Berlin, Germany
Phone: +49(0)30 / 204 59 12, Fax.: +49(0)30 / 204 59 50
E-mail: zellbiologie@mci-group.com

Abstract submission

Abstract submission closing: January 31st, 2012

Maximum number of characters: 2000 characters including spaces

Abstracts for the poster exhibition can be on any area of cell biology. The short talks to be held in the minisymposia will be chosen on the basis of the abstracts. For further information see **www.zellbiologie2012.de**

The abstract itself is free of charge!

Please note that abstract registration does not include conference registration. Register online through the webpage **www.zellbiologie2012.de**

For any questions concerning the abstract procedure please contact:

MCI Deutschland GmbH
Markgrafenstr. 56, 10117 Berlin, Germany
Phone: +49(0)30 / 204 59 0, Fax.: +49(0)30 / 204 59 50
E-mail: zellbiologie@mci-group.com

For any questions concerning the abstract topics please contact the Scientific Committee / Meeting

President:
Prof. Dr. Elisabeth Knust
Max-Planck-Institute of Molecular Cell Biology and Genetics
Pfotenhauerstr. 108, 01307 Dresden, Germany
Phone: +49 (0)351 / 210-1300, Fax: +49 (0)351 / 210-1309
E-mail: knust@mpi-cbg.de

Travel grants for young DGZ members

Young researchers and students with no or only half-time positions are eligible to apply for a DGZ travel grant for participation in the DGZ annual meeting. Prerequisites are active participation at the meeting with a poster or oral presentation and DGZ membership. Grants will be giro transferred to the account given by the applicant.

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

Please refer to the following points in your application:

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

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

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

Deadline for applications: January 31, 2012

Applications received after the deadline cannot be considered anymore.

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
6. „Other"

We are looking forward to seeing you in Dresden.
The DGZ executive board

Walther Flemming Medal 2012

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

The Walther Flemming Medal is awarded annually for outstanding scientific merits from all fields of cell biological research. Eligible are researchers up to an age of 38 years. The award consists of the medal itself and a prize money of EUR 4000, partly sponsored by the European Journal of Cell Biology.

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

Both individual applications and nominations are accepted. Applications will be reviewed by an independent commission of the DGZ. The award ceremony takes place at the Annual Meeting of the DGZ, which will be held on March 21-24, 2011 in Dresden.

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

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

Deadline for applications:
January 15, 2012

Werner Risau Prize 2012 for Outstanding Studies in Endothelial Cell Biology

Together with the German Society for Cell Biology (DGZ) the prize comittee will award a prize for "outstanding studies in endothelial cell biology" to candidates within the first 5 years after obtaining their PhD or MD (except in the case of maternal leave). The Prize will be awarded for an article already published or in press, and consists of a personal diploma and a financial contribution of EUR 4000.

Applicants are requested to send a letter of motivation together with their CV and one copy of the article by e-mail (preferably in pdf format) to the

Werner Risau Preiskomitee
c/o Prof. Dr. rer. nat. Rupert Hallmann
Institute of Physiological Chemistry and Pathobiochemistry
Westfälische Wilhelms-Universität Münster
Waldeyerstr. 15
D-48161 Münster, Germany
E-mail: hallmanr@uni-muenster.de

Deadline for applications: January 15, 2012

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

Binder Innovation Prize 2012

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A Novel Role for Retromer in Epithelial Cell Polarity

Shirin Meher Pocha and Elisabeth Knust

Epithelia form sheets of cells that surround tissues and organs and function as a selectively permeable barrier, separating the outside and inside of an organism. To perform these functions, epithelial cells adopt a polarized morphology that allows them to a) form tight contacts between neighbouring cells, vital for their barrier function, and b) form specialised apical and basal surfaces that orient the cell, enabling directed transport into or out of tissues (reviewed in St Johnston and Ahringer, 2010).

Common to all epithelia is an adhesion belt, called *zonulae adherens* (ZA), which encircles the apex of the cells. A major component of this adhesive junction is the Ca^{++}-dependent homophilic adhesion molecule E-Cadherin, conserved from sponges to mammals (Fahey and Degnan, 2010). E-Cadherin is connected by α- and β-catenin to the actin cytoskeleton, thus providing mechanical strength to cell-cell contacts. Beside adhesion contacts, epithelia have to be tight, in order to prevent uncontrolled paracellular diffusion of molecules. In vertebrates, this function is mediated by tight junctions (TJs), localised apical to the ZA, with the major constituents occludins, claudins and Junctional Adhesion Molecules (JAMs). In *Drosophila*, this function is mediated by septate junctions (SJs), which are localized basal to the ZA. Interestingly, claudin-like molecules have been identified as constituents of *Drosophila* SJs (Furuse and Tsukita, 2006). Although *Drosophila* epithelia do not have TJs, they develop a highly specialised membrane region at the equivalent site, apical to the ZA, called subapical region (SAR), which harbours the Crumbs (Crb) complex (see below). Strikingly, the corresponding complex localizes to the TJs in vertebrate epithelia.

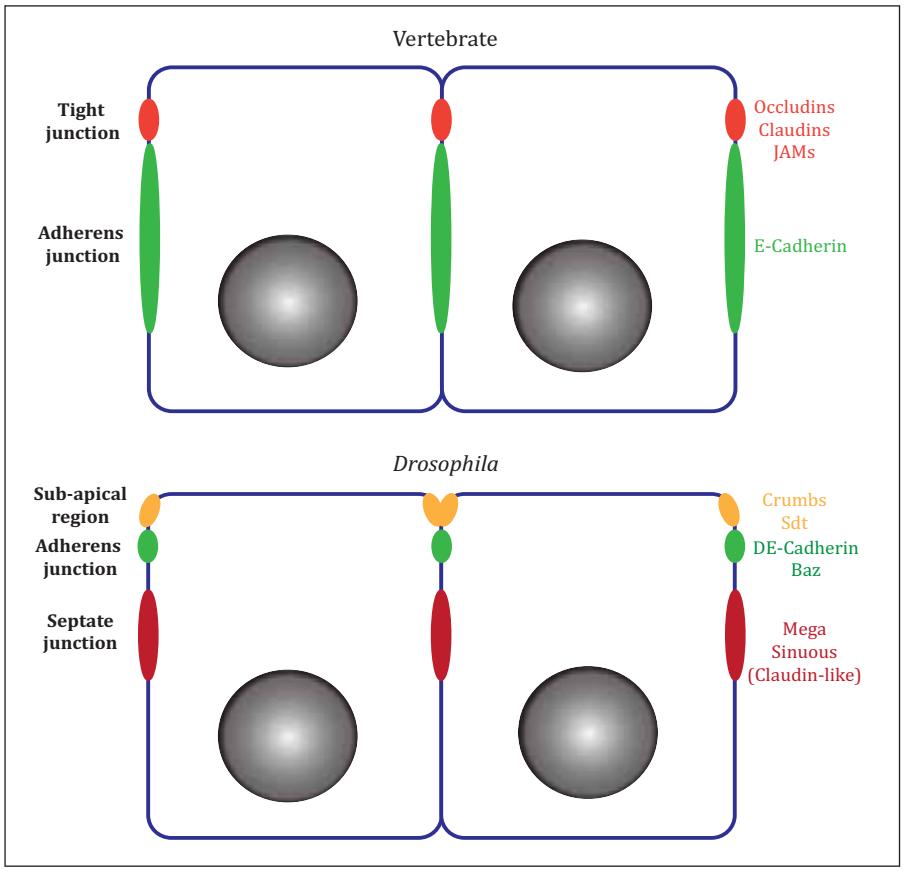

Figure 1: The junctional and apical complexes of vertebrate and invertebrate epithelia.
A scheme showing the arrangement and composition of the tight and adherens junctions of vertebrate epithelia (top) and the sub-apical region, adherens and septate junctions of *Drosophila* epithelia (bottom).

Three mutually antagonistic protein complexes/networks have emerged as main regulators of epithelial cell polarity, the Par complex (consisting of Par-3 (Bazooka, Baz in *Drosophila*), Par-6 and atypical protein kinase C, aPKC), the Crumbs and the Scribble complexes. These complexes show polarized distribution within the cell, which is vital for their function. The Crumbs complex is restricted to the sub-apical region in most epithelia (Tepass et al., 1990; Wodarz et al., 1995). Par-complex members can be found apically, but do not always co-localise, since Bazooka is often associated with the ZA (Harris and Peifer, 2005a). The Scribble complex localizes to the baso-lateral membrane (Bilder et al., 2003).

Establishing and maintaining the distinct localizations of the polarity complexes is in part achieved by antagonism between them, but it is also heavily dependent on a polarized cytoskeleton and polarized trafficking. Indeed, the initial polarization of Baz in the cellularizing *Drosophila* embryo is dependent

on both apical actin and dynein-dependent polarized transport (Harris and Peifer, 2005b). Mechanisms of polarized transport vary greatly depending on the developmental stage and cell type. However, most of the current molecular details are based on cell culture models of polarized cells such as the Madine Darby canine kidney (MDCK) cell line (Fölsch et al., 2009; Weisz and Rodriguez-Boulan, 2009). Therefore, there is a need for more extensive studies in developing organisms and tissues to understand the contribution polarized trafficking machineries play in the establishment and maintenance of epithelial cell polarity. This is particularly true if we are to gain full insight into the ways in which different cell types exploit different trafficking itineraries throughout development.

The Crumbs complex localizes exclusively to the apical domain of epithelial cells. Crumbs is an evolutionarily conserved type I transmembrane protein that was initially identified as an apical determinant in *Drosophila* embryonic epithelia (Tepass et al., 1990; Wodarz et al., 1993; Wodarz et al., 1995). The highly conserved intracellular domain of Crumbs recruits a plasma membrane-associated protein scaffold, composed of the membrane-associated guanylate kinase (MAGUK) protein Stardust (Sdt) and the PDZ domain-containing proteins DPATJ and DLin-7 (reviewed in Bulgakova and Knust, 2009). In addition to embryonic epithelia, a role for Crumbs in apico-basal polarity has been reported in follicular epithelial cells (Morais-de-Sa et al., 2010; Tanentzapf et al., 2000), imaginal discs (Lu and Bilder, 2005) and the retina (Izaddoost et al., 2002; Pellikka et al., 2002). In all cases, the strict apical localization of Crumbs is vital for its function. Strikingly, little was known about the mechanisms of Crumbs trafficking, nor how its strict apical localization is achieved and maintained. Therefore we designed a strategy to identify components of trafficking machineries that bind to the cytoplasmic domain of Crumbs (Pocha et al., 2011).

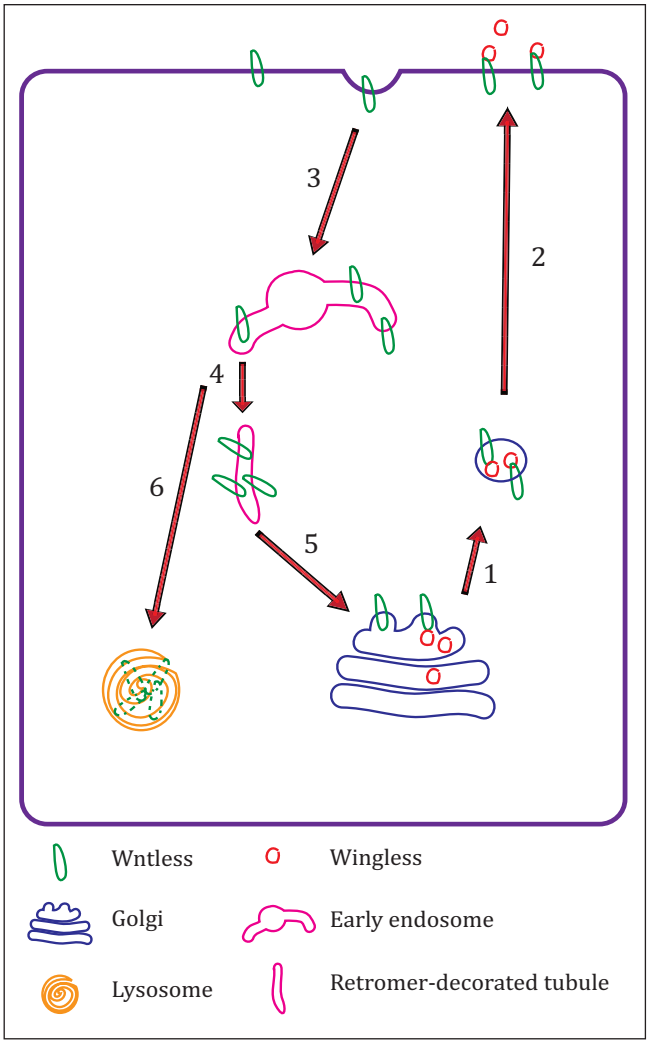

Figure 1: Retromer-mediated recycling of Wntless.
In *Drosophila* and *C. elegans*, Wingless/Wnt is transported to the plasma membrane for secretion by the transport receptor Wntless (1 and 2). Wntless that has released its cargo undergoes endocytosis (3) and enters the early endosome. From there, retromer mediates its sorting (4) and transport back to the TGN (5), where it can pick up another Wingless molecule for secretion. In the absence of retromer, Wingless is not recycled and instead is targeted for lysosomal degradation (6).

We displayed the murine Crb2 intracellular domain on liposomes, thus mimicking its native configuration at the interface between membrane and cytosol, and analyzed proteins that were recruited specifically by the Crb2 tail from brain cytosol. Using this technique, we identified Crumbs as a target of the retromer trafficking machinery.

Retromer was first identified in yeast (*Saccharomyces cerevisiae*) as a pentameric complex responsible for the recycling of a vacuolar hydrolase receptor, Vps10p, from endosomes to the Golgi (Seaman et al., 1998). This retrograde transport returns Vps10p back to the Golgi, where it can then bind another hydrolase molecule for delivery to the vacuole. Structurally, yeast retromer is composed of two sub-complexes, the cargo-selective subcomplex (composed of Vps26p, Vps29p and Vps35p) and a membrane-inter-

acting subcomplex composed of two sorting nexins, Vps5p and Vps17p. Loss of function of any retromer subunit in yeast results in the secretion of carboxypeptidase Y, a hydrolase of the vacuole and cargo of Vps10p. More recently, additional cargoes for yeast retromer have been identified, including the reductive iron transporter Fet3p-Ftr1p and the endoprotease Kex2p (reviewed in Attar and Cullen, 2010). To date, all of the functions described for retromer in yeast involve the sorting of proteins for retrograde transport to the Golgi.

The cellular importance of retromer is underscored by the high level of conservation observed both in subunit composition and function, between organisms as simple as yeast to humans. Although conserved, the composition of the mammalian retromer is somewhat more complex, as there are two genes that encode Vps26p orthologues, Vps26A and Vps26B. In addition, there are four (possibly five, based on sequence homology) sorting nexins that can form heterodimers to make up the membrane interacting subcomplex; SNX1, SNX2 (orthologues of Vps5p), SNX5, SNX6 and potentially SNX36 (orthologues of Vps17p) (McGough and Cullen, 2011). Functionally equivalent to Vps10p, the cation-independent mannose-6-phosphate receptor (CI-MPR) was the first cargo described for the mammalian retromer (Arighi et al., 2004; Seaman, 2004). As in yeast, mammalian retromer transports the CI-MPR from endosomes to the *trans*-Golgi network (TGN), where it binds mannose-6-phosphate-tagged hydrolases that are destined for the lysosome. In the absence of retromer, CI-MPR is not retrieved from endosomes and is degraded as these compartments mature into lysosomes.

In the last 10 years some important clinical implications have been associated with the retromer by the identification of several additional cargoes. These include sortillin 1 and the sortillin related protein SorLA. The latter regulates amyloid precursor protein (APP) trafficking, and mutations in SorLA have been reported as risk factors for Alzheimer's disease (Rogaeva et al., 2007). Furthermore, retromer is required for trafficking β-Site APP-cleaving enzyme (BACE; also called β-secretase), the protease responsible for APP processing to Aβ (the main component of plaques found in the brains of Alzheimer's disease patients)(He et al., 2005). Loss of retromer results in an accumulation of Aβ and thus contributes to the progression of neurodegeneration. Retromer has also been implicated in cancer progression, with SNX1 shown to be downregulated in numerous colon cancer samples (Nguyen et al., 2006). It has been proposed that the involvement of retromer in EGF receptor (EGFR) trafficking is responsible for this. However, as the exact relationship between EGFR and retromer is unclear, the link between retromer and cancer progression is yet to be understood at a molecular level.

More recent work in *Caenorhabditis elegans* and *Drosophila* has identified an important role for retromer in embryonic development. Studies that showed defects in Wnt (Wingless or Wg in *Drosophila*) signaling paved the way for the identification of Wntless, a transport receptor of Wnt/Wg, as a retromer cargo (Fig. 2) (reviewed in Eaton, 2008). Very recently, this transport step was shown to be mediated by the retromer cargo-selective subcomplex in concert with SNX3 rather than the "classical" SNX1,2 and SNX5,6 heterodimers (Harterink et al., 2011). This was the first report to show that the cargo-selective subcomplex functions with different combinations of sorting nexins, and raised questions about the uniqueness of Wntless in this pathway. Interestingly, the role of retromer in Wnt signaling is not limited to its role in recycling Wntless, as in *Xenopus laevis* Wntless independent requirements for retromer have been described (Kim et al., 2009). The mechanism of this Wntless-independent regulation of Wnts by retromer is yet to be elucidated. However, this, together with insight into the role of SNX3, may provide valuable insights for the Wnt field.

While the vast majority of studies in other systems reinforce the findings from yeast, that retromer is involved in the retrograde

Figure 3: : Crumbs is lost from Vps35 mutant follicular epithelial cells.
Part of a *Drosophila* follicle epithelium containing clones mutant for *Vps35*$^{[MH20]}$ (null allele, cells marked by GFP), stained for Crb (magenta). Mutant cells show a marked reduction of Crb in the absence of retromer function. In addition, multilayering is seen in the two cells at the edge of the mutant clone (arrows). Scale bar 5 µm.

traffic of membrane proteins from endosomes to the TGN, two groups have proposed alternative functions. The Mostov group observed trafficking defects for the polymeric immunoglobulin receptor (pIgR), which is usually transported from the basolateral to the apical surface of epithelial cells and hepatocytes by the process of transcytosis (Verges et al., 2004). Korolchuk et al identified Vps35 in a screen for endocytosis defects using *Drosophila* S2 cells (Korolchuk et al., 2007). A genetic interaction between Vps35 and Rac1 led them to propose that regulation of endocytosis by Vps35 was due to its regulation of Rac, however no mechanism was proposed suggesting how this interaction could occur. Interestingly, the involvement of retromer in both transcytosis and endocytosis has not been shown to be direct and the question remains whether or not these processes are perturbed as consequences of defects in retrograde transport by retromer in its classical pathway. Further work will be required to establish mechanistic details of this in the future.

Our study identifying Crumbs as a cargo for retromer identified a novel role for retromer in controlling epithelial cell polarity. This has since been corroborated by work from another group, which showed that retromer also traffics Crumbs in *Drosophila* embryos (Zhou et al., 2011). As described for classical retromer cargoes, in the absence of retromer, Crumbs is lost from *Drosophila* embryonic, larval, imaginal disc and follicle epithelia (Fig. 3) (Pocha et al., 2011; Zhou et al., 2011). In addition, Vps35 mutant tissues display two striking phenotypes: 1) multilayering of the follicular and embryonic epithelium, indicating that there are gross defects in cell polarity, and 2) the specific loss of Sdt and DPATJ and a reduction in apical aPKC and Par6. Strikingly, all of these defects phenocopy the loss of Crumbs phenotype. Moreover, the latter could be rescued by Crumbs overexpression, indicating that the polarity defects caused by loss of retromer are due to the loss of Crumbs.

Together, these finding show that Crumbs localization at the apical domain is regulated to a large extent by membrane trafficking, in particular, by sorting decisions at the early endosome. Retromer is responsible for this step in retrieving Crumbs from endosomes to enable recycling rather than degradation, however the destination of this retrograde transport is ill-defined. Does Crumbs travel back to the TGN or does it enter other or additional sorting compartments on its way back to the plasma membrane? Rab11-positive recycling endosomes might be an interesting candidate for this, as newly synthesized Crumbs has been suggested to travel via these compartments (Roeth et al., 2009; Schluter et al., 2009). However, it remains to be established whether this holds true for only newly synthesized protein or if recycled proteins converge with it in the same compartment.

Finally, a very interesting point that remains to be elucidated is the reason for Crumbs to be recycled by retromer. Is the retrograde transport of Crumbs purely a means of regulating levels of Crumbs at the plasma membrane? Or is there a special significance to the recycling by retromer as opposed to by other trafficking machineries? If the latter is the case, then it opens the door to a whole range of new questions about the function of Crumbs itself. Is Crumbs transported back to the Golgi so that it may aid the apical sorting of other molecules? It has been shown that the TGN can be the site of sorting for the polarized delivery of proteins (Fölsch et al., 2009). This would therefore be an exciting hypothesis that fits well with the defects in apicalization observed in Crumbs mutants (Wodarz et al., 1993; Wodarz et al., 1995). Crumbs contains an extremely large extracellular domain, binding partners for which are yet to be identified. It is plausible that Crumbs, like many of the classical retromer cargoes described above, is acting as a transport receptor, ensuring the apical secretion of proteins from the TGN. These possibilities will be the focus of future work, the findings of which will greatly enhance our understanding of the role of Crumbs in epithelial polarization.

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Shirin studied at the University of Bristol, where she obtained both her undergraduate degree and Ph.D. in Biochemistry. During her postgraduate studies, Shirin investigated the role of phosphorylation in regulating the activity of the WASP family of actin regulators. Towards the end of her Ph.D., she started work in Drosophila to assess the physiological relevance of WASP protein phosphorylation. In 2008 Shirin moved to the lab of Elisabeth Knust at the Max-Planck Institute of Molecular Cell Biology and Genetics in Dresden, to pursue a postdoc using Drosophila as a model organism and to study the mechanisms of maintaining epithelial cell polarity and retinal degeneration.

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Elisabeth Knust studied genetics, zoology and physical chemistry at the University of Düsseldorf, where she also got her PhD in genetics. For her PhD, she analysed the lampbrush loops of the Y-chromosome of Drosophila hydei, the formation of which is crucial for male fertility. During her postdoctoral trainings in virology at the Institute of Virology at the University of Erlangen-Nürnberg she worked on the transcriptional program of the oncogenic Herpesvirus saimiri during lytic infection and and persistent transformation. She continued her career at the Institute of Developmental Biology at the University of Cologne, first as assistant professor, then as a Heisenberg fellow, spending also some time at the University of Boulder, Colorado, before she became Associate professor at the Institute of Developmental Biology at the University of Cologne, and full professor and head of the Department of Genetics at the Heinrich-Heine University, Düsseldorf. Since 2007, she is director at the Max-Planck Institute of Molecular Cell Biology and Genetics in Dresden. Her lab studies the genetic, cell biological and molecular mechanisms controlling epithelial cell polarity and retinal integrity.

Get Your Motors Running: Sex, Self-organization, and Oscillations

Iva M. Tolić-Nørrelykke

Introduction

A cell is not a container full of randomly dispersed molecules, molecular assemblies, and organelles. The cell interior is instead neatly organized in a dynamic yet controlled manner. Dynamic organization of the cell interior requires constant exploration of the intracellular space to adjust the position of cell components in a response to changes such as cell growth, progression through the cell cycle, and signals from the environment. To this aim the cell uses microtubules and actin filaments, motor proteins, and other cytoskeleton-associated proteins.

Microtubules are dynamic polymers, which can be in a growing or a shrinking state. Growth and shrinkage are more pronounced at one end of the microtubule, which is called the plus end, compared to the minus end. The switch from growth to shrinkage is termed catastrophe, while the switch from shrinkage to growth is rescue. Catastrophe and rescue are stochastic events, which together with growth and shrinkage belong to intrinsic properties of microtubules [1].

Cells use microtubules to carry out a variety of functions, such as segregation of chromosomes, transport of organelles and molecular assemblies, and positioning of the nucleus and of the mitotic spindle. To perform these different tasks, the cell regulates the intrinsic properties of microtubules according to the specific task, by using microtubule-associated proteins.

Organization of the cell interior requires forces to move and position organelles [2]. Force is generated when microtubule growth and shrinkage is hindered by objects such as the cell cortex and organelles. Microtubules growing against an obstacle produce pushing force [3], while shrinking microtubules pull on structures that remain attached to the microtubules as they shrink [4]. In addition, microtubules transmit the force generated by motor proteins [5].

Fission yeast: A model cell ideal for studies of the cytoskeleton

Microtubule dynamics and microtubule-based organelle movements are extensively studied in the fission yeast *Schizosaccharomyces pombe* as a model system. An advantage of this system is its genetic amenability, which allows for fluorescent tagging, deletion, mutation, and changes in the level of expression of proteins involved in microtubule behavior. Moreover, tracking and manipulation of single microtubules is possible due to the small number of microtubules in these cells [6].

Fission yeast cells are rod-shaped and the microtubules typically lie along the main axis. Hence, one-dimensional models are appropriate theoretical descriptions of microtubule-based movements. Because of the low number of important degrees of freedom in the theoretical models, the underlying mechanisms can be easily understood. A combination of modeling and experimental work has recently provided understanding of several mechanisms driving nuclear movements, from nuclear centering [7] to pole-to-pole nuclear oscillations [8].

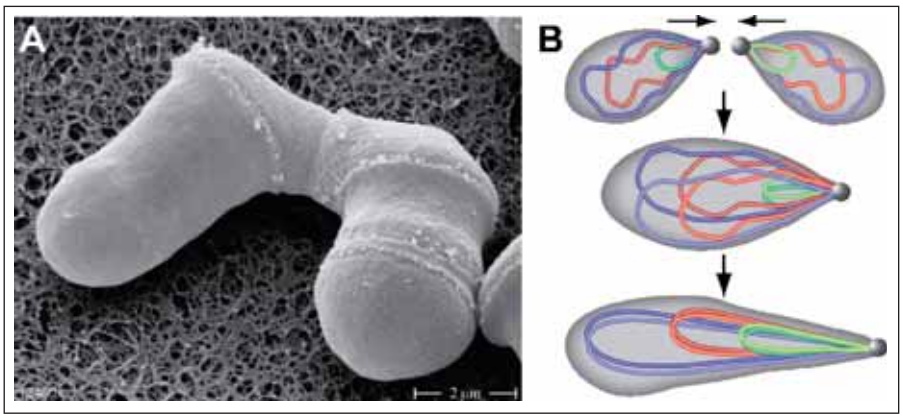

Figure 1: **A**, An electron micrograph of a fission yeast zygote. **B**, Two nuclei fuse at the beginning of meiotic prophase. The chromosomes (blue, red, green) are attached by their telomeres to the SPB (small grey sphere). The oscillatory movement of the SPB and thus of the nucleus helps homologous chromosomes (e.g., dark and light blue) to pair with each other and recombine.

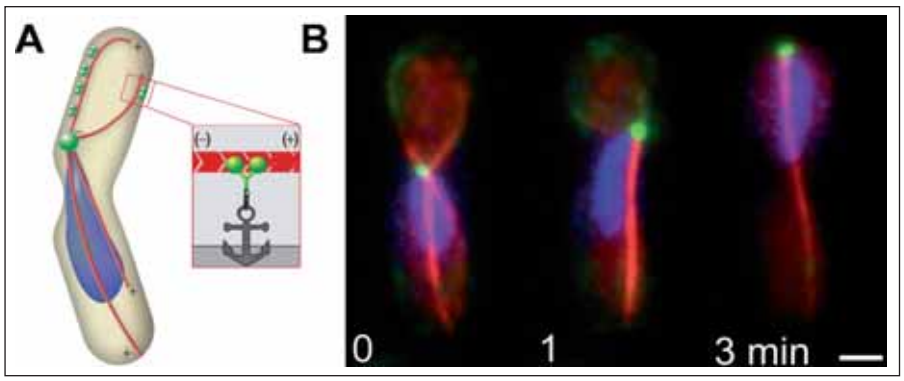

Figure 2: **A**, Scheme of a fission yeast cell and an enlarged region where a microtubule (red) is close to the cell cortex. Dynein (green) links the microtubule to a cortical anchor protein. **B**, Time-lapse sequence of nuclear oscillations. The cell expresses dynein heavy chain labeled with GFP (green), tubulin labeled with mCherry (red), and the nucleus is stained with Hoechst dye (blue). Scale bar represents 2 µm.

Meiotic nuclear oscillations: Shaking helps chromosome pairing

The nuclear oscillations in the meiotic prophase of fission yeast provide an excellent system to study oscillatory movement dependent on cytoplasmic dynein and microtubules [8, 9]. These oscillations occur after two cells of opposite mating types melt together (Fig. 1A). Shortly after that, the nuclei of these cells fuse into one. The joint nucleus then travels from one cell pole to the other and back with a period of about ten minutes, lasting for several hours [10].

Why does a cell use so much time and energy to move the nucleus back and forth? The answer comes from the studies showing that without nuclear oscillations, chromosome pairing, recombination, and spore viability is reduced [9, 11]. The idea here is that dragging the chromosomes from one end of the cell to the other and back helps homologous chromosomes to align with each other, thereby promoting their pairing and recombination (Fig. 1B).

Similar chromosome movements have been observed in meiotic prophase in a variety of model organisms, from budding yeast to mouse [12, 13], and the role of these movements in chromosome pairing and recombination has been confirmed [14]. Thus, extensive chromosome movements in meiotic prophase, which are important for chromosome pairing, are most likely a conserved phenomenon.

Mechanism of the oscillations: Mechanically regulated self-organization

Which physical mechanism underlies this striking oscillatory movement of the nucleus? Let us first consider the main molecular players and the geometry of the system (Fig. 2A). The movement of the nucleus is led by the motion of the spindle pole body (SPB) [10], from which microtubules extend with their minus ends at the SPB and the plus ends pointing towards the opposite cell poles (Fig. 2B) [15, 16]. These microtubules are being pulled on by dynein motors, as shown by genetic studies [9] and by laser ablation of the microtubules [8, 17, 18]. In order to exert force on the microtubule, dynein motors are anchored to the cell cortex by the membrane protein Mcp5/Num1 [19, 20].

As the SPB moves towards one cell pole, dynein is found mainly along the microtubules extending in front of the SPB, whereas microtubules trailing behind the SPB have fewer dyneins (Fig. 3) [8]. This asymmetry of dynein distribution results in a stronger pulling force by the leading than by the trailing microtubules and, consequently, in persistent nuclear movement towards one cell pole. As the SPB moves, the leading microtubules shrink and eventually disappear. Afterwards, the microtubules directed towards the opposite cell pole start to lead the SPB movement and the oscillations are established.

A key question is how is the asymmetry of dynein distribution and thus of the pulling forces generated. There are several possibilities. First, it has been proposed that the SPB sends an inhibitory signal [15]. When the SPB reaches one cell pole, it may deactivate a cortical component required for force production, e.g. the cortical anchor proteins for dynein, in that region. The anchors are in the meantime re-activated at the opposite cell pole, resulting in pulling towards that side [15].

A different scenario may be envisioned based on the measured speeds of microtubule growth and of the SPB movement [15]. The assumption here is that microtubules interact only with dynein motors anchored at one cell pole. At the same time, microtubules pointing towards the opposite cell pole are not able to reach this pole, because microtubule growth is slower than the speed of the SPB movement. Only when the SPB pauses at one cell pole, microtubules reach the opposite pole and start pulling the SPB in the opposite direction. Though these scenarios seem plausible, they lack experimental support.

Dyneins playing tug of war: Detachment depends on the load force

In contrast to the possibilities described above, oscillations may be driven by mechanical cues [21], where dynein detachment under high load forces plays a key role [8, 22, 23]. In general, an external pulling force

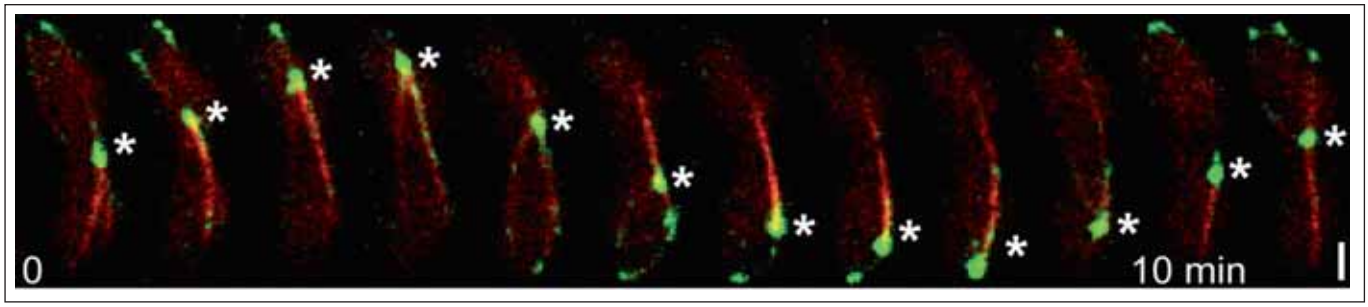

Figure 3: A time-lapse sequence of a cell during one oscillation period. Dynein is more abundant on the leading than on the trailing microtubules. Dynein heavy chain is labeled with GFP and shown in green, while tubulin is labeled with mCherry and shown in red. Asterisks indicate the position of the SPB; scale bar represents 2 µm. The figure is adapted from Ref. [8].

increases the probability of breaking a bond between two objects, e.g., between a motor protein and a microtubule, which is known as load-dependent detachment [24, 25]. This process for motor proteins has been studied theoretically [26], *in vitro* [27], and has been suggested to play a crucial role in bidirectional transport processes and spindle and chromosome oscillations [28-31].

Microtubule-based motor proteins walk either to the plus end or to the minus end of a microtubule, depending on the motor type. The walking velocity depends on the load force exerted on the motor. As the load force increases, the motor velocity decreases; when the load exceeds a certain value the motor moves in the opposite direction. If the tail of the motor is fixed to a surface and its head attached to a microtubule, the direction and the velocity of the motor can be determined from the movement of the microtubule. Hence, the load force on the motor depends on the movement of the microtubule.

How does microtubule movement control the load force experienced by dyneins during nuclear oscillations? Two groups of cortically anchored dyneins can be defined: those on the leading microtubules walk toward the minus end of the microtubules, while those on the trailing microtubules are forced, by the movement of the trailing microtubules, to walk towards the plus end of those microtubules. Therefore, during the SPB movement, the dyneins on the trailing microtubules are under higher load force and thus may have a higher probability to detach from the microtubules (Fig. 4).

A minimal model of nuclear oscillations

To test whether load-dependent detachment of dynein is important for the generation of SPB oscillations, we have developed a minimal one-dimensional model [8]. In our model, motors attach to dynamic microtubules and link them to the cortex. The change in the total number of attached motors depends on microtubule length and motor concentration; the detachment rate is load-dependent. The linked motors generate a force on the microtubules described by a force-velocity relationship. We found that, for typical parameter values, our model accounts for the experimentally observed end-to-end SPB oscillations with a triangular waveform and the key features of the dynein dynamics: positive feedback of the SPB movement on the number of dyneins at the leading microtubules, and the absence of dyneins at the trailing microtubules [8].

How does our model generate oscillations? As the SPB moves, the load on the motors on the leading microtubules is low. Thus, the probability of their detachment is also low. Because of the different detachment probability on the leading and the trailing side, the asymmetry in the number of motors on the leading and the trailing microtubules grows, resulting in a faster SPB movement. The faster movement further increases the asymmetry in the load on the motors, creating a positive feedback between the SPB

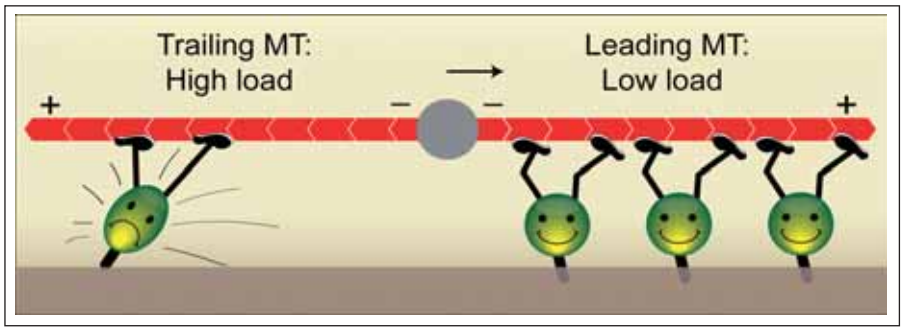

Figure 4: Nuclear oscillations in meiotic prophase are based on preferred detachment of motors under high load force. During the SPB movement, the load on the cortically anchored dynein motors on the leading microtubule is low. Thus, these motors remain attached and keep pulling the SPB. Simultaneously, the load on the motors on the trailing microtubule is high, which enhances their detachment. The drawing is modified from Ref. [22].

movement and the number of motors on the leading microtubules. However, as the SPB moves, the leading microtubules shrink and thus lose motors. When the number of motors on both sides of the SPB is equal, the SPB stops.

When the movement of the SPB stops, which occurs near a cell pole, the microtubules extending towards the opposite cell pole are longer than those pointing to the closer cell pole. Because the number of attachment sites for dynein on the microtubule depends on microtubule length, more motors will accumulate on the longer microtubules. Thus, the SPB starts to move in the direction of the longer microtubules. Once the movement has started, the motors on the longer microtubules experience lower load forces, the motor detachment rate thus decreases and the motors accumulate on the longer microtubules, thereby the change of direction is completed. Afterwards, during the SPB movement, the load on the motors rather than the microtubule length plays a key role in dynein accumulation and the resulting force.

Conclusion

A key aspect of life is sexual reproduction, which involves the mixing of genetic material during meiosis. In fission yeast and other organisms, the successful mixing and recombination of the chromosomes requires concerted movement of the nucleus. This phenomenon is driven by dynein motors that move the nucleus back and forth inside the cell with the aid of microtubules.

Nuclear oscillations in fission yeast occur as dyneins redistribute from the trailing to the leading microtubules, generating an asymmetric distribution of motors and, consequently, of pulling forces. This dynamic motor redistribution occurs purely as a result of changes in the mechanical strain sensed by the motors. Thus, spatio-temporal pattern formation within a cell can appear as a result of self-organization driven by mechanical cues, rather than via the more commonly observed mechanisms of conventional molecular signaling or self-organization driven by biochemical reactions and diffusion. Concepts such as preferred detachment of motors under high load force, which are emerging from a combination of experimental and theoretical work, can explain the basis of how the cell positions and moves the nucleus, mitotic spindle, and chromosomes on the spindle.

Our results prompted us to explore what happens to dyneins in the cytoplasm and to those bound to the microtubule or to the cortex, as well as in which order the respective binding and unbinding processes of dynein occur. We are now able to track single dyneins on the microtubule and, surprisingly, also those diffusing in the cytoplasm. Observation of single dyneins will allow us to identify key steps of the binding process and to measure the kinetics of the reactions, as well as to uncover the search strategy by which dyneins find cortical anchors in order to generate large-scale movements in the cell.

Acknowledgments

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The SR protein family: functional specificity or redundant functions? *In vivo* studies show the way

Michaela Müller-McNicoll & Karla M. Neugebauer

Introduction:

Cells express an astounding variety of messenger RNAs (mRNAs) from a limited pool of genes. Transcripts produced from the same gene can differ at their 5' and 3' ends as well as throughout the transcript body. During its lifetime each mRNA is complexed by numerous RNA-binding proteins (RBPs) forming messenger ribonucleoprotein (mRNP) particles. RBPs can change the output of gene expression at several steps, including transcription, 5' capping, splicing, polyadenylation, RNA export, RNA stability and translation, in this way enabling the differential expression of several distinct protein products per gene. During their journey from transcription in the nucleus to translation in the cytoplasm, mRNPs undergo a constant remodeling, manifested by the loss and gain of different RBPs. On the other hand, continuous binding of particular RBPs can assist to couple subsequent steps in gene expression. Many RBPs are subjected to post-translational modifications (PTMs), providing another level of mRNP regulation. At present, the *in vivo* binding specificity and function(s) of most RBPs are not well understood. How do nascent and mature mRNPs assemble? What is their composition? What are the specific functions of mRNP components in gene expression? These questions currently represent a black box in our knowledge of gene expression.

The SR protein family

Our lab studies the SR protein family as an example of regulatory RNA-binding proteins comprising seven canonical members. SR proteins are evolutionarily conserved and

structurally related RBPs, which play essential roles in pre-mRNA splicing, both as general splicing factors and as regulators of alternative splicing (AS). Through their additional pre- and post-splicing activities, SR proteins are important players in connecting nuclear and cytoplasmic gene expression.

The first "classical" mammalian SR protein was characterized by two different groups in 1991, and was named ASF or SF2 (now SRSF1; [1, 2]). In the same year it was reported that a monoclonal antibody (mAb104) recognizes a conserved phospho-epitope on a group of proteins, with molecular masses of 30, 40, 55 and 75 kDa, [3]. One year later these proteins were identified to be members of a family of closely related splicing factors that included the previously characterized SRSF1 and SC35 (now SRSF2; [4]). They were named SR proteins, because all members contained a domain with consecutive serine (S) and arginine (R) dipeptides [5]. SR proteins are encoded by separate genes and named SRSF1 to SRSF7 [6]. The numbers reflect the chronological order in which the genes/proteins were discovered. All members are highly related in primary sequence and protein domain organization; they contain one or two RNA recognition motifs (RRMs) at their N-termini, a glycine/arginine-rich spacer region and the region of repeated serine-arginine dipeptides, called the RS domain, at their C-termini (Figure 1). The second RRM is also called RRM-homologous sequence (RRMH) and contains an invariant and evolutionarily conserved SWQDLKD heptapeptide. The structure of this domain revealed an atypical RRM fold that binds to RNA in a novel manner [7]. The RS domain is the defining feature of the SR protein family, but it is also present in over 40 additional SR-related splicing factors [8]. It is through these domains that protein-protein interactions among splicing factors often occur [9]. The RS domain is extensively phosphorylated at most serine residues by several SR protein-specific kinases [10], and phosphorylation was shown to be important for subcellular localization and activity of SR proteins in early steps of splicing [9, 10]. Conversely, SR protein dephosphorylation, occurring during splicing, is important for catalysis and the release of the splicing machinery [9, 11]. The RS domain can also function as a nuclear localization signal (NLS) by mediating the interaction with the SR protein-specific nuclear import receptor, called transportin-SR [12]. The glycine/arginine-rich spacer domain is the most divergent region between the different SR protein family members and is the binding site of the mRNA export receptor, Nxf1/TAP [7, 13]. Other differences between SR proteins include the absence or presence of the RRMH domain, the length of the RS domain and the presence of a zinc knuckle in SRSF7 (Figure 1).

Classical SR proteins can be purified from all other cellular proteins by a two-step salt precipitation method using ammonium sulfate followed by magnesium chloride [3]. Magnesium mediates ionic cross-linking between phosphoserines on adjacent SR proteins leading to the formation of supramolecular complexes of SR proteins, which can then be isolated by centrifugation. *In vitro* splicing assays and complementation of the residual splicing-deficient cell extracts (S100) were used to identify and characterize all members of the SR protein family [9].

SR proteins and their roles in pre-mRNA splicing

Most eukaryotic pre-mRNAs contain introns, which have to be removed, and exons, which have to be ligated together, before mRNAs can be translated into proteins. Pre-mRNAs can be alternatively spliced, providing a major source of transcriptome and proteome diversity in cells. It has recently been estimated that transcripts from 95% of human multi-exon genes undergo alternative splicing (AS), with the majority of them being differentially expressed between cells and tissues [14].

SR proteins have important functions in both constitutive and alternative splicing (AS). They are primarily located in the nucleus and concentrated in nuclear speckles [15], highly dynamic subnuclear domains enriched in pre-mRNA processing factors. From there, SR proteins are recruited via phosphorylation to sites where RNA polymerase II (Pol II) is actively transcribing genes [16]. SR proteins interact with nascent transcripts as they protrude from Pol II via their RRM in a sequence-specific manner. Both the canonical RRM and the RRMH contribute together to sequence specificity and binding affinity [17]. SR proteins bind mostly to exonic splicing enhancer sequences (ESEs). Binding of ESEs usually leads to the activation of flanking splice sites, which otherwise would be

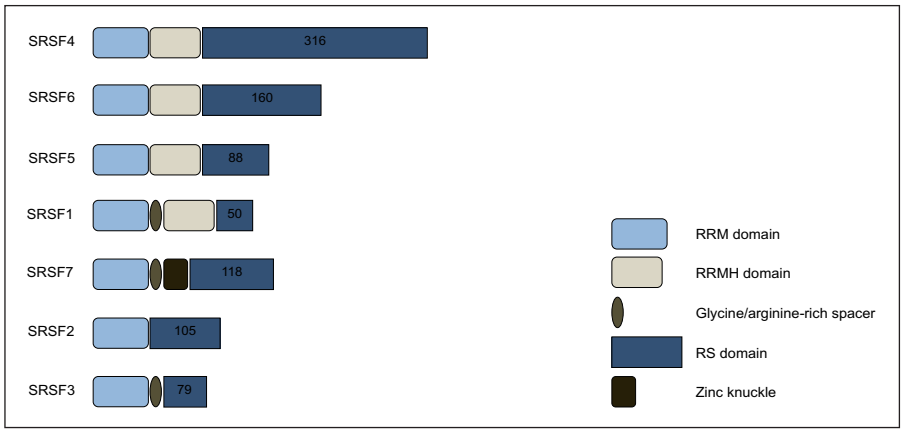

Figure 1: Domain structure of canonical SR proteins

inefficient. It also prevents the skipping of exons and may ensure their correct linear order in the spliced mRNA. Hence, SR proteins facilitate exon definition [18]. Because of differences in their affinity for ESEs, individual SR proteins activate splice sites differently or have even antagonistic effects [19, 20]. Using their RS domains as protein-protein interaction modules, SR proteins subsequently recruit other components of the splicing machinery to flanking 3' and 5' splice sites (ss). They can also bridge pairs of 5' and 3' ss via interaction of their RS domains, looping out the intronic sequence and juxtaposing both splice sites during early steps of spliceosome assembly [21]. The general consensus is that SR proteins stimulate exon inclusion in constitutive and alternative splicing; there are however, some reports where SR proteins bind to introns and act as splicing repressor [9]. Interestingly, a genome-wide study on AS events recently conducted in our lab revealed that targeted depletion of individual SR proteins leads to distinct changes in AS with a similar proportion of exon inclusion and skipping events (Änko, de Jesus Domingues, Müller-McNicoll & Neugebauer, unpublished).

SR proteins - redundant functions or functional specificity

The strict size, number and sequence conservation of individual SR proteins throughout evolution suggests that each SR protein has a distinct and essential function *in vivo*. Although it was shown that SR proteins are differentially expressed among tissues and cannot compensate for other members of the family in alternative splicing assays *in vitro* [19], it was believed for a long time that SR proteins have redundant functions and are largely interchangeable [18]. On the other hand, numerous studies that explored the functions of the prototypical member SRSF1 have made generalized conclusions implicating the whole SR protein family. This view is slowly changing, since evidence has accumulated showing that individual SR proteins are not functionally equivalent. For example, SRSF1 and SRSF2 were shown to be essential for cell viability and their depletion could not be rescued by overexpression of other SR protein family members [22, 23]. Furthermore, knockout mice for SRSF1, SRSF2 and SRSF3 show early embryonic lethality, indicating that SR proteins are not redundant [24]. Yet, the extent to which other SR proteins are required for different steps of gene expression is largely unknown. One obstacle has been the lack of specific antibodies specifically detecting individual SR proteins. To overcome this restriction and investigate the functions of individual family members *in vivo*, our lab has established the physiological expression of individual GFP-tagged SR proteins from bacterial artificial chromosomes (BACs) that are stably integrated into somatic or multipotent cell lines (Figure 2).

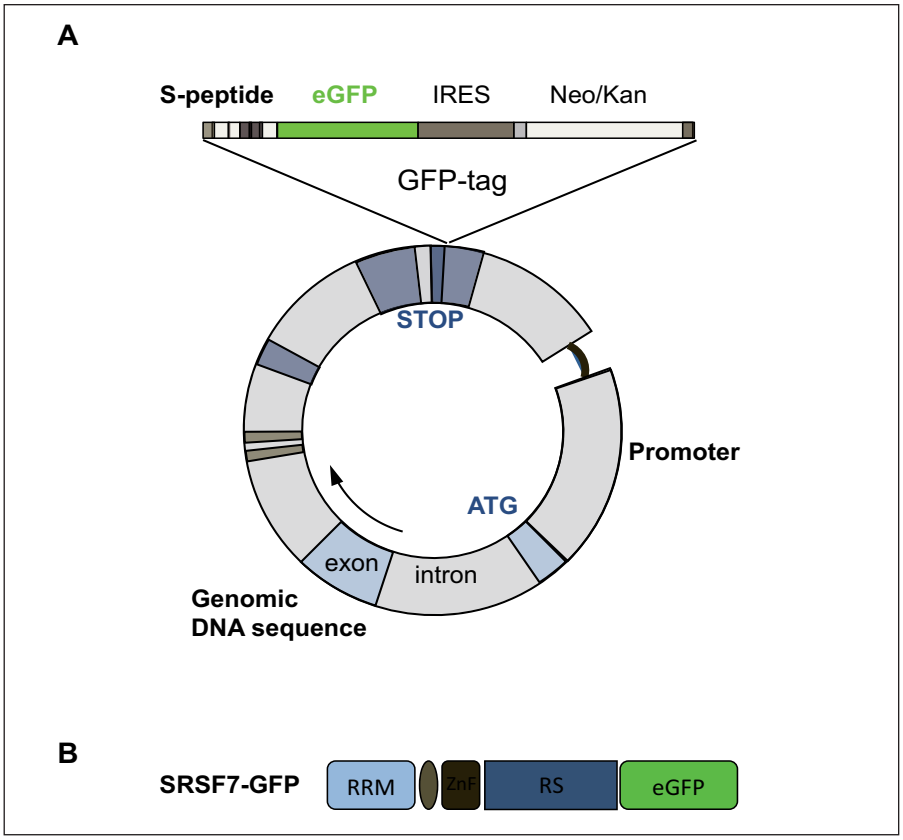

Figure 2: **A**) Bacterial artificial chromosome (BAC) recombineering to express tagged proteins at physiological levels under endogenous regulation. **B**) Example of GFP-tagged SRSF7

Multipotent P19 cells can be differentiated into neural cells showing distinct patterns of gene expression. Due to a tight co-regulation of the endogenous and stable transgenes, total SR protein levels are maintained in P19 cells [25]. The uniform GFP-tag on each protein facilitates purification of SR protein-containing mRNPs, from which protein and RNA components can then be analyzed. Using this approach, we found that each SR protein is associated with a distinct subset of nuclear and cytoplasmic polyadenylated mRNAs after splicing [26]. We furthermore identified endogenous mRNA targets of two different SR proteins in undifferentiated and neural P19 cells and found that mRNA targets of individual SR proteins change upon differentiation. Moreover, we showed that many target mRNAs required the associated SR protein for their expression [25]. In line with this, a current microarray study on

genome-wide AS events revealed that knockdown of individual SR proteins causes discrete changes in AS on largely non-overlapping targets (Änko, Domingues, Müller-McNicoll & Neugebauer, unpublished).

To further study the widespread functions of individual SR proteins in gene expression, the identification of endogenous RNA targets, their binding sites and binding motifs is necessary. *In vitro* binding specificities have been determined for some SR protein family members revealing that they have specific, yet degenerate RNA-binding specificities [27]. Recently, a novel technique named CLIP (crosslinking and immunoprecipitation) followed by high-throughput sequencing was developed to identify *in vivo* RNA binding sites [28]. One advantage of this method is the *in vivo* crosslinking step (e.g. UV light), which induces a covalent protein-nucleic acid bond. Consequently, protein-RNA interactions that occurred in intact cells are preserved during isolation of mRNPs. CLIP was used to define *in vivo* mRNA targets of SRSF1. Thousands of SRSF1 target sites were identified, and the rather degenerate binding motifs resembled sequences derived *in vitro* [29]. Our lab has used an improved technique called iCLIP [30], performed with GFP-tagged SR proteins expressed at physiological levels, to identify *in vivo* mRNA targets of SRSF3 and SRSF4 in a genome-wide manner with high resolution. GFP-tagged SR proteins recapitulated the binding patterns of endogenous proteins and could functionally rescue the endogenous SR proteins [25, 26]. Our analysis revealed that SRSF3 and SRSF4 bind to distinct target RNAs, including non-coding RNAs (ncRNAs), and have completely different binding motifs [31].

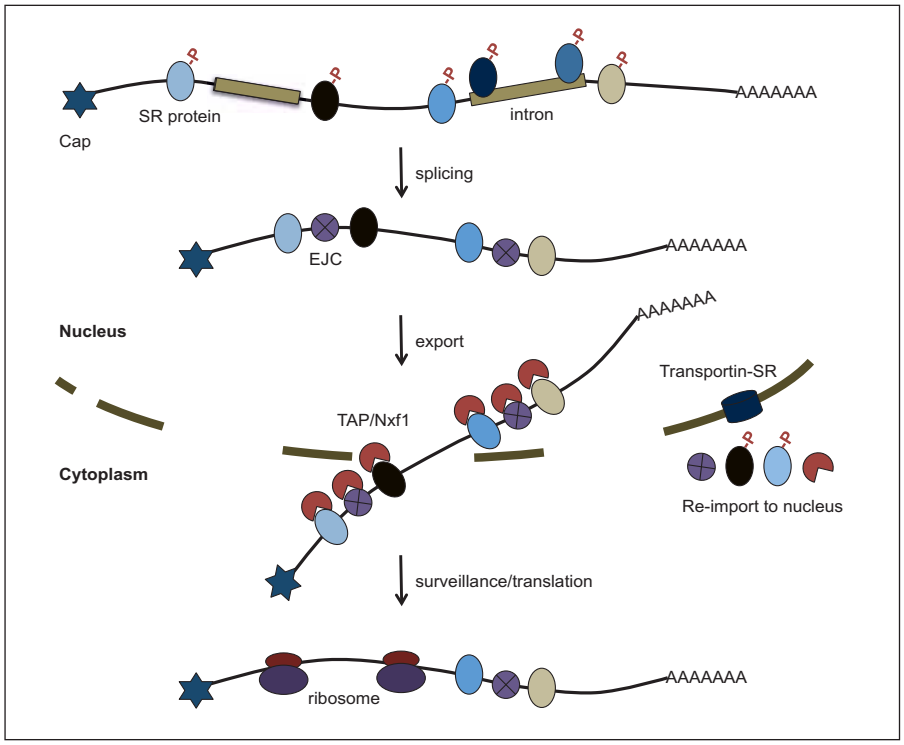

***Figure 3*. Model for the Multiple Roles of SR Proteins in mRNA Metabolism** (modified from [11])
Hyperphosphorylated SR proteins are recruited to pre-mRNA molecules, where they participate in splicing. Splicing results in the dephosphorylation of bound SR proteins and in the deposition of exon junction complexes (EJC) in the spliced RNA. Hypophosphorylated forms of the shuttling SR proteins recruit the mRNA export adapter TAP/Nxf1, which coordinates passage of the mRNP through the nuclear pore. Upon entry into the cytoplasm, a pioneer round of translation proceeds, and messages that contain PTCs are subjected to decay. Subsequent rounds of translation follow accompanied by mRNP remodeling. Rephosphorylation leads to the release of SR proteins, TAP/Nxf1 and the EJC. Phosphorylated SR proteins are re-imported by transportin-SR.

Regulation of SR proteins

Although SR proteins are abundant and ubiquitously expressed in nearly all cells and tissues, their expression levels are tightly regulated [19, 27]. Even small perturbations in the concentration of SR proteins or their phosphorylation status can change AS patterns of many pre-mRNAs [32]. Misregulation of SR proteins is further associated with developmental defects and numerous human diseases such as cancer, lupus or spinal muscular atrophy [27]. For example, SRSF1, SRSF2 and SRSF3 are proto-oncogenes with abnormal expression in many tumors [27]. Moderate overexpression of SRSF1 was shown to be sufficient to transform fibroblasts, which then rapidly form tumors in mice [33]. However, how the concentration or activities of SR proteins are regulated in cells is still not fully understood.

One example for the tight regulation of SR protein expression is the so-called unproductive splicing of SR genes. In mammalian cells, aberrant splicing often results in the inclusion of a premature termination codon (PTC) in spliced transcripts, which are then quickly degraded by the nonsense-mediated decay (NMD) pathway. SRSF3 is known to strictly regulate its own expression through inclusion of a PTC-containing exon [20, 25]. A similar feedback regulation has been suggested for all SR protein family members, as ultraconserved elements that overlap the alternatively spliced exons are present in all SR genes [34]. Using iCLIP, our lab confirmed that both SRSF3 and SRSF4 regulate their own expression through unproductive alternative splicing. Unexpectedly, we found that SRSF3 can also regulate the expression of several other SR protein family members.

Overexpression of SRSF3 led in all cases to alternative splicing introducing a PTC into the mRNA. Accordingly, transcripts levels were decreased due to degradation via the NMD pathway [31]. Remarkably, negative auto-regulation of SRSF1 expression is achieved by diverse mechanisms, including unproductive splicing, nuclear retention and translational repression. Here co-expression of three other SR proteins, including SRSF3, did not affect expression levels of SRSF1 [35]. Multi-level regulation might have evolved to control SRSF1 homeostasis more precisely.

Some SR proteins themselves were shown to modulate the NMD response. Specifically, for pre-mRNAs with a duplicated 5' ss, overexpression of several SR proteins lead to changes in splice site choices, resulting in PTC insertion into the transcript. Overexpression also significantly promoted degradation of these alternatively spliced mRNAs via the NMD pathway [36]. Again, different SR proteins differed in their ability to enhance NMD, with SRSF1 having the strongest effect.

Another way through which the various activities of SR proteins could be regulated is by posttranslational modifications (PTMs). It is well established that the reversible phosphorylation at multiple serine residues within the RS domain is regulated by a combination of various protein kinases and phosphatases [10]. Interestingly, two recent studies provided evidence that a long noncoding RNA, MALAT1, can also influence the cellular ratio and subcellular localization of phosphorylated versus dephosphorylated SR proteins [37]. MALAT1 was shown to bind to a subset of SR proteins in nuclear speckles and control their expression level, localization and activity [37, 38]. MALAT1 depletion compromised the recruitment of SR proteins from speckles to the sites of Pol II transcription, where splicing occurs [38], and also lead to changes in AS of a subset of transcripts. Another PTM, arginine methylation was so far only shown to be important for the functions of SRSF1. Methylated residues are located in the glycine/arginine-rich spacer region and influence AS, subcellular localization and post-splicing activities of SRSF1 [39].

Pre- and post-splicing activities of SR proteins

Apart from their role as constitutive and alternative splicing factors, unexpectedly, recent studies suggested additional roles for SR proteins in nuclear and cytoplasmic gene expression. Some activities are shared by several members of the SR protein family, but most are specific to one or two particular SR proteins, suggesting functional specificity of individual SR proteins.

SRSF1, the best-studied member of the SR protein family, has been implicated in several processes including transcription [40], mRNA stability [41], microRNA processing [42], mRNA translation [43], protein degradation [44] and maintenance of genomic stability [22], essentially influencing the fate of mRNAs from transcription in the nucleus to translation in the cytoplasm (Figure 3). Consistent with a role in transcription, depletion of SRSF1 and SRSF2 led to a dramatic decrease in the production of nascent RNA. In addition, SRSF2 was shown to promote Pol II elongation in a subset of genes [40]. It has been suggested that rapid recruitment to nascent RNA may only be possible if SR proteins are sitting directly on transcribing Pol II or are bound to chromatin. However, our lab has recently demonstrated that SR proteins are not directly bound to Pol II [26]. It remains to be seen whether SR proteins can bind to transcriptionally active chromatin. The involvement of SR proteins in transcriptional elongation might be related to their effect on the maintenance of genome stability. It has been shown that *in vivo* depletion of SRSF1 and SRSF2 induced double-stranded DNA breaks (DSBs) and DNA recombination, triggering the DNA damage response and cell cycle arrest [22, 23].

While SRSF3 was shown to be involved in 3'end processing and polyadenylation of pre-mRNA as a splicing-related function [45], a splicing-independent modulation of mRNA stability was demonstrated for SRSF1 and one single transcript, PKCI-1. Sequence-specific binding of SRSF1 destabilized PKCI-1 mRNA by an unknown mechanism [41]. It is currently unclear whether SR proteins have a general role in controlling polyadenylation or mRNA stability.

SRSF3 and SRSF7 are associated with poly(A)+ RNA in the nucleus and the cytoplasm and are required for the nuclear export of the intronless histone H2a transcript [11]. Both SR proteins bind to a specific element in the coding region of H2a and recruit the main mRNA export receptor TAP. Interestingly, only the dephosphorylated form of SR proteins can bind to mRNA and TAP [11]. It was proposed that SR proteins could serve as adapters for TAP-dependent mRNA export. Although export of mRNAs by SR proteins has been only demonstrated using intronless reporter RNAs, dephosphorylation of SR proteins, which occurs during splicing, could represent one mechanism for the selective export of spliced mRNAs versus unspliced pre-mRNAs [11]. Consistent with their proposed role as mRNA export adapters, most members of the SR protein family remain bound to the spliced mRNAs in post-splicing mRNPs isolated *in vitro* [46] and *in vivo* [26, 47], and shuttle continuously between the nucleus and the cytoplasm [26, 48]. Shuttling is conferred by the RS domain and is affected by phosphorylation [48]. Interestingly, individual SR proteins exhibit differences in their nucleo-cytoplasmic shuttling activity [26]. Although the basis for these differences is unknown, shuttling efficiency correlates with protein size and RNA-binding capability [29, 31]. Once in the cytoplasm, shuttling SR proteins are rapidly re-phosphorylated, which triggers their disassembly from TAP and the exported mRNA cargo. In addition, re-phosphorylation stimulates their nuclear re-import via transportin-SR [11, 48] (Figure 3). The exception is SRSF2; its inability to

shuttle is related to its unusual RS domain. SRSF2 contains a dominant nuclear retention signal (NRS) within its RS domain [49], which confers phosphatase resistance. As a consequence, SRSF2 is resistant to dephosphorylation normally occurring during splicing [50] and cannot bind to TAP [11].

A direct role for SRSF1 in mRNA translation was postulated because SRSF1 was found associated with 80S ribosomes and polysomes, and because its overexpression in human cells stimulated translation of an ESE-containing reporter mRNA *in vivo* and *in vitro* [43]. This direct effect of SRSF1 in translational control is mediated by the recruitment of components of the mTOR (mammalian target of rapamycin) signaling pathway, which causes the phosphorylation and release of the cap-binding protein 4E-BP1, a competitive inhibitor of cap-dependent translation. As a result, cap recognition and translation initiation are enhanced [51]. SRSF3 and SRSF7 have also been implicated in translational control. SRSF3 was shown to function in internal ribosome entry site (IRES)-mediated translation of a viral RNA [52], whereas SRSF7 plays a role in the translation of unspliced mRNAs containing a constitutive transport element (CTE), which is bound directly by TAP [53].

Finally, the multifunctional protein SRSF1 was recently reported to have another unexpected role in regulating the sumoylation pathway. Specifically, it is involved in heat shock-induced sumoylation and promotes conjugation of SUMO (small ubiquitin-related modifier) to several RNA-processing factors [44]. Interestingly, SRSF3 and SRSF5 failed to stimulate protein sumoylation under the same conditions.

Conclusions

Shuttling SR proteins constitute a group of multifunctional regulatory proteins that may have evolved to perform different functions in the nucleus and the cytoplasm, hence controlling gene expression in both compartments and coupling gene expression machineries. An outstanding question is how individual SR proteins influence the fate of mRNAs in cells and couple pre-mRNA splicing in the nucleus to mRNA translation in the cytoplasm. SR proteins provide an opportunity to systematically determine the role of RNA-binding proteins in each step of gene expression. Using our BAC-tagging approach, we can compare and contrast individual family members that are structurally highly related in a genome-wide manner. Recent studies from our and other labs have revealed mostly non-overlapping mRNA targets of SR proteins and differences in alternative splicing patterns, nucleo-cytoplasmic shuttling activities and post-splicing functions, suggesting essential non-redundant functions of each member of the SR protein family in all phases of gene expression. However, many open questions remain. Little is known about the composition of shuttling mRNPs, the endogenous mRNA targets of many individual SR proteins and their sequence specificity. Also, the mechanisms of nucleo-cytoplasmic shuttling by SR proteins have been largely unexplored.

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The mechanochemistry of cellular polarization

Stephan W. Grill

Introduction

The establishment of developmental form rests on an interplay between chemistry (1) and mechanics (2, 3). Biochemical regulatory pathways direct the active deformation and reshaping of cells and tissues. Components of these regulatory pathways are transported by flow and deformation arising from active mechanical processes inside cells (4). In some instances one can successfully decouple the biochemistry from the mechanics (1), but we are learning more and more that generally this is not possible (4). The actomyosin cortex is responsible for much of the reshaping and mechanical restructuring that proceeds at both the intracellular and the tissue scale. We must seek to find an appropriate mechanical description of the actomyosin cell cortex and then integrate with biochemical regulation if we ever want to understand the fundamental mechanisms by which patterning, structure and form arises in development.

The process of establishment of cellular polarity in *Caenorhabditis elegans* zygotes is a classical example of the coupling of mechanical and biochemical processes for the purpose of enacting morphogenetic change. The system is well described. Indeed, a large amount of what we know about polarity establishment in general comes from studies in *C. elegans* (5, 6). In the zygote, PAR proteins (for partitioning-defective, originally identified in *C. elegans* (7)) segregate into two antagonistic groups that inhabit mutually exclusive membrane domains (Figure 1). These are responsible for the spatial regulation of downstream polarity pathways, including those that maintain the stem-cell character of the germ-line cell lineage. Pattern formation in this conserved cell polarity pathway, in terms of the formation of an anterior and a posterior PAR domain, comes about through transient and actively generated flows of the actomyosin cell cortex (8). We have studied this system over the past several years in a quantitative and biophysical approach. To a certain degree this mechanochemical problem can be uncoupled, and I will review the mechanical and biochemical processes separately before discussing their coupling.

The *C. elegans* zygote

I will begin by describing the general chain of events that leads to the establishment of cell polarity in *C. elegans* embryos. Polarization occurs in a highly stereotyped and reproducible fashion, which is ideal for quantitative investigations. For the purpose of this discussion, the first embryonic cell cycle can be broken down into three phases (5, 6, 9, 10). There is an initial establishment phase during which anterior PAR proteins (PAR-3, PAR-6, and atypical protein kinase C) are segregated to the anterior membrane. Simultaneously, a small posterior membrane domain of posterior PAR proteins (PAR-1, PAR-2, and LGL) forms and expands to occupy approximately half of the cellular surface. This is followed by a maintenance phase during which the two domains persist in a stable state - each occupying roughly one half of the embryo's surface. Finally, there is a correction phase that coincides with cytokinesis, during which the boundary between the PAR domains is displaced to match the ingressing cleavage furrow, thereby ensuring differential inheritance of PAR proteins by the two daughter cells.

In the establishment phase, the initial segre-

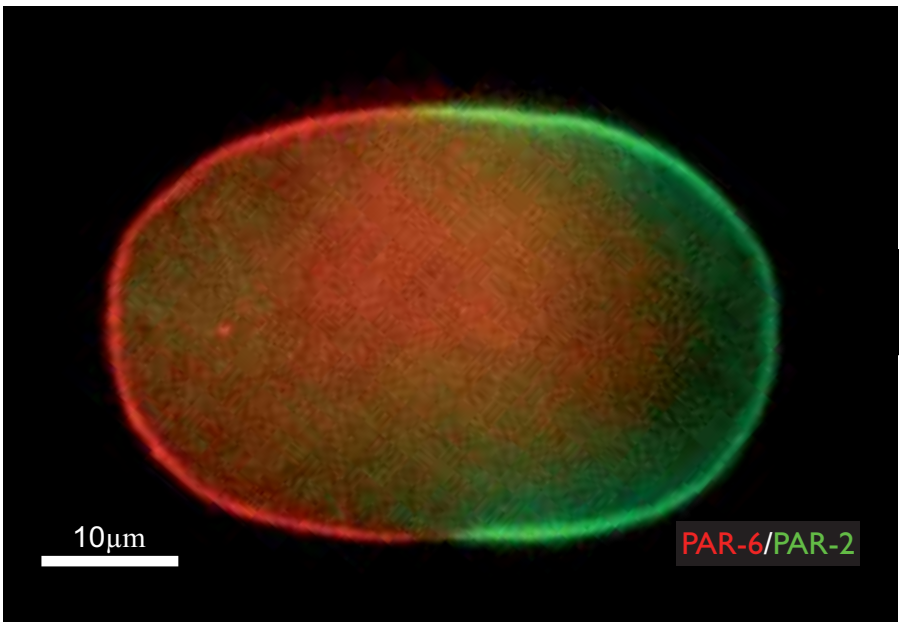

Figure 1: A single cell stage *C. elegans* embryo that has undergone polarization. The anterior PAR domain is characterized by the localization of PAR-6 (red), the posterior PAR domain by the location of PAR-2 (green). Courtesy Nathan Goehring.

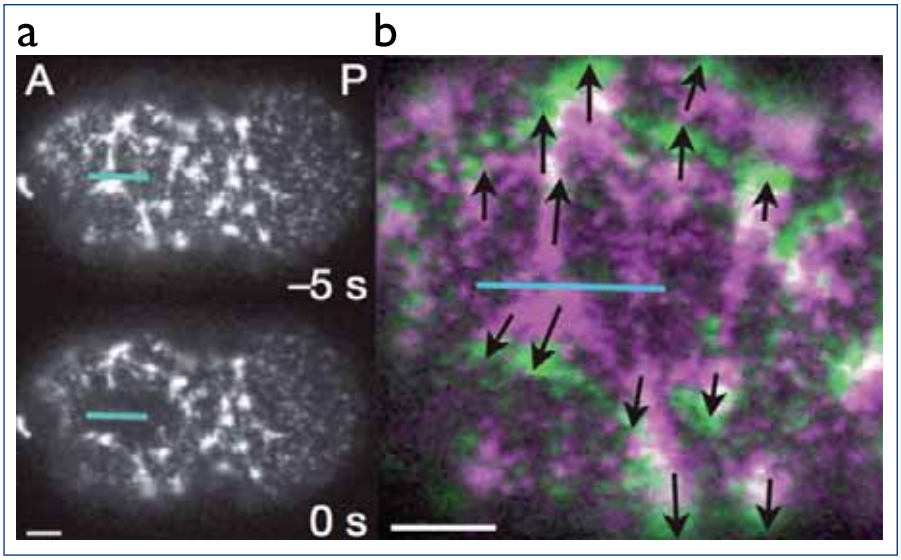

Figure 2: Lasercutting of the *C. elegans* cortex is used to measuring cortical tension, for the purpose of characterizing its local mechanical state. The cortex is cut along the blue line. **a**, Pre- (top) and post-cut image (bottom) in an NMY-2-GFP zygote. A, anterior, P, posterior. **b**, enlarged overlay, Arrows indicate the resultant recoil and show displacements between pre-cut (purple) and post-cut frame (green). Scale bar, 5 µm. Adapted from (12).

gation of the anterior PAR proteins depends on a dramatic and coordinated reorganization of a thin, contractile, and membrane-associated actomyosin network (8, 9, 11). Asymmetries in contractility of this network, due to spatial differences in myosin activity along the A-P axis, result in an anterior-directed and long-ranged flow of cortical actomyosin (8, 12). These flows appear to transport anterior PARs into the anterior independent of the biochemical interactions between anterior and posterior PARs (8, 13). However, flows are transient and contractility of the actomyosin cortex is greatly reduced at the transition from establishment to maintenance phases, with the consequence that long-ranged flows cease. At this point, the cortex becomes dispensable for the maintaining the segregation of the PARs (9, 10). Instead, maintaining asymmetry over the maintenance phase depends on biochemical interactions between anterior and posterior PARs (5, 6, 9, 10). Finally, during the correction phase, actomyosin contractility and cortical flows reappear to drive cytokinesis and alignment of the PAR boundary with the ingressing furrow.

Mechanics

To understand the generic coupling between mechanics and biochemistry in *C. elegans* polarity establishment, it is first necessary to understand how *C. elegans* cortical flow comes about. From a physical point of view, the cortex is a thin film (1-2 µm thick) of a highly dynamic viscoelastic gel of cross-linked semiflexible polar polymers, which actively generates forces due to the presence of ATP-consuming myosin motors and ATP-dependent treadmilling (14, 15). The cortex can be described at different length, time and force scales (16). Although its molecular constituents are very small (≈5 nm for an actin monomer, the microscopic scale), individual actin filament length is of intermediate order (hundreds of nm to a few µm, the mesoscopic scale), and collectively the components interact to form a higher-order network that spans the entire cell (several tens of µm, the macroscopic scale). At a sufficiently coarse-grained level, the cortex can be described as a bulk material using a continuum description, omitting reference to the underlying details of molecular origin and structure. The active polar gel theory (12, 15, 17-19) represents such a coarse-grained physical description. This theory represents a versatile tool to understand the physical principles governing the cortex as a whole. Most importantly, it has brought understanding concerning the mesoscale biophysical laws for the mechanics of actomyosin deformation and flow in development (20).

For the *C. elegans* zygote, the identification of the physical mechanisms that are responsible for generating cortical flow necessitated measurements of the sub-cellular distribution of cortical tension (12). We achieved this through the usage of a pulsed UV laser to cut the cortex (cortical laser ablation, COLA, Figure 2) and measure its recoil. This provided us with a position- and direction-dependent map of cortical tension during cortical flow. Importantly, these experiments revealed an anisotropy in cortical tension in the anterior domain. This anisotropy is the result of a balance of active and viscous stresses, and can be explained through a hydrodynamic description of the cell cortex in terms of a thin film of an active and viscous fluid, as described above. Through this, we identified two prerequisites for large-scale cortical flow: a gradient in actomyosin contractility to drive flow, and a sufficiently large viscosity of the cortex to achieve a long-ranged effect, to drive cortical flow along the entire length of the zygote for efficient PAR protein segregation.

To conclude, this work (12) has brought a simple mesoscale physical description of the thin and mechanically active actomyosin cortex, a kind of *Navier-Stokes equation* for the cortex that describes by which biophysical laws active and myosin-driven contractions drive large scale deformation and flow.

Chemistry

Much has been learned in the past decade

concerning the molecular constituents of both the anterior and the posterior PAR complex, and their modes of interaction (5, 6, 9, 10). Key to the ability to form domains appears to be the ability of these two groups of proteins to form anterior and posterior PAR complexes which exclude each other from the membrane, This yields a cell membrane that tends to exist in one of two states: an anterior like state with anterior and a posterior-like state with posterior PARs. To reveal the physical mechanisms by which this exclusion tendency allows for the generation of a PAR polarity pattern, it was necessary to investigate PAR protein dynamics, to characterize their mobility. We achieved this through a quantitative Florescence Recovery After Photobleaching (FRAP) technique that we developed, by which it is possible to measure the coefficient of lateral diffusion in the plane of the membrane together with their rate of spontaneous detachment and entry into the cytosol (21). This is achieved by photobleaching molecules in the plane of the membrane in squares of varying sizes, and recording the spatiotemporal characteristics of the return of fluorescence into the bleached area. Through this technique we were able to show that PAR proteins are free to diffuse within their domain and also across the domain boundary, which gives rise to a continuous loss of PAR proteins across the domain boundary by diffusion (13). There is overlap between the two domains (Fig. 1), and the extent of this overlap region quantitatively matches with what is expected from the measured rates of lateral diffusion and detachment (13). To conclude, this work suggested that the stably polarized embryo reflects a dynamic steady state in which PAR proteins undergo continuous diffusion between their respective domains. Placing both PAR complexes in a mathematical framework that takes into account the measured motile behaviors allows to generate stable PAR polarity patterns that are similar to the experimentally observed ones

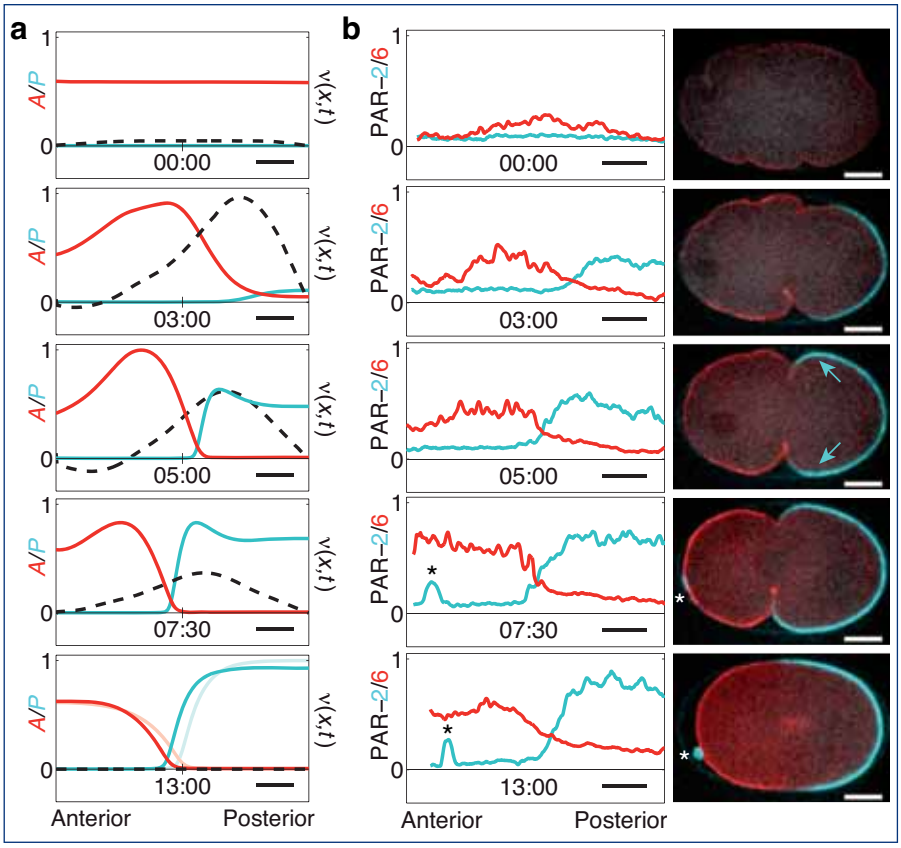

Figure 3: PAR polarization in *C. elegans* proceeds by advective triggering of a pattern-forming system. This figure provides a comparison between flow-triggered polarization in theory and experiment. **a**, Theory, calculated anterior (A, red) and posterior (P, cyan) PAR profiles with the unpolarized embryo subject to measured wild-type flow velocities (dashed black line; positive velocities are toward the anterior). Shaded lines in last time point indicate the final (steady state) distributions that are achieved when flow ceases. **b**, Experiments, GFP::PAR-6 (red) and mCherry::PAR-2 (cyan) fluorescence profiles (left) and still images (right) of a single cell stage *C. elegans* embryo undergoing polarization. The small PAR-2 peak in the anterior (*) is due to the polar body. Adapted from (23).

(Figure 3, bottom graphs) (22, 23). This capability relies on an antagonistic activity that allows both PAR complexes to induce the detachment of the other species, and this feedback needs to be sufficiently strong (23). This capability also relies on limiting pools of PAR protein amounts (23). Limiting pools are important for determining the size of the respective PAR domains, the more anterior PARs or posterior PARs present in the zygote, the larger the size of the respective PAR domain. We were able to test this model prediction using RNAi rundown experiments together with over- and under-expression studies, which revealed that PAR amounts indeed specify domain size in the embryo (23). To conclude, these motile behaviors and interactions suggest a mechanism by which PAR patterns can be generated, and describe how PAR domains are stably maintained. However, they do not address how the embryo switches from a stably unpolarized to a stably polarized state.

Mechanochemistry

In the zygote, switching to a stably polarized state relies on the transient period of cortical flow. Before explaining how flows enable this switching, I will first describe a thought experiment that discusses in fundamental

terms how biochemistry and mechanical flow could interact for the purpose of mechanochemical pattern formation (24). Imagine a class of molecules that are bound to the membrane and that locally up-regulate contractility of the adjacent cell cortex. These molecules are assumed to diffuse freely in the plane of the membrane. Now if, by chance, there are a few more of such regulators in a particular region, this will cause the cell cortex in this region to contract more than the cortex elsewhere. This in turn drives cortical flow towards this region, which will bring in more of the regulator through what is known as advective transport (similar to a swimmer carried downstream in a fast-flowing river). This then enhances contractility even more, resulting in a positive feedback that can destabilize the system towards the formation of steady state patterns. These patterns are special, because they continuously rely on cortical flow to counteract diffusion, to keep concentrating the regulator in those regions towards which the cortex flows (24). To summarize, this novel pattern forming mechanism relies on a two-way feedback: first, the chemical species up-regulates local contractility, and second, cortical flow transports the species by advection.

In light of this thought experiment, let us revisit the *C. elegans* zygote. First of all, cortical flow is transient, and the PAR pattern persists even after cortical flows cease (9, 10). The PAR pattern is therefore distinct from patterns generated in the thought experiment. In *C. elegans*, maintenance relies on mechanisms that are PAR intrinsic, as described above. There is a key similarity, however, which is that anterior and posterior PARs diffuse slow enough in the plane of the membrane so that they can be efficiently transported and segregated by cortical flow, through the process of advection. Indeed, advective transport can account for the transient and cortical-flow driven pattern of segregation of anterior PARs that is observed in embryos that are depleted of posterior PAR protein PAR-2 (23). If we now add the ability to form PAR patterns by adding the posterior PAR complex to the model, this transient and cortical-flow driven segregation can trigger polarization of the system, and cause it to switch from a stably unpolarized to a stably polarized state. Figure 3 shows that the spatiotemporal behavior of PAR segregation during flow-triggered polarization in the model matches what is seen in the experiment under normal conditions. Thus, by inducing a transient segregation, advection by cortical flow serves as a mechanical trigger for the formation of a PAR pattern within an otherwise stably unpolarized system (23).

To conclude, much remains to be learned about how biochemical pathways interact with mechanical ones. For example, the *C. elegans* mechanocemical polarization mechanism is complemented by a rescue mechanism that appears to operate through microtubule-induced formation of a posterior PAR domain that can serve to polarize the zygote under conditions where flows are absent (25). This indicates a requirement for including microtubule dynamics and mechanics in future models. One thing is clear today as much as it was clear to D'Arcy Thompson nearly a century ago (2): mechanics needs to be included in descriptions that seek to explain the ways by which patterning, structure and form arises in development.

Acknowledgements

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Stephan Grill, born in Germany, studied Physics at the University of Heidelberg. He switched to a topic in Cell Biology for his PhD, where he joined the European Molecular Biology Laboratory (EMBL) in Heidelberg. Since then he has been interested in combining theory and experiment to understand the underlying physical mechanisms of biological processes. After postdoctoral studies in Berkeley, where he was a postdoctoral fellow of the Helen Hay Whitney Foundation, he went to Dresden to head a research group that is jointly appointed to the Max Planck Institute for the Physics of Complex Systems and the Max Planck Institute of Molecular Cell Biology and Genetics. The group consists to approximately equal parts of theorists and experimentalists, and focuses its work on the mechanochemistry of development and on the biophysical mechanisms of transcription. Stephan Grill was awarded the 2009 Minerva ARCHES Prize, became an EMBO Young Investigator in 2010, and was awarded the Paul Ehrlich- und Ludwig Darmstaedter-Nachwuchspreis in 2011.

Building Brains with More Neurons by Manipulating G1 Length of Neural Stem Cells

Federico Calegari

Introduction

Mammalian neural stem cells of the developing central nervous system form a polarized epithelium, named the ventricular zone, whose apical domain delimits the lumen of the neural tube. As development proceeds, an increasing proportion of neural stem cells switch from divisions that generate additional apical stem cells (expansion) to divisions that generate more committed progenitors or postmitotic cells (differentiation)[1, 2]. Specifically, the switch to differentiation in the cerebral cortex leads to the generation of neurons as well as of basal progenitors that leave the ventricular zone and form a second germinal region at its basal boundary, the subventricular zone, which was suggested to be important for increasing cortical surface area during evolution[3-5] (Figure 1A). Both apical and, to a lesser extent, basal progenitors can expand or can differentiate to generate neurons through asymmetric or symmetric divisions, respectively. This leads to the self-renewal of apical progenitors and consumption of basal progenitors. In essence, it is the balance between expansion, self-renewal and consumption that establishes the cellular output of the precursor pool and, as a result, brain size[1, 2].

While most neural stem cells are consumed during development to generate neurons or glia, a minor proportion persists in the adult subventricular zone of the lateral ventricle and the subgranular zone of the hippocampus where they become a source of new neurons and glia throughout life[2, 6]. Adult neurogenesis is thought to be important for learning, memory and emotional behaviour[6-8] and it is generally believed that the potential of adult neural stem cells to unlimitedly expand and serve as a source of differentiated neural cell types may be important for developing novel approaches of regenerative medicine[9, 10]. Thus, great efforts are invested worldwide to understand the mechanisms controlling the switch from proliferation to differentiation of neural stem cells and to manipulate this process for therapy of neurodegenerative diseases.

Controlling the expansion of neural stem cells in the adult mammalian brain has proven particularly difficult. In fact, in contrast to embryonic development, the vast majority of adult neural stem cells in vivo is quiescent. Moreover, the few neural stem cells that divide do so to generate more differentiated progenitors giving rise to neuro-

blasts that are soon consumed to generate postmitotic neurons[2, 6] (Figure 1B and 2A). In essence, neural stem cells in the adult mammalian brain are few, cycle at extremely low rate, and are soon consumed to generate postmitotic cells and, until very recently, no methodology has been developed to transitorily promote their expansion to ultimately increase neurogenesis in vivo.

The role of cell cycle length

For over five decades mathematical analyses have been used to measure cell cycle parameters upon DNA-labeling during S phase of neural precursors in various species, from rodents to primates[11]. These studies have consistently shown that cell cycle lengthens as development proceeds and that this is mostly due to a lengthening of G1[12]. Within each developmental stage, cell cycle length was found to be heterogeneous with cells undergoing differentiation having a longer G1 than those undergoing proliferation[13], an effect that is due to a lengthening of G1 in basal progenitors[14]. In essence, lengthening of G1 in neural precursors was shown to correlate at the temporal[12], spatial[15] and cellular[13, 14] level with their switch from expansion to neurogenesis. Typically, this correlation has been explained as if lengthening of G1 should be a consequence, among many, of differentiation. Alternatively, and perhaps more intriguingly, G1 lengthening may itself be a cause of neurogenesis.

During my postdoctoral work, I investigated this possibility by pharmacologically lengthening, without blocking, the G1 phase of neural stem cells in embryos developing in a culture system and found that this alone was sufficient to induce premature neurogenesis[16]. To explain how cell cycle length can influence the fate of somatic stem cells, we proposed *the cell cycle length hypothesis* according to which a long G1 may allow the accumulation of factors, and their triggered effects, until the threshold needed for differentiation to occur[16]. Interestingly, *the cell cycle length hypothesis* suggested that expansion of neural, and perhaps any other somatic, stem cells could be achieved by shortening, instead of lengthening, G1, which would allow us to overcome a major obstacle in the study of somatic stem cell and they potential use in therapy.

Shortening of G1 was attemped in my laboratory at the Center for Regenerative Therapies Dresden (CRTD) by manipulating the expression of positive regulators of G1 progression in neural stem cells of the developing mouse cortex using in utero electroporation. We found that acute overexpression of the cyclin-dependent kinase4/cyclinD1 (referred to as 4D) complex i) inhibited the physiological lengthening of G1, ii) decreased neurogenesis, and iii) promoted the generation and expansion of basal progenitors, thus, resulting in iv) mice born with an increased cortical surface area due to a transitory expansion of the progenitor pool. Conversely, 4D RNAi induced a lengthening of G1, promoted neurogenesis, and depleted the progenitor pool[17]. Thus, lengthening of G1 is both necessary and sufficient to induce the differentiation of neural stem cells and a transient 4D manipulation during embryonic development can be used to increase the cellular output and size of the mammalian brain[18].

For this finding to be relevant for future stem cell-based therapies, it was fundamental to investigate whether a similar principle would apply beyond the nervous system of the developing embryo such as in adult neural, or even non-neural, stem cells. Using our knowledge of neural stem cells of the developing mouse embryo, we thought

that by overexpressing 4D in adult neural stem cells in a transient and temporally controlled manner we would be able to achieve this goal. Combining 2A peptides[19], Cre-recombination, and viral infection, we first developed a method that allowed us to acutely overexpress 4D (On) by stereotaxic injection of lentiviral particles in the adult hippocampus of transgenic mice expressing Cre-recombinase. After the desired period of time, mice were administered tamoxifen, which deleted the ectopic, loxP-flanked, 4D viral cassette (Off). We found that 4D overexpression inhibited neurogenesis while triggering a 2-fold expansion of adult neural stem cells (Figure 2B vs. A). After tamoxifen-dependent deletion of the 4D cassette, neural stem cells could resume physiological neurogenesis, thus, doubling the number of

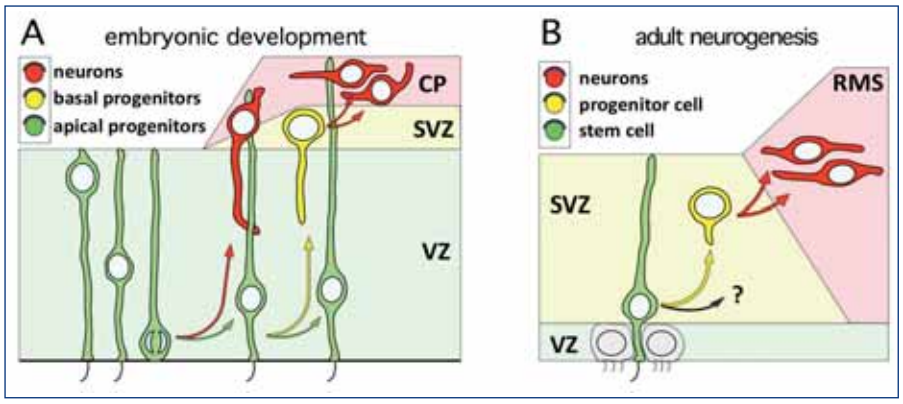

Figure 1: (**A** and **B**) Embryonic apical progenitors and adult neural stem cells (green) are highly elongated, polarized cells with radial morphology that expand through symmetric proliferative divisions or, alternatively, generate intermediate progenitor types (yellow) that, adopting a rounded morphology, lose polarity and are soon consumed to generate neurons (red). VZ=ventricular zone; SVZ=sub-ventricular zone; CP=cortical plate; RMS=rostral migratory stream. Gray cells represent ependymal cells of the adult VZ. Figure adapted from Salomoni and Calegari[18].

neurons generated in the adult hippocampus[20] (Figure 2C vs A).

To the best of my knowledge, this represents the first temporally controlled expansion of somatic stem cells performed in an adult mammal, which may have a huge potential for better understanding the role of adult neurogenesis in cognitive function and, perhaps, using neural stem cells for therapy.

Conclusions

The correlation between cell cycle lengthening and differentiation is not limited to neural precursors of the developing cortex but seems to hold true for almost any paradigm of stem cell differentiation[18, 21]. For example, extraordinarily fast cell cycles, even shorter than 30 minutes and without G phases, are characteristic of pluripotent embryonic stem cells in early phases of animal development in all phyla[22]. It is with gastrulation that cell cycle progressively lengthens and differentiation ensues with the formation of ectoderm, mesoderm and endoderm. Remarkably, induced pluripotent stem cells also display a very short G1 and removal of growth suppressive checkpoints greatly accelerates reprogramming[23].

Moreover, even if direct validation of a role of G1 length in the differentiation of non-neural stem cells has not yet been provided, Orford and Scadden have proposed a model for adult hematopoietic stem cells whereby cell cycle progression and quiescence are integrated with stem cell expansion versus differentiation. Equivalent to *the cell cycle length hypothesis*, early-G1 is considered a sensitive period during which differentiation may occur. Thus, increasing its length would favor stem cell differentiation and exhaustion while shortening G1 would promote their expansion[24].

The concept that solely time may influence the outcome of a chemical reaction should hardly surprise. Yet this notion was rarely applied to the differentiation of somatic stem cells. This is not to say that a differentiation program will necessarily require a change in cell cycle length. Clearly, *time* (G1 length) may not be the major limiting factor when a differentiation signal is sufficiently potent. Yet, under certain conditions, *time* alone may make a critical difference.

Five decades of cell cycle measurements in the developing brain and our recent tools to acutely and transitorily manipulate G1 length of neural stem cells during embryonic development or adulthood have allowed us to demonstrate the validity of this principle in vivo[17, 18, 20, 21]. The potential applications of this finding for basic research and therapy can be enormous and I hope that this may stimulate other laboratories to investigate *the cell cycle length hypothesis* in other paradigms of stem cell differentiation.

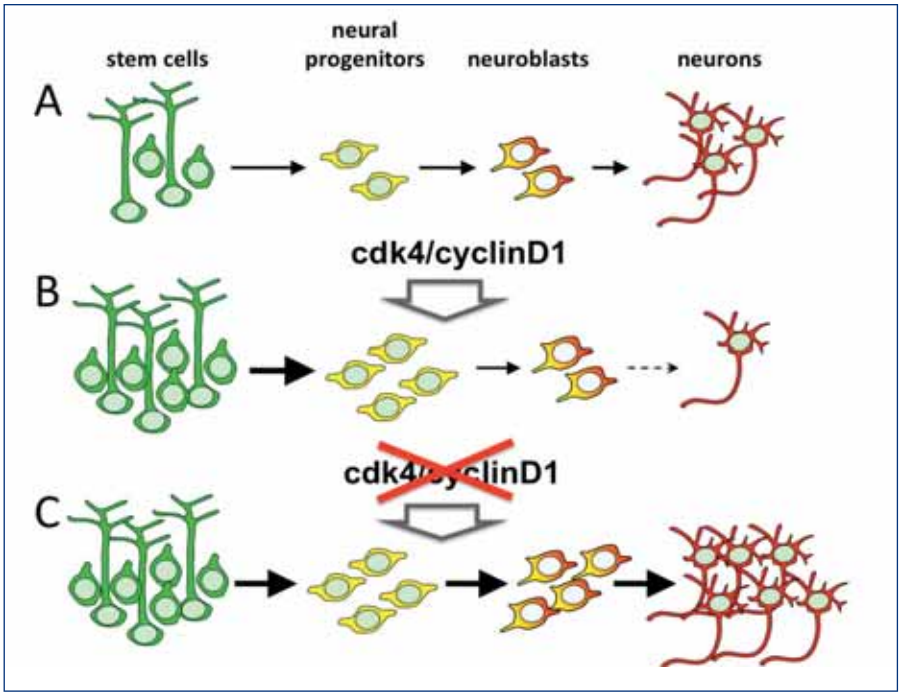

Figure 2: (**A**) Cell types of the adult hippocampus represented according to their relative abundance under physiological conditions. (**B**) Cdk4/cyclinD1 overexpression for 3 weeks induced a 2-fold expansion of stem and progenitor cells with concomitant reduction in neurogenesis. (**C**) 3 weeks of cdk4/cyclinD1 overexpression followed by removal of the cdk4/cyclinD1 cassette for additional 2 weeks allowed manipulated stem cells to resume a physiological balance between proliferation and differentiation, thus, doubling the number of neurons generated in the adult brain. Figure adapted from Artegiani et al.[20].

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Federico Calegari was born in 1971 in Buenos Aires, Argentina. At the age of 12, he moved to Milano, Italy; where he completed his academic training under the supervision of Patrizia Rosa at the CNR-National Institute of Neuroscience. In 2001, he relocated to Dresden to start his postdoctoral work in the laboratory of Wieland Huttner, director of the Max Planck of Molecular Cell Biology and Genetics. In 2007, Federico Calegari become group leader at the newly founded DFG–Research Center and Cluster of Excellence for Regenerative Therapies Dresden. His laboratory studies embryonic and adult neurogenesis and the role of the cell cycle in stem cell decisions.

Seeing is believing: Visualizing intercellular signalling at the cell biological level

Adam P. Kupinski and Christian Bökel

Eukaryotic cells communicate with the help of a limited number of dedicated and widely conserved signalling cascades. Over the last few decades, genetic analysis using model organisms such as flies and nematodes greatly contributed to identifying the components of the different pathways. In contrast, biochemical methods, mainly using cultured cells, were instrumental in identifying the molecular functions of these signal transduction molecules. However, these methods yield information at specific but different scales. Much less is understood about how intercellular propagation and intracellular transduction of these signals is organized at the subcellular to tissue level. Consequently, we are still far away from comprehensively and quantitatively understanding the complex interplay of intercellular signalling events that govern developmental processes and the function and homeostasis of mature organs.

Our lab is interested in signal transduction within stem cell niches. Such niches are specialized regions within a tissue, where a specific growth factor microenvironment provided by niche stromal cells allows stem cells to acquire and maintain their fate. Stem cells or their progeny that leave the range of the niche signals will differentiate by default, thus protecting the organism against uncontrolled proliferation of the self replicating stem cell population. Clearly, the generation, propagation, range, and interpretation of these niche signals must be tightly regulated to control the size of the stem cell pool. This makes stem cell niches an ideal subject for studying the subcellular-to-tissue level middle ground mentioned above.

We are using the stem cell niches of the *Drosophila* gonad as model systems to study the interplay between niche organisation and signalling. In the fly testis, germ line stem cells (GSCs) and somatic stem cells surround a cluster of differentiated, somatic cells called hub that serves as the major source of niche signals (Figure 1). Hub cells produce the BMP family ligands Dpp and Gbb as well as the cytokine-like growth factor Upd that activates the Jak/Stat signalling cascade via its receptor Domeless (1). While BMP signalling directly affects GSC stemness by repressing the germline differentiation factor Bag-of-marbles (Bam) (2), Upd seems to primarily act indirectly via the somatic stem cells (3). GSC maintenance is strictly depended on contact to the hub: GSCs detaching from the hub following loss of Cadherin mediated adhesion begin to express Bam and lose stem cell fate (4). Since BMP growth factors are prototypic long range morphogens this contact dependence of the niche signals is surprising. However, the situation is reminiscent of the mammalian bone marrow. There, N-cadherin mediated adhesion is thought to orchestrate and restrict communication between individual hematopoietic stem and niche cells. This idea has been formalized as the stem cell niche synapse hypothesis (5).

We therefore decided to test whether a similar, cadherin based stem cell niche synapse might also operate in the fly testis: Restriction of the BMP niche signal to the junctions between individual hub cells and GSCs might explain its unusually short range. However, simply mapping the distribution of ligands and other pathway components, e.g. via GFP tagged receptors, is not sufficient to test this hypothesis. Instead, one needs a tool that allows distinguishing the occupied and actively signalling receptors from the inactive pool with subcellular resolution.

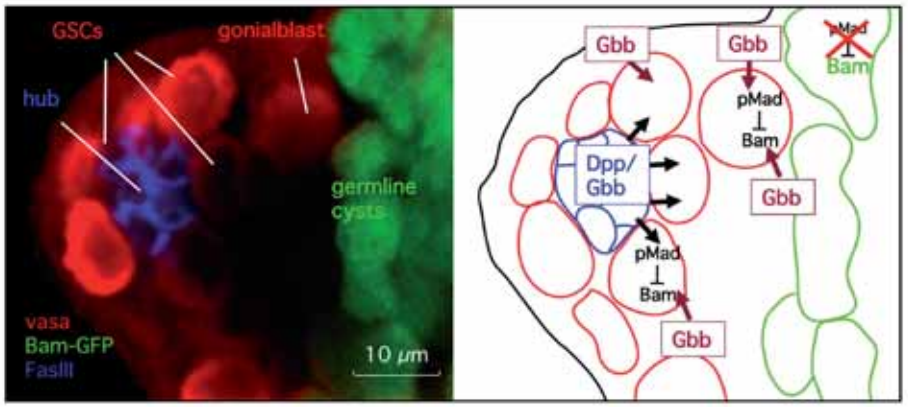

Figure 1: In the Drosophila testis tip, germline cells (vasa, red) surround a group of somatic cells termed hub (FasIII, blue). Only cells in contact with the hub retain GSC fate. Expression of the germline differentiation factor Bam (green) is repressed in GSCs and gonialblasts via activation of the BMP pathway.

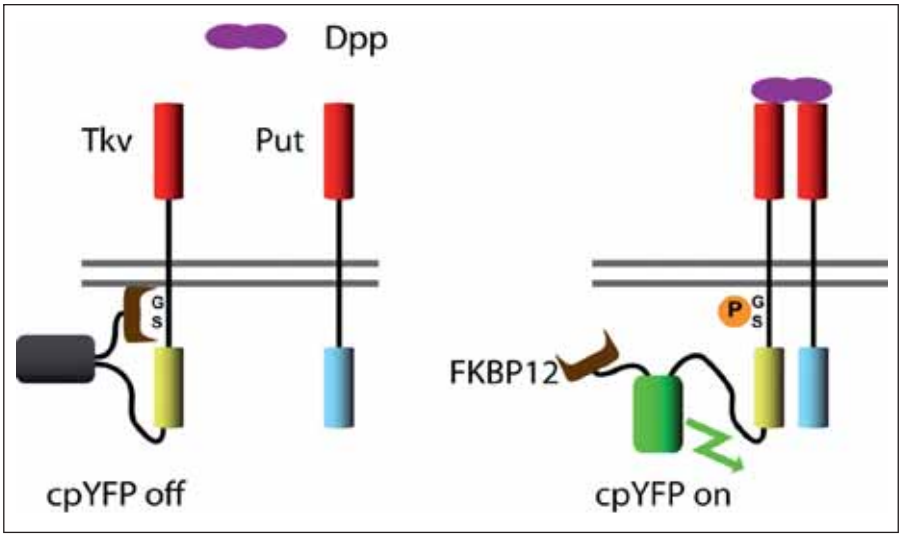

Figure 2: The TIPF reporter is based on a conformationally sensitive, circularly permutated YFP. Phosphorylation of the Tkv GS domain by Punt following ligand binding displaces FKBP12 and allows the TIPF reporter to relax into a fluorescent state.

For this, we turned to a method pioneered by the Miyawaki lab with their Pericam Ca^{2+} sensors (6). Nagai and colleagues used a circularly permutated YFP (cpYFP), i.e. a YFP molecule whose original N- and C-terminal halves had been swapped and linked by a short spacer. This rearrangement still allows the beta barrel structure to form, although it is destabilized and nonfluorescent presumably due to protonation of the chromophore. In the FlashPericam sensor, Ca^{2+} - mediated interaction between a tropomyosin peptide and a Calmodulin domain fused to the ends of the cpYFP core stabilizes the barrel and causes the protein to become fluorescent. Conversely, the InversePericam sensor, which mainly differs in the linker between the two YFP halves, is fluorescent on its own, but becomes dark when the ends of the barrel are forced together by the presence of Ca^{2+}. Importantly, these sensors do not detect the presence of Ca^{2+} ions as such, but instead react to conformational changes in the cpYFP barrel structure that are modulated by the opening or closing of a protein interaction. There was thus no *a priori* reason why these fluorescence switches could not also be adapted to detect other protein-protein interactions.

BMP receptor signal transduction is triggered by ligand induced heterodimerization of the type I receptor subunit, in flies encoded by *thickveins (tkv)*, with the type II subunit Punt (Put). In the absence of ligand, type I BMP receptors are inactivated by the binding of the inhibitor protein FKBP12 to the membrane proximal, glycine and serine rich (GS) domain. Phosphorylation of the GS domain by the type II receptor following ligand binding displaces FKBP12, and generates a binding site for the R-Smad transcription factor. This inhibitor to substrate binding switch defines the activated state of the receptor, and is thus the modification that identifies the site of signalling (7).

We therefore generated a triple fusion construct, consisting of Tkv, the cpYFP core of InversePericam, and FKBP12. This reporter was named TIPF, the acronym of its components (4). Analogous to the InversePericam Ca^{2+} sensor, FKBP12 binding to the nonphosphorylated GS domain suppresses TIPF fluorescence in the inactive state, while its displacement following phosphorylation allows the cpYFP core to adopt a fluorescent conformation (Figure 2). The TIPF reporter retains signalling activity, and its fluorescence faithfully reflects the pattern of BMP activation in the wing imaginal disc, where BMP pathway activation is well understood quantitatively. When expressed in the germline of the fly testis, TIPF reporter fluorescence is concentrated at punctuate, subcellular patches at the interface of hub and germline stem cells (Figure 3). We confirmed that these patches coincide with DE-Cadherin containing adherens junctions that are also the sites of ligand secretion by the hub cells. The localized receptor activation is therefore a consequence of the local generation of the niche signals. Finally, we could show that this intracellular polarization of Dpp trafficking towards the junctions is achieved via the hitchhiking of the ligand molecules on exocyst tagged vesicles that also direct Cadherins to preexisting junctions (4).

Thus, niche communication in the fly testis resembles the stem cell niche synapses in that signalling is organized by and restricted to adherens junctions between niche and stem cells. We have identified the cell biological processes involved in local signal release, and successfully visualized the similarly locally restricted activation of receptors at the target cell surface. However, there are still many questions that remain open. For example, adherens junctions do not constitute permeability barriers. What, then, prevents the secreted BMP niche ligands from diffusing away from their site of secretion? Genetic evidence points at an involvement of ECM proteoglycans, but the models currently discussed in the literature are unconvincing. We believe that understanding ligand propagation behaviour at the quantitative level will require reliable affinity data for ligands, receptors, and ECM components. We have therefore started to measure these parameters in vivo using fluorescence cross-correlation spectroscopy (FCCS) (8).

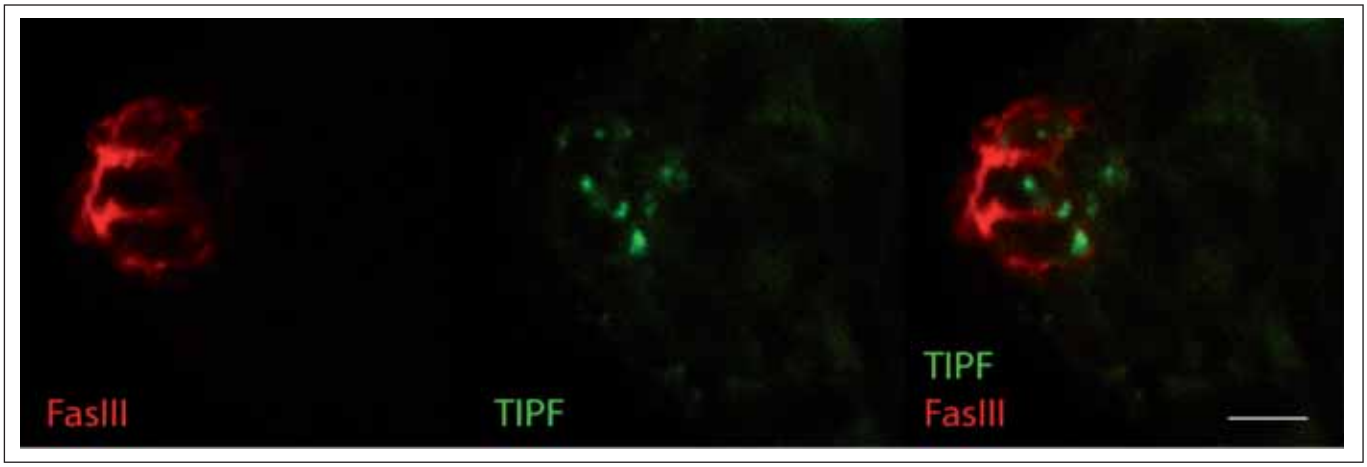

Figure 3: Fluorescence of a TIPF reporter expressed in the germline (green) is restricted to small foci at the interface of GSCs and hub cells (marked in red using FasIII, scale bar 5 µm)

The strategy we used to visualize BMP receptor activation only requires the formation or dissolution of a protein interaction that can be tracked by a suitably positioned conformation sensitive fluorophore. We can therefore in principle envisage similar reporters also for other, molecularly divergent signalling pathways, or even unrelated processes like the regulation of adhesion.

For example, in the Hedgehog signalling cascade we are interested more in the cell biological basis of signal transduction within the receiving cells rather than the control of signal propagation within the tissue. We have therefore generated a similar construct for studying the connection between the subcellular localization and activation state of the Hh signal transducer Smoothened. We are also validating reporters for the activation of cytokine receptors that we study in collaboration with Thomas Weidemann and Petra Schwille at the BIOTEC Institute of the TU Dresden (9).

We are confident that the direct visualization of signalling pathway activation states will be instrumental for the further analysis of the fundamental cell biological processes underlying intercellular signalling, whose complex interactions we are only now beginning to understand.

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Fluctuations and symmetries in biology and physics

Sebastian Koth, Michael Krahe, Claus Fütterer

Abstract

Fluctuations can be found in physical as well as biological systems. Are they related? They also appear to be important for symmetry breaking. We compare common aspects of structural transitions in biological and physical systems: *Hydra vulgaris* tissue fragments always reconstitute a symmetric spherical shape prior to symmetry-breaking regeneration. Breaking this spherical symmetry is accompanied by strong specific fluctuations. Cells also show distinct lamellipod fluctuations. Structural change in such different phenomena as magnetism, liquid freezing and evaporation, cytoskeletal rearrangement and morphogenesis of tissues shows symmetry breaking scenarios accompanied by fluctuations. We are questioning their common purpose and consequences.

Townes & Holtfreter wrote in 1955: "One of the most striking features of early vertebrate development is the transformation of a spherical egg into a body of about equal size in which groups of cells have shifted into specific arrangements."

Introduction

How do micro-reactors crowded with an incredibly large number of macromolecules get self-organized to realize such complex spatial structures as the lung, the brain or the eye? We introduce some aspects from purely physical systems and pose questions about cells and tissues from a physicist's point of view. The focus is mainly symmetries and self-organization and less gene regulation and signaling pathways. Of course, the big picture has to comprise both.

Novel developments in nano-technologies, micro-systems and microfluidics have completely changed today's situation [1] as we can manipulate single molecules, cells and cell assemblies in freely shaped tiny micro-systems. On a molecular scale, the cell is biochemically organized. However, on a larger scale coarse-grained passive and active properties of complex materials dominate. The relation of microscopic and macroscopic properties of matter has been a major issue in physics for a long time: How do atomic spins generate magnetism? How does water form ice crystals when freezing?

A further issue are the cross-links and feed-back control loops which seem to couple everything to everything in a non-linear and dynamic way. Such loops enable stable adaptation to varying external or internal conditions (e. g. ion concentration) and are controlled by modulation of coupling parameters. Gene expression is co-controlled by factors which themselves are produced by gene expression – a feed-back loop. However, that makes reductionist's life difficult: suppression of any element in a feed-back loop, even if it appears neglectable, leads to a total break-down of the loop's function [2]. Hence a further reduction of function is not possible. Therefore, the loop has to be taken as the smallest functional unit in theoretical descriptions.

Feed-back loops are also very important in physics. The probably best known and simplest model is the harmonic oscillator. This model is applied to a large variety of systems as pendulums, oscillating quartz-crystals, vibrating molecules in vacuum, and even elementary particles in quantum field theory. When agitated by an external force the pendulum follows. Once the agitation meets the pendulum's resonance frequency the amplitude increases without limit which is called "resonance catastrophe". This feature explains the sharp and specific spectrum of the light emitted by de-excitation of isolated molecules e. g. in molecular clouds in the universe or the inverse effect in flame absorption spectroscopy. However, in real systems damping usually limits and smears out the resonances: A pendulum typically slows down due to air-friction (the energy of motion is transferred to heat) and the motion stops almost instantly once it is brought into a denser medium like water.

Cells are mainly composed of water, therefore, the harmonic oscillator model has limitations. An attentive observer may plead that there are also waves in water and in other over-damped ("dissipative") systems. Right! It is quite surprising that such systems, once permanently supplied with energy, can also create oscillations and, even more, a wealth of spatio-temporal structures like heat convection waves, spirals and even chaotic patterns. The different oscillating phenomena share a common requirement for oscillations and waves: a delayed back coupling. Inertia and gravitation forces of the pendulum are geometrical coupled by the string. In heat convection flow-inertia and gravitation/buoyancy are responsible for waves. The structures in dissipative systems are investigated by the science of pattern formation [3]. The question is how and which patterns, growing from a totally uniform state, are

selected and how very local interactions between the molecules lead to large scale ordering and patterning [2]. The uniform state has to be destabilized in favor of a different growing pattern. Therefore, small, e. g. thermal fluctuations permanently explore all possible states, though of very small amplitude, and finally, the system selects the fastest growing pattern.

In contrary to the harmonic oscillator, dissipative patterns are highly relevant in biology - like patterns on shells or skins of animals as well as morphogenesis of tissues [3]. Self-organizing systems add new features to the traditional toolbox of physics as creation ("birth") and suppression ("death") of patterns [3].

Symmetries need fluctuations

Cells deploy self-organizing sub-systems, like the cytoskeleton (fig. 1), and control them by only few parameters (e. g. the capping protein concentration). Lamellipod fragments from fish keratocytes illustrate this in an impressive way. Despite lacking nuclei, microtubules and most other organelles, some of these fragments are still capable of migration spontaneously. This points toward a two state system and a physical instability of the acto-myosin network [4]. Migration of the resting fragments can be induced by a short mechanical stimulation, e. g. a gentle hydrodynamic shear flow. It was found that the local detachment of the membrane leads to a local increase in acto-myosin density. This accelerates the transition into the migrating state. The involved actin turnover becomes asymmetric as well [4,5]. The cell's symmetry is clearly broken in function and shape. The need for external stimulation of some fragments intimates a stabilization mechanism. Apparently the threshold is not reached in all cases by internal random fluctuations; external stimulation is required. In contrary, the fluctuations may even degrade small asymmetries, hence stabilizing the resting state.

So far, only few physical models have treated the molecular mechanism of symmetry breaking in actin-myosin contractile networks [6,7]. To which extend are thermal and ATP driven fluctuations involved? Reliable experimental data is still lacking. It is known from physics that fluctuations are required to break symmetries. However, to analyze cellular fluctuations properly, cells have to be observed in large numbers and with reproducible shapes and conditions. Therefore, new techniques like surface pattering by micro-contact printing and microfluidics are currently being employed.

At the beginning of research on fluctuations was a simple experiment of the botanist Robert Brown. In 1827 he observed perpetual motion of pollen particles in water affronting energy conservation. Do these fluctuations violate one of this most fundamental doctrine of today's physics? Today, we know that his observation reflects the atomic and hitherto unexpected stochastic nature of the microscopic world. Moreover, it explains "fluidity" of liquids as well as mobility of molecules and particles (diffusion). The study of fluctuations is at the heart of today's condensed matter research, which started with the observation of a mindful botanist.

The strength of physics is the quantitative description of phenomena and to put the obtained numbers into relation by mathematical laws. How could a random movement be characterized? A. Einstein proposed in 1905: particle positions x after a given time interval have to be measured as well as the initial position x_0; both have to be squared before averaging (average is represented by $\langle\rangle$) since $\langle x\rangle = \langle x_0\rangle = 0$. The difference to the initial position allows to compare the distance to other particles, $\langle x^2\rangle - \langle x_0\rangle^2$, and is equivalent to $\langle (x-x_0)^2\rangle = (\Delta x)^2$. This reads as fluctuation strength.

Pulling a particle through the liquid with a constant force F, e. g. with an optical trap, results in a constant speed v - constant because of friction. This yields the so called mobility constant $\mu = v/F$. A surprisingly simple relation was theoretically found by Einstein and Smoluchowski: $\langle (x-x_0)^2\rangle \sim \mu\, k_B\, T\, t$. That means that fluctuation growth is simply proportional to temperature as well as the particle mobility despite the extremely high complexity of the microscopic interactions. With smaller mobilities the fluctuations slow down. Here, fluctuations are intimately linked to the particle mobility - an important statement. The experimental confirmation was honored with a Nobel prize (1926 J. B. Perrin) as these fluctuations finally solved the question of the atomistic and stochastic nature of matter. Maybe a second message can be learned: complex systems do not always require a complicated description.

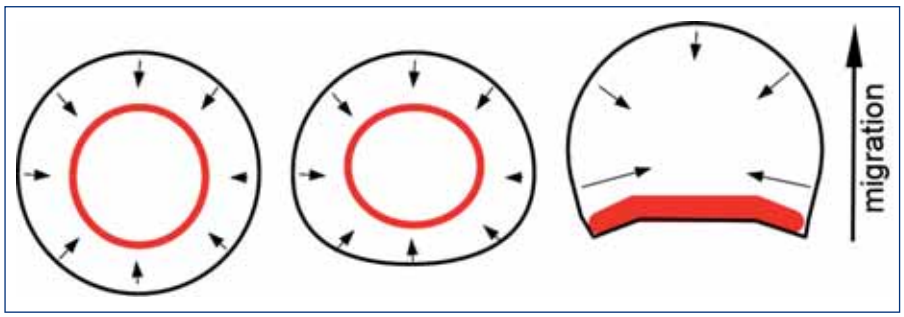

Figure 1: The resting and migration states of Keratocytes shows different cytoskeletal shape and structure (red = perinuclear/ transverse acto-myosin bundles, arrows = retrograde actin flow). Both states may be stabilized by hysteresis, which is not fully understood. Fluctuations may play a significant role for conserving the symmetry of the resting state against small perturbations (modified from [5]). Larger perturbations as induced by external shear flow may overcome the threshold and result in the stable migration state characterized by a strong polarized retrograde actin flow and cellular shape.

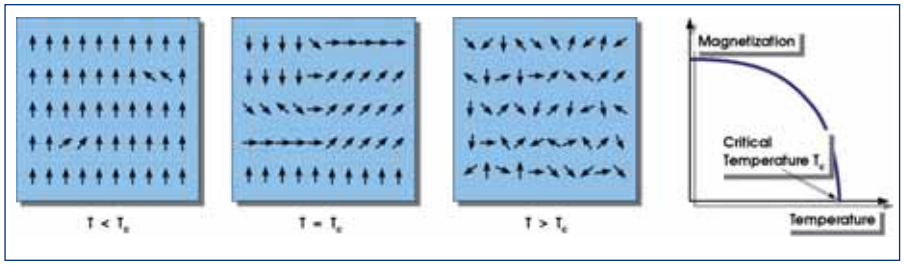

Figure 2: Atomic spin-spin interaction is local (spins in a ferromagnet are parallel in contrast to macroscopic magnets [8]). However, at low temperatures spontaneous spin alignment yields a macroscopic organization resulting in a magnetic field. At high temperatures the spins fluctuate strongly and become uncorrelated: the material loses magnetization. At the critical temperature (Curie temperature) fluctuations are found at all length scales up to the size of the system. The magnetization and the fluctuations follow power laws during the transition [8].

Critical phenomena and symmetry

It is a common strategy in physics to study matter under extreme conditions to understand more general properties. If the temperature is decreased, the fluctuations slow down more and more and suddenly gases liquefy and solidify in many cases. The related dramatic structural changes are called phase transitions and represent a major research topic of contemporary physics called "critical phenomena". Paul Ehrenfest classified them into mainly two categories: phase transitions of 1st and 2nd order. The 1st order transition is characterized by a constant temperature while heating. At this "critical temperature" the system stays partially in its new and old state, e. g. coexistence of liquid and vapor. Once the so called latent heat is added to the system, the transition is complete and further heating results in a rising temperature again. Examples are melting of ice, vaporizing of water, or crystal rearrangements. Phase transitions of 2nd order do not show coexistence of states but change them completely at once. A prominent example is ferromagnetism which is a collective phenomenon of interacting electronic spins (fig. 2). Passing the critical point from higher temperatures the local spin alignment results in increasingly large correlations and finally in one single distinguished macroscopic direction yielding instantly a macroscopic magnetic field. While approaching the critical point from lower temperatures the spin fluctuations spread over all length scales and the macroscopic magnetization disappears abruptly. Then, the system becomes isotropic, thus more symmetric. When passing below the Curie temperature again the symmetry is spontaneously broken and magnetism reappears, however, in a new random direction . This complex "phase transition" is described by surprisingly simple characteristic scaling laws [8].

Transitions between states of different symmetry were equally found in over-damped dissipative systems. They share with biological cells the requirement of permanent energy throughput ("non-equilibrium systems"): Warming up a hot chocolate drink can spontaneously develop honey comb patterns at the surface despite an initially homogeneous state by carefully stirring [3].

Can critical phenomena also be found in biological systems?

Cellular fluctuations on micro-patterns

Tissue cells in cell cultures surprisingly behave very individually and one may wonder how they realize the precisely orchestrated tissue organization and growth. The key is the shape of the cell. Microtechnologies developed for semiconductor physics were adapted to micro-fabricate chemically structured surfaces with extracellular matrix components [9]. Previously cell division of pattern-bound cells was studied, and it was found that the pattern shape strongly determines organelle orientation [10]. As a consequence the cell cycle of these cells became very precisely synchronized. This seminal observation permits a novel concept: the averaged cell [10]. It appears that geometry and function are tightly linked and it may be assumed that cells behave much less "erratic" under well controlled "boundary" conditions.

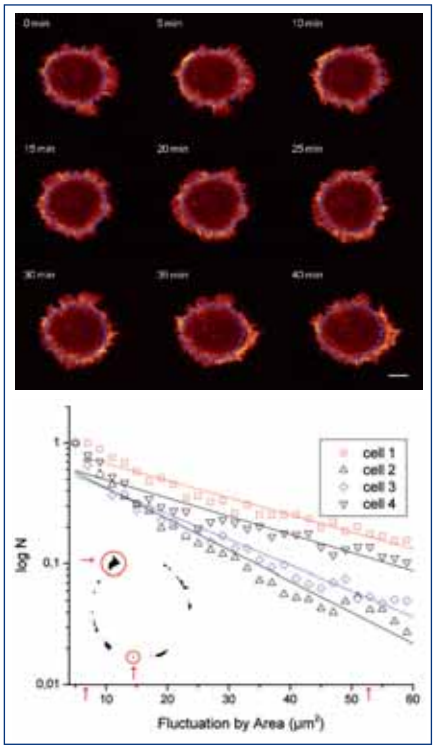

Fig. 3 a&b: Micro-patterns (blue circles) are used to regularize the cells (HEK293). The onset of the transition from stationary to migrating state is locked by the cellular shape due to the circular pattern (bar 10µm). The area difference of the binary threshold image and the micro pattern (inset) is measured and the corresponding normalized logarithmic size distribution of fluctuations reveals a linear decline. The applied micro-patterned cells significantly simplifies the analysis and improves the quality of measurement.

* More precisely: this applies only to isotropic single magnetic domains. Bulk material composed of many randomly oriented domains does not reconstitute magnetization.

The shape of cells embedded in tissues is determined by adhesion to other cells or surfaces. As the cell shape and symmetry depends on its environment, in particular in a tissue, this might explain the strong differences of coordinated behavior of cells in tissues and the more random behavior of cells on substrates.

Our hypothesis is that the circular pattern locks the cell between the resting and migration state. Can we expect phase transition – like properties? However, the analyzed fluctuations (fig. 3) revealed an exponential scaling of the lamellipod size fluctuations. In future more precise measurements are planned and the influence of stimulation with growth factors and stimuli and cytokines (Rac, Cdc42, EVH) will be studied: Does the distribution stay exponential or does it become algebraic which would be a clear sign of a phase transition? Further, we found that the exponents vary significantly between different cells of the same line. This may allow to identify different states in the cell cycle or to quantify healthiness. Further analysis into this direction is ongoing. The exponential scaling can be explained by the stochastic switching between the two, growing and shrinking, state of the actin bundles. The camera records a coarse-grained superficial fluorescence signal of the fluctuating features being, in fact, coarse grained linearly growing bundle-system. Therefore the features and their fluorescence also grow or shrink linearly in time. The found exponential distribution for the growth and shrinking time intervals is a feature of the Poisson process which only is found for statistically independent events and if its probability is constant over the observation time interval – the same as the statistics of the radioactive decay.

Tissues fluctuate actively when changing symmetry

On a higher level, cells self-organize to larger systems despite the interaction being local only. This is quite similar to magnetism or fluids and gases. In contrast to the intracellular complexity, tissues composed of repetitive cells are more uniform and better suited for physical continuum descriptions for fluids (Navier-Stokes equations) and elastic and plastic materials. But we have to stay careful as cells possess not only passive but also active properties. The ability of a tissue to regulate stiffness, shape and viscosity in response to external cues is by no means similar to known materials. It is clear that any model of cells remains incomplete as long as the molecular level and mechanical regulation are not integrated. As the next step, tissue models describing more complex than viscoelastic responses [11] are being investigated in collaboration with the theory department in Leipzig.

Fluctuations have been described for several tissues. During *Drosophila melanogaster's* apical constriction of the ventral furrow, cells fluctuate in a partially coordinated way [12]. The proposed ratchet model correlates contractions of the acto-myosin network of the apical cortex during the constriction process. It is suggested that these pulsed cortical fluctuations facilitate the "dynamic rearrangements of the actin network to optimize force generation as cells change shape" [12]. Fluctuations fluidize the system prior to rearrangement similar to magnetic systems. Furthermore, fluctuations where found during the dorsal closure process [13]. Fluctuations have been described in aspired cells from a cell cluster, an arrangement to measure surface tension [14].

During epiboly of Zebrafish embryos the development starts with a cell division sequence of the zygote cell attached to a giant yolk cell. After several division cycles the cell assembly forms a droplet called cell dome [15]. These cells start spreading rapidly over the yolk cell similar to a fluid droplet spreading over a hydrophilic surface. Latter is explained by the strong binding energy of hydrogen bridges overcoming the action of entropy.

Fluid-like behavior of cell assemblies was observed by J. Holtfreter already in the 1930s during large scale cellular rearrangement in developing tissues [16]. This led to the "Differential Adhesion Hypothesis": cells with the strongest adhesion collect in the center surrounded by less affine cells [17]. This yields even a quantitative linear relation between adhesion strength and surface tension. This purely passive description is surprising as cells are known to be very active in growing tissues. In the isotropic local environment of aggregates, cell-cell contacts may be reduced and polarity as well as forces may fluctuate isotropically and hence average out. Nevertheless, these random forces are important and keep the cells motile – an indispensable ingredient to avoid positional "crystallizati-

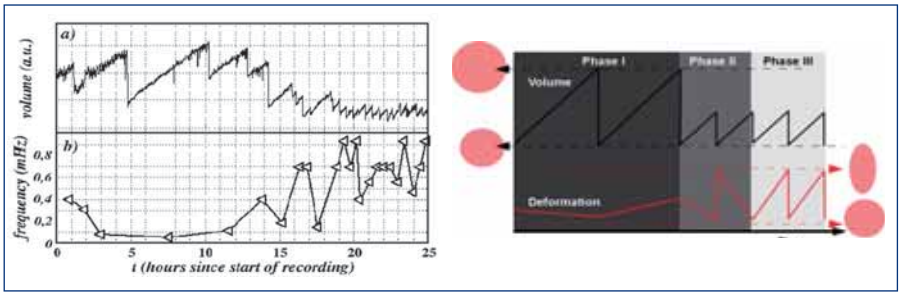

Fig. 4 a&b: a) The first two phases of shape fluctuations of a regenerating hydra cell spheroid are distinguished by characteristic fluctuations. The frequency increases, though varies strongly. b) The schematics shows all three phases. In phase I the slow fluctuations show no axis. The symmetry tracer gene ks1 is unstable against small temperature gradients. In phase II the spheroid starts showing deviation from the symmetric arrangement, ks1 is getting locked. In phase III the elastic properties of the spheroid become asymmetric and swelling leads to a deformation increase [19].

on" or "freezing". In fully grown tissues the cells are strongly bound and epithelial cells cannot displace anymore. These random forces could allow the cells covering the Zebrafish's yolk cell to flow rapidly and to perform the symmetry breaking transition needed for the further development [15]. To our knowledge, the nature of this phenomenon has not been investigated so far.

Hydra vulgaris cells and tissue fragments show extraordinary regeneration capabilities [18]. Fragments form spheroids after several minutes and develop an entire organism within 2 days. The axis formation is not biased by placenta attachment points and therefore shows a truly spontaneous symmetry breaking scenario in the early regeneration process. Two transitions have been described: From phase I to phase II and finally to phase III [19]. The phases are characterized by swelling fluctuations, increasing in frequency.

The slow osmotic driven [20] saw-tooth fluctuations observed in phase I are assumed to destroy any remaining memory of the previous axis (fig. 4). The fluctuations accelerate instantly on the passage from in phase I to phase II and clearly designate the transition. Prior to the first transition the axis still follows small temperature gradients. In phase II the axis becomes insensitive ("locked") [21]. Therefore feed-back loops are certainly responsible, however the mechanism is not understood. The second transition leads to an anisotropic elasticity distribution of the tissue and a visibly asymmetric morphology [19]. The symmetry breaking could be related to the gene ks1. The mechanism responsible for destabilization the previous state and stabilization the new state is not known. During the first transition this gene is expressed in patches of exponentially distributed sizes – the same as for magnetic and lamellipod fluctuations.

In many cases, the spherical form is adopted prior to development and regeneration. How is this possible? Surface tension in fact is not dominant for spheroid formation of regenerating *Hydra*. When starting with a dissected ring, we observed that the cells are actively striving for a spherical shape accompanied again by strong fluctuations (fig. 5). Apparently, a symmetric geometry is required as an initial situation for a proper regeneration. We are currently investigating the folding dynamics and involved mechanisms.

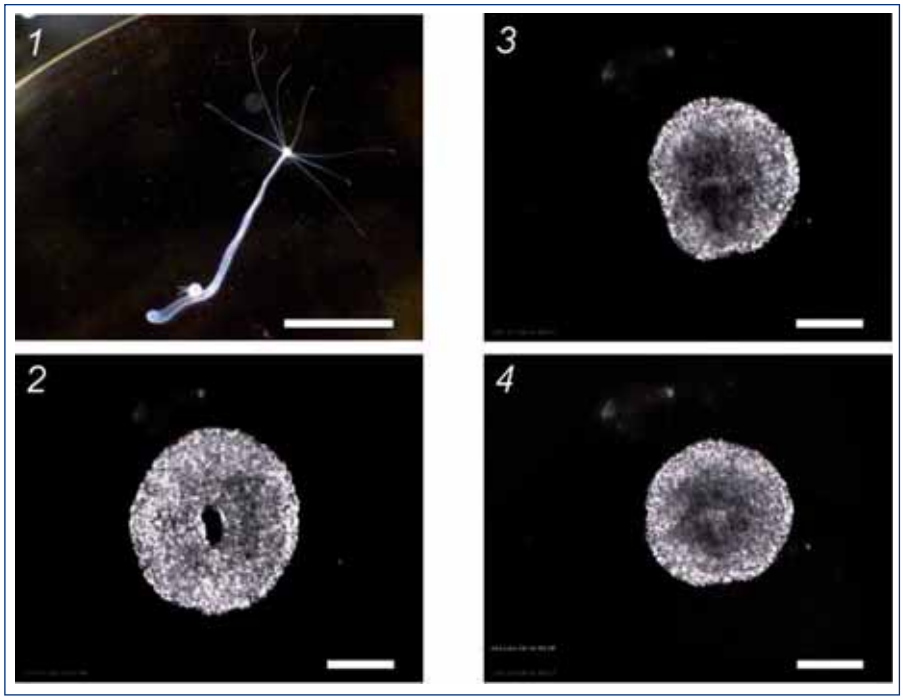

Figure 5: (1) An adult Hydra vulgaris (bar 5mm) (2-4) A dissected toroidal Hydra tissue fragment (bar 100µm) from the central column fluctuates strongly and rearranges to a sphere after approx. 10min. The hollow sphere enters the described symmetry breaking scenario.

Conclusions

Fluctuations, symmetry change and pattern-formation were discussed for biological and physical systems. Fluctuations are very large during phase transitions of melting ice crystals and magnetization. Large fluctuations are also found in toroid-spheroid transforming *Hydra* tissues, apical constriction and dorsal closure of Zebrafish embryos. Exponential fluctuation distributions are reported to occur in cellular lamellipods and expression of genes tracing symmetry breaking of Hydra spheroids which also undergo mechanical fluctuations.

Fluctuations help to conserve symmetries: Cells expend considerable energy to generate cytoskeletal fluctuations. We assume a similar role in all these cases, namely to realize a controlled fluidization of the system as a prerequisite to accelerate rearrangements as well as equilibration. Fluctuations allow to overwrite destabilizing feed-back mechanisms (low signal-noise ratio) and residues of previous asymmetries, thus, to establish and conserve symmetries. Keratocytes need a stimulation strong enough to trigger the transition to the migration state. Hydra tissue fragments realize prior to regeneration a symmetric arrangement by strong fluctuations. Fluctuations allow to establish a well defined initial condition presumably required to sustain uniform and reliable development afterwards.

Fluctuations break symmetries: Fluctuations permit the system to permanently deviate slightly from the perfect symmetric situation. This allows the system to start the transition to the new state characterized by a different symmetry. For example, changing the field direction of a ferromagnet is best

done, first, by suppressing the magnetization through heating, and second, by subsequent cooling which leads to spontaneous re-magnetization. A second example is a homogeneous mixture of differently adhering cell types in a spheroid. The different cells sort out passively due to "differential adhesion" relying on fluctuations. These fluctuations have not been characterized so far.

Cells can actively change the parameters of the regulation loops destabilizing the actual state, e. g. by changing the actin cap protein expression rate. They can not perform transitions to a different state but can amplify appropriate fluctuations which guide to the new state.

Universality in phase transitions? It is not clear, if the described fluctuations reveal the same characteristic power laws as known to be universal for phase transitions in physics. In case of a positive answer it would be a fundamental cornerstone in research of complex systems crossing the fields of physics and biology. Maybe biological and physical systems are closer than expected?

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Great lessons told by little cells: The model organism *Dictyostelium discoideum*

Annette Müller-Taubenberger

Introduction

Since the dawn of cell biological research, scientists have made use of easily accessible systems to advance their studies. 'Model organisms' became extremely valuable tools not only for investigating fundamental biological questions, but also increasingly influence clinical research. The use of such model systems is thought to be beneficial as they may help to overcome: (1) ethical and experimental limitations; (2) reduce animal death and/or adverse experimentation; (3) help to optimize and standardize analytical methods; and (4) the findings may allow more general conclusions or are representative for a larger class of organisms relating to a variety of biological processes.

The selection and use of model organisms has changed over time depending upon the methods available for the model and the objectives of the research. One of the oldest models is the medusozoan *Hydra*, which was described already 300 years ago in microscopical studies by Antoni van Leeuwenhoek. Today, *Hydra* is used to explore the mechanisms underlying regeneration in the adult organism and in understanding the various signal cascades involved. Other commonly used models have been sea urchins that were explored for instance by Theodor Boveri for elucidating the basic principles of cell division more than hundred years ago, or amphibian embryos for the ontogenetic studies of Hans Spemann which led to the description of the *organizer* in 1924. A few years later, Thomas Hunt Morgan showed that *Drosophila melanogaster* genes are encoded in chromosomes and that these provide the basis for heredity and thus confirmed earlier evolutionary theories. These examples exemplify the role of models in helping to decipher fundamental biological questions. More recently, simple organisms like yeast, worms, or flies are employed to investigate a wide range of processes including for instance gene- and signaling-pathway-regulation, and the resulting knowledge helps to understand the biology of more complex species including humans that are more difficult to study directly. Today's work with model organisms generally provides a number of experimental advantages as these species have been widely studied and are easy to maintain and to manipulate under laboratory conditions.

Nonetheless, the different model organisms are not without shortcomings as their use is sometimes limited to specific questions. In addition, a major contention lies over the question of whether research using model organisms allows scientists to achieve the ultimate goal – understanding human diseases and developing cures. This requirement cannot always be fulfilled and a universal model organism does not exist. However, by using specific model systems, a range of common biological processes can be studied which helps to gain a deeper insight into the understanding of disease etiologies and treatments.

Currently, the National Institutes of Health lists twelve model organisms for biomedical research (http://www.nih.gov/science/models). The mammalian models are mouse and rat even though the rat is much less frequently used since it became obvious that its genome is less tolerant of foreign DNA insertion compared to the mouse and thus makes the rat model less suitable for genomic manipulations. The non-mammalian models comprise chicken, zebrafish, *Xenopus*, *Drosophila melanogaster*, the worm *Caenorhabdidtis elegans*, the filamentous fungus *Neurospora*, the water flea *Daphnia*, the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and the amoebozoan *Dictyostelium discoideum*.

Dictyostelium discoideum – a model used for many reasons

D. discoideum belongs to the social amoebae (*Dictyostelia*). Phylogenetically, *Dictyostelia* form a different branch in the tree of life and are separated in evolutionary terms as far from yeast as from humans, and they are positioned at the borderline between uni- and multicellularity. The genome sequence of *D. discoideum* has been published in 2005 (Eichinger et al., 2005), and more recently the genomes of three other amoebozoans, *Dictyostelium fasciculatum*, *Dictyostelium purpureum* and *Polyspondylium pallidum* became available for comparative genome studies (Sucgang et al., 2011; Heidel et al., 2011).

D. discoideum lives in the soil and feeds on bacteria and other microorganisms that are taken up by phagocytosis (Figure 1A). During the vegetative stage, the single-celled amoebae divide by simple mitotic divisions (Figure 1B). In times of starvation, a developmental program is initiated, which is accompanied by major changes in gene expression. By chemotaxis towards extracellularly released cAMP, cells signal and attract each other, and up to 100,000 cells form a

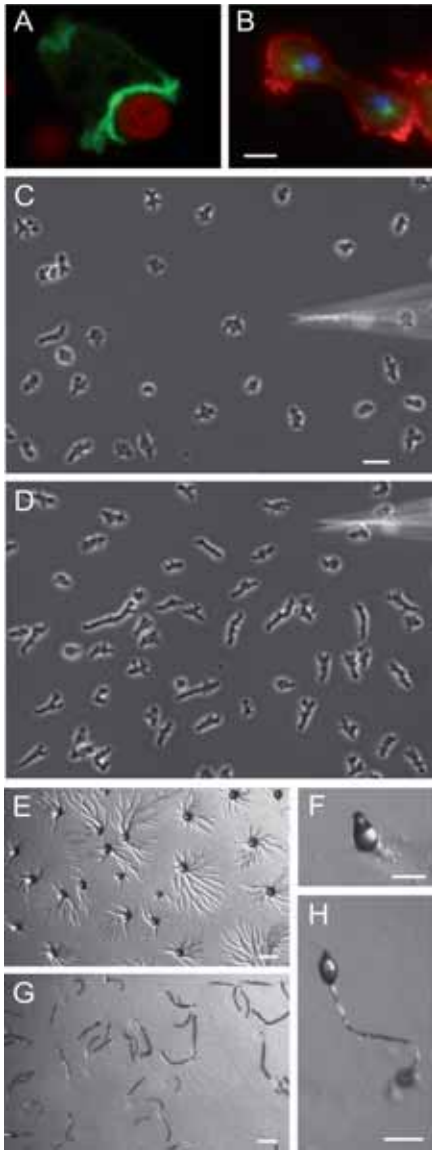

Figure 1: Different stages of the Dictyostelium life cycle. (A) A *Dictyostelium* cell phagocytosing a TRITC-labeled yeast (red). The formation of the phagocytic cup is visualized by the expression of GFP-LimEΔ (green), which corresponds to filamentous actin. (B) A *Dictyostelium* cell during a late stage of cytokinesis. Fixed cells were labeled for filamentous actin (red), microtubules (green) and DNA (blue). (C, D) *Dictyostelium* cells chemotaxing towards cAMP released from a micropipette. Cells that have not yet sensed cAMP are shown in (C). Within one or two minutes the cells polarize (note the elongated cell shape) and migrate towards the source of chemoattractant (D). (E) Formation of aggregation centers on an agar plate. (F) Culmination stage. (G) Slugs moving on an agar surface. (H) Fruiting body. The bars correspond to 5 µm in B, 10 µm in C, 1 mm in E, F and G, and 0.5 mm in H.

multicellular aggregate (Figure 1 C-E). This aggregate undergoes differentiation and morphogenetic changes (Figure 1F, G) and finally forms a fruiting body (Figure 1H) that consists of two main cell types, spore and stalk cells. The stalk cells are dead vacuolated cells, while the spore cells are resistant to extreme temperatures or drought. More favorable environmental conditions enable the hatching of new amoebae from the spores. It is this peculiar life cycle (which is responsible for the term 'social' because it includes a multicellular phase) that has intrigued scientists for decades since the first description of *D. discoideum* by Kenneth Raper (Raper, 1935). Lots of pioneering studies have been performed that strengthened the use of *Dictyostelium* as a model, and a few examples are highlighted in the following.

(1) Studies on cell adhesion during the early aggregation state of *Dictyostelium* led to the identification of a membrane glycoprotein, the contact sites A (csA) that mediates specific cell-cell contacts. The strategy and the methods to identify csA were path-breaking for the analysis of cell adhesion molecules (CAMs) in many other organisms (Müller and Gerisch, 1978).

(2) Another ground-breaking finding was the detection of cAMP as the chemotactic agent (Konijn et al., 1967) that is produced and sensed by the cells themselves, and central to the relay system that mediates aggregation of single cells into multicellular bodies during development (Devreotes and Steck, 1979; Gerisch et al., 1975). These innovative studies provided the basis for the understanding of chemotaxis.

(3) *Dictyostelium* amoebae are highly motile cells both during the vegetative phase as well as during aggregation. Motility is dependent on actin-driven processes (Figure 2). Directed movement involves actin polymerization in pseudopods at the front of cells in combination with a myosin-II-dependent retraction at the rear. However, cytoskeletal activities cannot be reduced only to the interplay of actin and myosin and the microtubule system, but involve a whole arsenal of interactors including actin-binding proteins and many other regulators like protein kinases or GTPases. One of the outstanding advantages of the *Dictyostelium* model system is the fact that *Dictyostelium* has a huge number of cytoskeletal proteins in common with higher eukaryotes. Some cytoskeletal proteins, like for instance coronin, have been described first in *Dictyostelium* and only later in mammals. Moreover, by studying the *Dictyostelium* homologs the functional activities of many cytoskeletal regulators could be defined. In recent years, the combination of fluorescently labelled cytoskeletal proteins with live-cell imaging employing different microscopical techniques has revealed and visualized so far undisclosed details of actin cytoskeleton dynamics (Bretschneider et al., 2009; Bretschneider et al., 2004) (Figure 2).

(4) From the advent of the GFP technology the method was employed in *Dictyostelium* for the visualization of specific compartments and organelles or the localization of specific proteins. One of the first studies that has nicely emphasized the potential of the GFP technique was using GFP-coronin in live-cell imaging studies of *Dictyostelium* amoebae during phagocytosis of yeast cells (Maniak et al., 1995). To this day, the technique has been constantly improved and used to study a great variety of biological processes in the *Dictyostelium* model.

(5) *Dictyostelium* mutants lacking non-muscle myosin-II revealed an unexpected essential role of this motor protein in cytokinesis (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987) and showed that it is involved in formation of the contractile ring. It later became clear that *Dictyostelium* is also able to complete cytokinesis in the absence of myosin-II through an adhesion-dependent process, and these and other experiments have confirmed *Dictyostelium* as a useful model to study different aspects of cytokinesis (Figure 3).

Other key aspects using *Dictyostelium* as a model

Dictyostelium is a genetically tractable organism, and the molecular tools available to manipulate and investigate the cells are manifold. One of the great advantages of the system is the ease of generating mutants. In addition, biochemical studies can be performed because isogenic cells can be grown in large quantities, and lots of cell biological tools are available. But beyond that *Dictyostelium* plays an important role in studies of comparative phylogenetics and social evolution.

Studies on social amoebae help to explore social interactions at physiological, genetic, and genomic levels. The social stage of *Dictyostelium*, which begins with the aggregation stage of the organism, is analogous to a social group and is thus vulnerable to internal conflict. *Dictyostelium* has become a new model for social evolution helping to understand multicellular organization from developmental pathways to cell adhesions, and in particular how social cheaters are controlled (Strassmann and Queller, 2011; Strassmann et al., 2000). In these studies, cheaters were shown to be limited from exploiting other clones by mechanisms such like high relatedness, kin discrimination, pleiotropy, noble resistance, and incidental role assignment (Foster et al., 2004; Khare et al., 2009; Mehdiabadi et al., 2006). The active nature of these limits is reflected in the elevated rates of change in social genes compared with non-social genes (Santorelli et al., 2008). Despite control of cheaters, some conflict is apparent in chimeras that show slower movement of slugs, and different contributions to stalk and spore cell populations.

Another unique behavior of *Dictyostelium* that has been recently reported is a primitive form of farming (Brock et al., 2011). When food becomes scarce, about one third of *Dictyostelium* clones stop feeding early and incorporate bacteria into their fruiting bodies. This has been interpreted as a way that the 'farming' *Dictyostelium* can seed a new bacterial colony as a food source in case the new habitat should be lacking edible bacteria. This genetically built-in behavior is costly for the individual, but provides also benefits for the population to persist in nature. Interestingly, some amoebas carry bacteria that are not used as food, and this type of symbiosis will be central for further studies.

Research using *Dictyostelium* also has helped to understand the evolution of epithelial polarity in metazoans. During its multicellular stage, *Dictyostelium* forms a polarized epithelium. Epithelial polarity in metazoans requires α- and β-catenin, and homologs of both proteins are present in *Dictyostelium*. Recent data suggest that the catenin complex appears to form the ancient functional module that mediates epithelial polarity in the absence of cadherins, Wnt-signalling components and polarity proteins (Dickinson et al., 2011).

Dictyostelium and medical research

Studies using *Dictyostelium* provide excellent options for addressing disease-related processes or helping to understand the principles underlying pathological aberrations (Williams et al., 2006). Basic research on disease mechanisms using *Dictyostelium* as a model is constantly expanding into new research areas and just some examples can be mentioned here.

The capacity to respond and to migrate directionally towards external cues is crucial for a variety of vitally important processes like angiogenesis, immune cell trafficking and inflammatory responses, wound healing, nerve growth, and embryogenesis. *Dictyostelium* cells are intrinsically motile and move in a fashion that is generally known as amoeboid movement just like as seen in leukocytes and some tumorigenic cell types, and the molecular principles of the motile activities are studied both

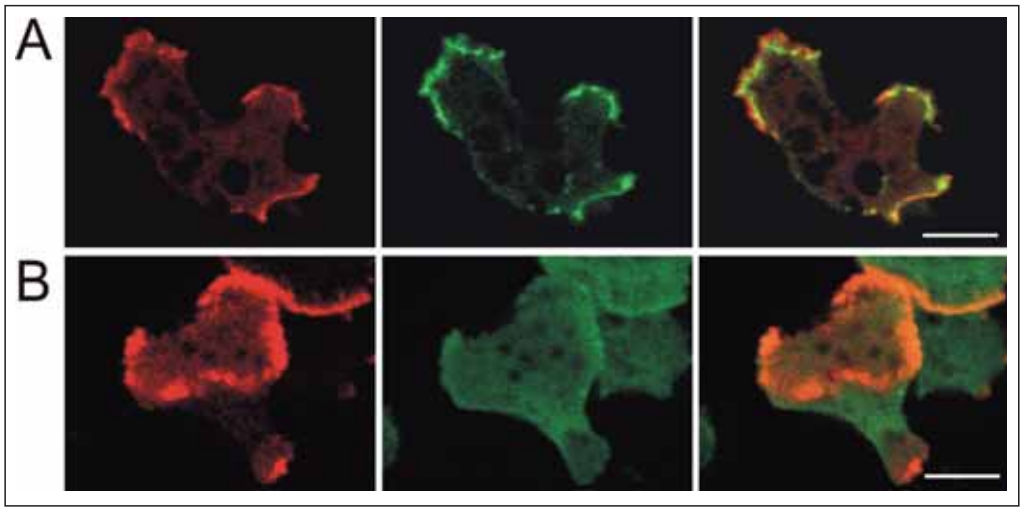

Figure 2: Visualization of actin cytoskeleton dynamics. (A) A *Dictyostelium* cell coexpressing RFP-LimEΔ (red, left image) in combination with GFP-coronin (green, middle image) demonstrates the sequential enrichment of cytoskeletal proteins (merge, right image). While LimEΔ, which visualizes filamentous actin is localizing to the front, coronin, an actin-depolymerizing protein, enriches further back from the assembly site of actin and promotes the disassembly of F-actin. B. A *Dictyostelium* cell coexpressing RFP-LimEΔ (red, left image) in combination with GFP-cortexillin (green, middle image) captured during actin wave formation. While actin is assembled in highly dynamic waves, cortexillin, an actin-bundling protein, is excluded from the waves (merge, right image). Bars, 5 µm.

in *Dictyostelium* and in neutrophils (Parent, 2004). Many fundamental aspects of chemotaxis have been deciphered in *Dictyostelium*, for instance how chemoattractants bind to specific receptors coupled to heterotrimeric G proteins and causing a range of biochemical responses that finally lead to polarization, redistribution of cytoskeletal components and migration of the cell (Swaney et al., 2010). There are a number of examples that exemplify the substantial similarity of the signaling pathways in *Dictyostelium* and neutrophils, and often the pioneering study was conducted using *Dictyostelium*. The evolutionarily conserved S/T kinase complex TORC2 (target of rapamycin complex 2) has been shown to be involved in regulating the actin cytoskeleton in various systems. Consistent with preceding findings in *Dictyostelium* (Lee et al., 2005) it has been recently established that mTORC2 is a key regulator of neutrophil polarity and chemotaxis (Liu et al., 2010).

A nice example how research using *Dictyostelium* and lymphoblasts can complement each other is given by a study on the Shwachman-Diamond syndrome (SBDS). This is a rare autosomal disease characterized by ineffective hematopoiesis, increased risk for leukemia and pancreatic insufficiency. Conditional *Dictyostelium* SBDS mutant cells revealed a defect in maturation of the 60S ribosomal subunit that is fundamental to the pathophysiology of the disorder and indicated that the Shwachman-Diamond syndrome is a ribosomopathy (Wong et al., 2011).

During their vegetative phase, *Dictyostelium* amoebae are professional phagocytes and engulf bacteria or yeast cells by a similar mechanism like macrophages (Clarke et al., 2006). *Dictyostelium* can be infected with a variety of intracellular pathogens, including *Mycobacteria* (Hagedorn et al., 2009), Legionella (Solomon and Isberg, 2000), and *Salomonella* (Steinert, 2011). In order to understand the infection mechanisms from host cell entry to establishment of replication niches, studies using *Dictyostelium* may also offer insights into new directions of research of infections in mammalian cells. In addition, a previously unknown cell type termed sentinel (S) cell, which appears to provide detoxification and immune-like functions has been described (Chen et al., 2007). S cells were observed to engulf bacteria and sequester toxins while circulating within the slug. A Toll/interleukin-1 receptor (TIR) domain protein, TirA, was shown to be required for some S cell functions and for vegetative amoebae to feed on live bacteria. The data suggest that this apparent innate immune function, and the use of TirA for bacterial feeding, developed from an ancient cellular foraging mechanism that may have been adapted to serve defense functions before the diversification within the tree of life (Chen et al., 2007).

Dictyostelium is also an emerging model to study the molecular basis of mitochondrial and neurodegenerative diseases. Mitochondrial dysfunction is commonly linked to neurological or neurodegenerative defects and many neurodegenerative diseases are accompanied by mitochondrial disorders, enabling the use of *Dictyostelium* to examine mitochondrial dysfunction by down-regulation or knockout of genes encoding various mitochondrial proteins (Barth et al., 2007).

MND (motor neuron disease) or ALS (amyotrophic lateral sclerosis) and HSP (hereditary spastic paraplegia (see Cell News 2/2011) are severe neurodegenerative diseases caused by the degeneration of motor neurons. Current work with *Dictyostelium* focuses on the questions whether mitochondrial dysfunction is causal in MND by investigating Sod1 (Cu/Zn superoxide dismutase) homologs (personal communication Sarah Annesley and Paul Fisher, La Trobe University), the molecular function of strumpellin which is mutated in HSP or Strumpell-Lorrain disease (personal communication Ludwig Eichinger and Christoph Clemen, University of Cologne; Clemen et al., 2010), and on the cell biological and biochemical analysis of a spastin homolog

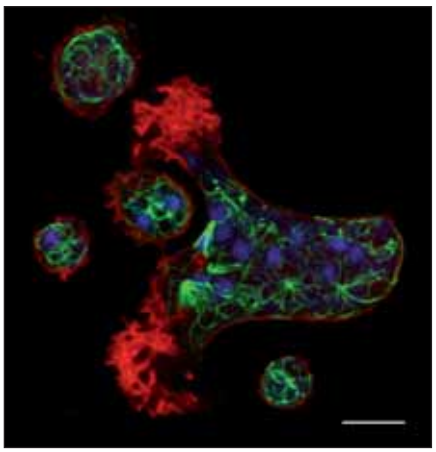

Figure 3: Mutants lacking the actin regulators coronin and Aip1 are defective in cytokinesis. *Dictyostelium* cells lacking the actin-regulators coronin and Aip1 are multinucleate due to defects in cleavage furrow formation (Ishikawa-Ankerhold et al., 2010). Fixed coronin-Aip1-null cells were labeled for F-actin (red), α-tubulin to visualize microtubules (green), and DNA (blue). Due to impaired actin disassembly, F-actin enriches in cortical patches. Bar, 10 µm.

(Müller-Taubenberger and Günther Woehlke, work in progress).

Another degenerative disorder is Parkinson's disease. Pathogenic mutations in the Roco family protein LRRK2 (leucine-rich-repeat kinase 2) have been linked to idiopathic Parkinson's disease. LRRK2 kinase activity provides a therapeutic target, but the enzyme is very difficult to study in mammalian systems, and therefore the biochemical and structural understanding is very limited. *Dictyostelium* encodes 11 Roco proteins, and in particular Roco4 has the same domain structure and biochemical characteristics as LRRK2 (Marin et al., 2008). *Dictyostelium* is currently used as a model to elucidate the intramolecular regulatory mechanisms of Roco proteins in order to understand LRRK2-mediated Parkinson's disease (personal communication Arjan Kortholt, University of Groningen).

Dictyostelium can be also employed to study the mode of action of neurological drugs such as lithium or valproic acid that are widely used current treatments for bipolar dis-

order and epilepsy, including both identifying novel uptake mechanisms (Terbach et al., 2011) and defining drug action and potential better treatments (Chang et al., 2011).

This list could be extended by a number of interesting studies ranging from understanding pathological processes underlying lissencephaly (Rehberg et al., 2005) to the misregulation of the actin cytoskeleton causing a variety of disease pathologies, including compromised immunity, neurodegeneration, and cancer spread (Carnell and Insall, 2011). Taken together these examples show that by exploiting the genetic advantages and the simplicity of the *Dictyostelium* system compared to a variety of specialized mammalian cells, a lot can be learned about the principles important for cellular homeostasis. Thus, in the world of biosciences, the simple eukaryote *Dictyostelium* has legitimated its important position in research over the last decades and will continue to serve as a valuable model for major biological questions in the future.

Acknowledgments

This short review article exemplifies just some of the landmark findings using *Dictyostelium* as a model. More information can be found at http://www.dictybase.org/.

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2009	Habilitation in Cell Biology

Newton meets Cells: Forces of Life

Cells that bump into stiff obstacles, are squeezed through tiny channels or perform a "dance" on a small circular area they are restricted to: When physicists perform cell research, unconventional methods come into play. Atomic force microscopes, microfluidics and micropatterning, often in combination with state-of-the art optical imaging techniques and usually accounting for statistical variations are employed and give rise to fascinating new approaches to cancer research.
The meeting "Physics of Cancer" was held at the University of Leipzig, October 13 to 15 2011. During two days filled with an ambitious agenda, twenty invited speakers – both well known and established ones as well as young researchers early in their career - showed their newest results and findings. In between, talented young researchers contributed ten oral and 26 poster presentations many of which were at a surprisingly high quality level.
The topic seems to be very timely: the room was filled to the last seat throughout the conference. Matthias Schwarz, Vice Rector for Research and Young Academics at the University of Leipzig, who opened the conference, found the enthusiastic attendance remarkable and exceptional. The meeting was supported by the University of Leipzig, the German Research Foundation (DFG), "ibidi - cells in focus" and the DGZ.
The topics of the meeting ranged from theoretical models and simulations to applications of the newest research results in medicine, covering mathematics, physics, cell and molecular biology and biochemistry. Novel experimental techniques such as the optical laser stretcher, "FLAP" (Fluorescence Localization After Photobleaching) and variations of atomic force microscopy (AFM) clearly were one focus. The types of experiments presented covered in vitro setups, cell studies and in vivo investigations. One field where this becomes particularly clear is cytoskeletal mechanics, which is important for, e.g., cell invasion into tissues, adhesion and division. A profound knowledge of the individual components (microtubules, intermediate filaments and microfilaments (actin filaments)) as well as their interactions and the influence of other, smaller molecules is a precondition for understanding more complex systems, like whole cells or organisms. Researchers nowadays move away from the classical notion of 2D cell culture on dishes and try to mimic the physiological conditions as well as possible by using 3D cell culture in polymer matrices and micro- and nano-structuring the substrates. Thereby, molecular models for force transduction are being developed and - very important in the context of the meeting - differences concerning mechanical properties, motility and cell plasticity between healthy and cancerous cells can be detected.
It is one common aim of the presenting and attending researchers to develop approaches to detect and cure cancer, possibly be using their "unconventional" ways to develop model systems for cell motility, invasion and growth. In any way, it is now a solid fact that physicists have joined cell biologists to tackle complex phenomena of mechanobiology such as self-organization, pattern formation and tissue simulation. Certainly, we will hear more about "Physics of Cancer" in the years to come. The planning for the meeting 2012 in Leipzig is under way (http://www.uni-leipzig.de/poc/).

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The multi-talented nuclear envelope proteins nesprins regulate cellular architecture and signaling events

Sascha Neumann

The nuclear envelope

The nuclear envelope (NE) is a continuous membrane system that encloses the genetic material in eukaryotes. It consists of two lipid bilayers, an inner nuclear membrane (INM) and an outer nuclear membrane (ONM). Both are separated by the perinuclear space (PNS), which forms an evenly and regularly spaced entity of 20-30 nm between both membranes. INM and ONM meet at the nuclear pore complexes (NPCs), macromolecular protein assemblies that traverse the NE to allow passive diffusion of particles with a molecular weight of less than 40 kDa and selective transport of proteins. The ONM is continuous with the endoplasmatic reticulum (ER). Even though there are no physical barriers between the different NE membrane "compartments", most NE proteins specifically localize to the inner or the outer nuclear membrane, respectively, by specific mechanisms. Here, I will give a compact overview of nuclear biology and discuss the functions of the nesprins as important nuclear envelope components in integrating the nucleus into the cytoskeleton and as regulators of nuclear morphology and cytoskeletal organisation. Additionally I will refer to the role of nesprins in controlling signaling events.

Nesprins

Nesprins (Nuclear envelope spectrin repeat proteins) belong to a group of about 80 unique integral membrane proteins of the nuclear envelope (NE) (Schirmer et al., 2003). Currently, four different mammalian nesprins have been described, nesprin-1, -2, -3 and -4 (Mellad et al., 2011). Each nesprin is encoded by a single gene that due to differential initiation of transcription and splicing gives rise to a plethora of isoforms, differing in size and domain composition. The largest isoforms are the so called giant isoforms of nesprin-1 and -2 that have molecular weights of 1014 and 796 kDa, respectively. Nesprin-3 and -4 differ in respect to their sizes with molecular weights of approximately 100 to 120 kDa.

Nesprins connect the nucleus along the NE to the various cytoskeletal components, like the actin filament system, microtubules and the intermediate filament system. These functions are based on the presence of individual protein domains in the nesprins (Figure 1). Nesprin-1 and -2 giant harbor at the N-terminus paired calponin homology domains that mediate the binding to F-actin. At the C-terminus is the KASH domain, with a transmembrane domain that is sufficient for anchoring the nesprins to the NE. The name KASH is derived from the presence of this domain in *Drosophila* (Klarsicht), *C. elegans* (ANC-1) and mammalian (Syne) (homology) NE proteins. N- and C-terminus are separated by a stretch of more or less conserved spectrin repeats (SR) amounting to 74 in nesprin-1 and 56 in nesprin-2 (Simpson and Roberts, 2008). Spectrin repeats are bundles of three antiparallel α-helices that are known sites for protein-protein interactions. SRs can be found in cytoskeletal organizer-proteins like α-actinin and spectrin where they allow the homodimerization of these proteins. Nesprin-3 harbours instead of an N-terminal actin binding domain (ABD) a plectin binding domain. Plectin is a cytoskeletal crosslinker protein that mediates the binding to intermediate filament proteins. Nesprin-4 interacts with the plus end directed motor protein kinesin-1, and thus connects the nucleus with the microtubule network (Roux et al., 2009). Taken together, the different domains confer the ability of nesprins to connect the NE via different cytoskeletal acceptor sites to all cytoskeletal systems of a cell. A loss of nesprin results in a disorganization of the actin, microtubule or intermediate filament network (Figure 2).

Mechanisms that anchor nesprins along the NE

The C-terminus of the nesprins reaches into the lumen of the PNS whereas their N-terminus extends either into the cyto- or nucleoplasm. Even though nesprins could freely diffuse into the ER, they remain along the INM and the ONM. This is achieved by interacting with type II integral INM proteins of the SUN-domain family. SUN proteins share a highly conserved C-terminal SUN domain (Sad1 and UNC-84 homology domain) that encompasses around 150 amino acids. They have at least one transmembrane domain and a less conserved N-terminus facing the nucleoplasm. SUN domain proteins are evolutionary conserved and have been found in plants (*Arabidopsis thaliana*), amoebozoa (*Dictyostelium discoideum*), yeast (*Saccharomyces cerevisiae*) and mammals. In humans five SUN domain proteins have been described (SUN-1, -2, -3, SPAG4 and SPAG4L). Like other NE proteins SUN proteins function in a broad range of cellular processes like nuclear migration and anchorage, centrosomal positioning, tethering of meiotic telomeres to the

NE, mitotic spindle formation and function, and spermatogenesis. SUN domain proteins are retained at the INM by interacting with nuclear pore complexes or lamins that line the INM.

Lamins are Type V intermediate filament proteins that form a fibrous meshwork below the INM (Figure 1) with functions in maintaining nuclear architecture and the regulation of chromatin organization, transcription, cell cycle progression and nuclear migration. The interaction between the SUN domain of the SUN proteins and KASH domain of the nesprins in the lumen of the PNS anchors the nesprins along the NE and thus provides a physical connection between the cytoplasm and nucleoplasm called the LINC complex (Linker of Nucleoskeleton and Cytoskeleton) (Crisp et al., 2006; Padmakumar et al., 2005). This complex functions in the force transmission along the cytoskeleton into the nucleus to convert mechanical forces into biochemical signals like the activation of signaling pathways or the regulation of gene expression. One approach to explore the role of SUN proteins and nesprins in the force transmission between cytoskeleton and nucleus is to disrupt the LINC complex by using dominant negative SUN or nesprin peptides, as shown by Lammerding and colleagues (Lombardi et al., 2011). Nesprins can efficiently be displaced from the nuclear envelope to the ER by overexpressing the SUN luminal domain in the PNS. The competition between the mobile SUN protein and the endogenous SUN domains to bind the KASH domain of the nesprins, results in a displacement of nesprins from the NE to the ER. Vice versa ectopic overexpression of the KASH domain results in a saturation of SUN anchors along the nuclear envelope and a displacement of endogenous nesprins from the NE. The disruption of the LINC complex results in impaired force transmission from the cytoskeleton to the nucleus, which leads to a reduced nuclear deformation or displacement after cytoskeletal strain application in microneedle manipulation assays. Contrary to earlier studies that demonstrated a prolonged NF-κB signaling in response to cyclic strain in the absence of a functional LINC complex (Brosig et al., 2010), the authors did not observe the activation of exemplified mechanosensitive genes (e. g. *Egr-1*, *Iex-1*).

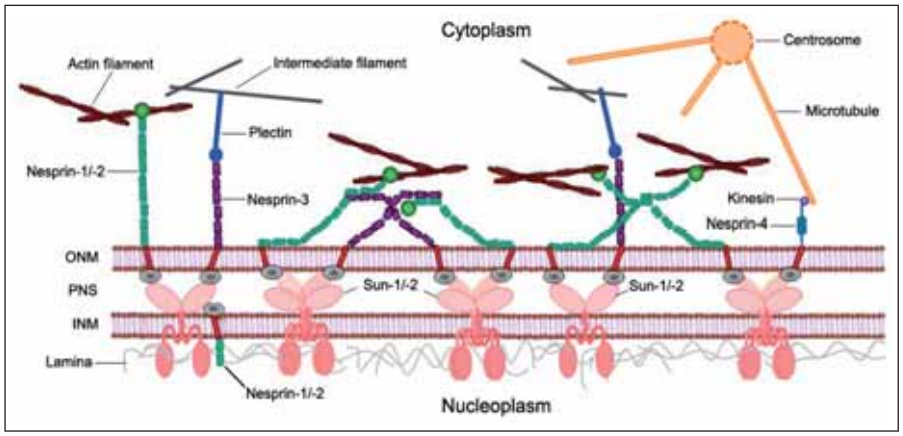

Figure 1: A Model depicting the various nesprins and nuclear envelope protein assemblies. Nesprin-1 and -2 connect the NE to actin filaments, nesprin-3 connects the NE via Plectin to intermediate filaments and nesprin-4 mediates via Kinesin-1 the connection to the microtubule system. The current textbook view of the nesprin topology predicts a projection into the cytoplasm. Our data support, however, a more sophisticated pattern with an interconnectivity between nesprin-1 and -3 and oligomerization via spectrin repeats to form a meshwork along the NE, probably in order to maintain proper nuclear structure. See text for detailed description (from Taranum et al., 2012, modified).

Nesprin assemblies along the nuclear envelope

The topology of nesprins along the nuclear envelope remains an open question. Based on their enormous molecular weights nesprin-1 and -2 giant might reach up to 300 – 400 nm into the cytoplasm. C-terminal isoforms of nesprin-1 are able to self-associate by forming antiparallel dimers through their spectrin repeats (Mislow et al., 2002), similarly nesprin-3α was shown to dimerize (Ketema et al., 2007). It remains to be determined which nesprin-3 spectrin repeats are involved in this process. Recent data from our lab suggest a more complex assembly of nesprins along the NE with additional dimerization sites within nesprin-1 and an interconnectivity between the N-termini of Nesprin-1 and -3, respectively. In our study, we used N-terminal nesprin-1 sequences (nesprin-1-165) corresponding to a nesprin-1 isoform that was previously known as Enaptin-165. Using GST tagged fusion proteins of the N-terminal nesprin-1 ABD and the following spectrin repeats we found that all proteins had the ability to precipitate their GFP tagged counterparts. To explore an interaction between SRs from different nesprins, we performed further binding studies between nesprin-1-165 and the N-terminal half of nesprin-3. Our data suggest that nesprin-1 and -3 interact with each other along the nuclear membrane. We therefore predict a more sophisticated assembly of nesprins along the nuclear membrane including self-associations and the connection of the nesprin-1 N-terminus to nesprin-3 that finally brings the nesprins in close proximity of the nuclear envelope (Figure 1). The interaction of different nesprins additionally is an important finding, since nesprin-1 and-2 are known to connect the nucleus to the actin filament system, whereas nesprin-3 connects it to the intermediate filament system. The

interconnectivity among the nesprins offers further possibilities on how the nucleus is integrated in the cytoskeleton (Taranum et al., 2012). The complexicity of NE protein assemblies continues on the level of the SUN proteins that are able to interact with the KASH domains of the different nesprins (Stewart-Hutchinson et al., 2008).

Nuclear envelope and disease

In recent years an increasing number of diseases have been attributed to mutations in NE components. The first disease that was related to mutations in NE components was the X-linked Emery-Dreifuss muscular dystrophy (XL-EDMD) that was found to result from mutations in STA, a gene encoding emerin. Emerin belongs to the LEM (Lap, Emerin, MAN) domain family of proteins and is primarily located in the INM and to lesser extent along the ONM. EDMD is a muscle disease that mainly affects muscles important for movement, as well as cardiac muscles. Characteristics of the disease are an early onset joint contractures and muscle weakness. Contractures become visible at early childhood or teenage years. The disease is characterized by slowly progressive muscle weakness and wasting and heart problems that lead to an increased risk of cardiac arrest. Most of the diseases due to mutations in NE components have been referred to mutations in *LMNA*, encoding lamin A/C. More than 300 mutations, covering almost the entire sequence of the LMNA gene have been described. Even though they are based on mutations in the same gene, one can observe strong phenotypic differences that range from muscle diseases and premature ageing (Progeria) to neuropathy and lipodystrophy (Zaremba-Czogalla et al., 2011). Even though many aspects of nuclear envelope protein functions have been explored, less is known about the mechanisms on how mutations in ubiquitously expressed proteins like lamins, nesprins or emerin give rise to phenotypically completely different and tissue-specific diseases.

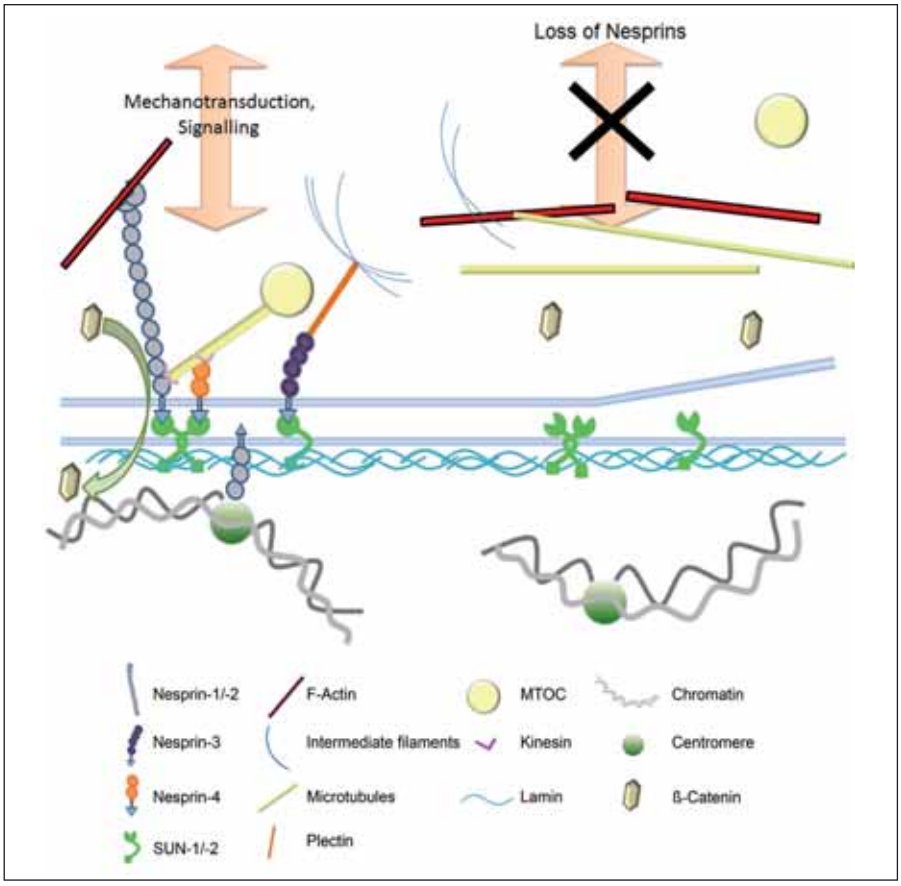

Figure 2: Illustration of nesprin assemblies and signaling functions. Nesprin connects the nuclear interior with the cytoskeleton thus forming the LINC complex (Linker of Nucleoskeleton and Cytoskeleton). Mechanical induced signals are transferred along the cytoskeleton and across the NE into the nucleus where they might induce gene expression. Additionally, nesprins are part of signaling cascades by controlling the nuclear access of transcription factors like ß-catenin. A loss of nesprin-2 results in disorganized actin and microtubule network and impairs proper gene expression (from Noegel and Neumann, 2011, modified).

Nesprins and signal transduction

Currently there are two non-exclusive hypotheses to explain the role of NE proteins in the formation of laminopathies. The first one is the structural hypothesis and refers to the role of NE proteins as structural reinforcers of nuclear morphology and the importance of an intact nucleo- to cytoskeletal connection in the transformation of mechanically induced signals. The second one proposes that mutations in NE proteins impair proper gene expression. This leads to the question how NE proteins can affect the expression of genes. Several possibilities are discussed. NE proteins might control the expression of genes by regulating the access of transcription factors in and out the nucleus trough nuclear pore complexes, by enabling the transduction of mechanically induced signals that reach the nucleus via different cytoskeletal structures via the LINC complex, by regulating the topology of chromatin to generate a surrounding that is favourable or unfavourable for gene expression or by the sequestration of transcription factors (Heessen and Fornerod, 2007) (Figure 2).

Recently, nesprin-2 was identified as a regulator of Wnt and ERK signaling (Neumann et al., 2010) (Warren et al., 2010). At the center of the Wnt signaling pathway is the pro-

tein β-catenin. β-catenin is a protein with dual functions. It is a component of plasma membrane protein complexes that maintain cell-cell contacts by forming adherens junctions between neighbouring cells and it acts as a transcription factor. When the Wnt pathway is not activated cytoplasmic ß-catenin pools are degraded. In the presence of a Wnt signal, cytoplasmic β-catenin levels increase and the protein passes the nuclear envelope and enters the nucleus where it acts as a transcription factor. Interestingly, nesprin-2 together with α-, β-catenin and emerin forms a quaternary protein complex, which suggests that beside the signal transduction along the cytoskeleton and the LINC complex, nesprins might also be involved in more dynamic signal transduction cascades including protein shuttling between plasma membrane and nucleus. Finally, loss of nesprin-2 impairs the accumulation of the transcription factor β-catenin inside the nucleus. Another example for the role of nesprin-2 in nuclear signaling is the regulatory function of nesprin-2 on ERK1/2 activity. It was shown that a KASH-less nuclear Nesprin-2 isoform scaffolds ERK1/2 kinases at promyelocytic leukemia protein nuclear bodies (PML NBs) and in this way negatively regulates the activity of these kinases. Loss of nesprin-2 leads to sustained ERK1/2 signaling and increased cell proliferation. Taken together these studies predict a role of nesprin-2 as a negative regulator of ERK- and Wnt-signaling.

With this report I give a compact summary about the nuclear envelope and especially focus on the nesprins. The challenge for the future will be to provide a detailed understanding of the various nesprin functions to elucidate the role of these proteins in organizing cellular structures or gene expression. Based on a better understanding of nesprin interactions and functions we hope to finally answer the question how NE proteins contribute to the formation of laminopathies.

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Sascha Neumann, studied Biology at the University of Cologne. In 2009 he received his PhD at the Center for Biochemistry, Medical Faculty, University of Cologne. Since 2010 he is group leader at the Institute of Biochemistry I, Medical Faculty, University of Cologne, working in the field of nuclear envelope proteins. The main aims of the group are to investigate the role of nuclear envelope proteins like nesprins, lamins or SUN proteins in maintaining cellular architecture and regulating signaling events in the nucleus. Finally the work aims to understand the biological function of nuclear envelope proteins on the cellular level and in the formation of human diseases that are collectively known as laminopathies.

International Meeting of the German Society for Cell Biology

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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

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- to enhance the acceptance and recognition of life sciences and scientists in our society.

How is VBio organised?

VBio is led by a president, who is supported by an executive committee with two vice presidents and six further members, an advisory board and two staff officers, Dr. Carsten Roller in Munich and Dr. Kerstin Elbing in Berlin. The new president, who was elected at the recent annual conference on November 4th, is Prof. Dr. Wolfgang Nellen (Dept. of Genetics, University of Kassel). New vice presidents are the former president Prof. Dr. Diethard Tautz (MPI Evol. Bio., Plön) and Prof. Dr. Bernd Müller-Röber (Dept. of Mol. Biol., University of Potsdam).

What is VBio doing for the interests of biologists?

VBio is struggling for the interests of biologists at many fronts. One hot issue is biosafety. VBio has proclaimed its protest against the broad scope of the proposed rules in the "Guidance Document for CWA 15793:2008 Laboratory Boris Management Standard" by the European Committee for Standardization (CEN). VBio has expressed our apprehensions of overregulation and hindrance of life science research. On the national level, VBio is struggling for an abolition of the S1 level in the German GenTG. Moreover, VBio has pointed out serious problems with regard to shipping of biological samples by airfreight due to the new EU-Directive 1069/2009, which regulates transport of animals, animal byproducts and side products. With regard to school education in life sciences, VBio, DdCh (Society of German Chemists) and DPG (German Society for Physics) have raised an objection against the cutback of biology, chemistry and physics lessons in high schools. VBio is active in many more hot topics for biology. Its current and past activities are listed and explained on their website at http://www.vbio.de/der_vbio/aktivitaeten/index_ger.html.

Why should we support VBio?

Threats against our working conditions are rising. VBio is our strongest voice to claim OUR interests as biologists toward governmental organizations. We have no better one. All specialized biological societies such as ours are (hopefully) good in organizing meetings and fostering scientific communication among their members, but they all have a far too little number of members to be heard and recognized by politicians and journalists. But even VBio needs more members to get a stronger voice.

How can we support VBio?

Go for a direct membership in VBio at www.vbio.de! As most other biological societies the DGZ supports VBio with 5 € per member, which accounts for the indirect members of VBio. Yet, this is not enough to support all the various tasks of VBio. Therefore, at their recent annual meeting, the delegates from the "Landesverbände" and biological societies came to a board decision to introduce a new kind of membership, the "*community membership*". Starting with 2012 indirect members from biological societies can get this new kind of direct membership for only 25 € per year. Community members have the

same rights and benefits as full members except the subscription to BiuZ (the VBio Journal "Biologie in unserer Zeist"), which is available for community members for an extra fee. Of course, as a DGZ member you can also go for a standard full membership for 70 € including subscription to BiuZ.

The delegates have also decided for a newly designed "*student membership*", which is a joint membership in VBio and a biological society of choice such as the DGZ. All bachelor and master students in biology will be able to apply for this type of membership starting with winter semester 2012/2013. It will cost only 25 €, which are shared 1:1 between VBio and the other society. If you are a lecturer go and advertise this new student membership, since it will foster both VBio and the DGZ. If you want to be active or participate in VBio visit their website at http://www.vbio.de and contact Dr. Kerstin Elbing (elbing@vbio.de)for issues regarding science and society or Dr. Carsten Roller (roller@vbio.de) for education and carreer.

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International Summer School on Actin Dynamics 2011

Eugen Kerkhoff & Klemens Rottner

In collaboration with the DFG priority programme SPP 1464 on actin nucleators, the German Society for Cell Biology has organized the second "International Summer School on Actin Dynamics", which took place from September 17th to 23rd, in Regensburg. The Summer School on Actin Dynamics intends to teach current knowledge and state of the art methodology in actin cytoskeleton research to young, distinguished scientists. An international group of 31 participants from 9 different nations came together with leaders in their respective fields, to listen to lectures in the morning and to attend experimental classes in the afternoon. In the evenings, exhausted students (and teachers) mostly gave in to the temptations of the Bavarian way of live and enjoyed "Bier" and "Schweinebraten" in the historical old city of Regensburg.

The school was kicked off with a keynote lecture by Laurent Blanchoin, University of Grenoble (France), who introduced to the students the fascinating world of in vitro actin filament dynamics illuminated by total internal reflection fluorescence (TIRF) microscopy, allowing to directly visualize and thus dissect biochemical activities as distinct as polymerization, bundling or disassembly. Laurent Blanchoin also offered a practical course on TIRF microscopy together with his coworker Christophe Guérin during the entire period of the summer school. The introductory lecture was followed by a students' symposium, held the day after, in which every student had the opportunity to present in a short lecture his/her current research project, and discuss most recent results with their colleagues and/or course teachers. The quality of these presentations was outstanding and made the students' symposium one of the many, very entertaining highlights of this year's summer school.

The rest of the week was organized as daily morning lectures, mostly by honourable, external guest speakers, followed by practical courses in the afternoons. Many guest speakers are also members of DFG priority programme SPP 1464 on actin nucleators, and thus covered many relevant aspects of research on the actin cytoskeleton. The lectures included Henry Higgs (Dartmouth, USA): actin biochemistry, Peter Lenart (Heidelberg): animal models for analyzing the cytoskeleton, Jan Faix (Hannover): formins, Margot Quinlan (San Francisco, USA): WH2 nucleators, Peter Hahn (Qiagen): DNA/siRNA transfections, Matthias Geyer (Dortmund): methods in structural biology, Kornelius

Zeth (Tübingen): structure of actin nucleator complexes, Beata Bugyi (Pecs, Hungary): in vitro actin polymerization assays, Andreas Bausch (München): biophysics of the actin cytoskeleton and Sven Vogel (Dresden): biomimetic membrane system and fluorescence correlation spectroscopy. The closing keynote lecture was given by Catherine D. Nobes from the University of Bristol (UK), known to all of us through her outstanding discoveries on the regulation of the cytoskeleton by Rho family GTPases in the laboratory of Alan Hall back in the early 90ies, who presented an overview on her most recent contributions to unravelling the molecular secrets of cell-cell attraction and repulsion.

The responses of both participants and teachers of the first summer school in 2010 were extremely constructive for shaping and organizing this year's school. Students were divided into five groups, who rotated through five independent experimental classes each repeated every day, which allowed every student to attend each of the experiments. Besides TIRF microscopy taught by Laurent Blanchoin and Christophe Guérin (Grenoble, France), more bulk assays for studying actin polymerization in vitro e.g. using pyrenylated actin were demonstrated by Henry Higgs (Dartmouth, USA). Advanced live cell imaging and photomanipulation techniques such as fluorescence recovery after photobleaching (FRAP) or photoactivation were demonstrated by Jan Faix (Hannover) and one of the authors (KR) together with their co-workers Jörn Lindner (Hannover) and Jennifer Block (Bonn), respectively. The experimental courses further covered bead motility assays (Beata Bugyi, Pecs, Hungary) and biophysical protein interaction studies using isothermal titration calorimetry (ITC), demonstrated by Margot Quinlan (San Francisco, USA), with expert support by Susanne Dietrich (University of Regensburg).

In spite of state of the art technology and fanciest equipment kindly provided by several companies listed in acknowledgements, it was a reasonably simple and historical experiment, which attracted significant attention among students. Henry Higgs showed, using a home-made apparatus, how actin assembly rates can be monitored by simply measuring the viscosity of solutions. This so called falling ball assay just requires a pipette, a little ball and a stop watch.

What makes the summer school a continuous event of enthusiasm is the fact that participants can bring their own reagents in the form of proteins or DNA expression vectors, to be assayed with the different methods, and exciting results being interpreted or discussed by colleagues in the field. This could not be achieved without the flexibility and readiness for action of all course teachers, especially those from abroad, and their helping lab members, both of which were again outstanding.

We feel that the summer school 2011 was a great success, and of course hope that the spirit can be maintained for the next one around. We would like to thank everyone involved! We are well aware this would not have happened without the many helping hands and brains operating in the background.

The Summer School 2012 will take place on September 29th through October 5th in Regensburg. To register, please klick www.actindynamics.com (registration will start in January, 2012).

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RNA meets Disease in Paradise

Julia Winter*, Anna Roth*, Sven Diederichs

Getting off the train in Jena-Paradies, 60 participants joined for the 12th Young Scientist Meeting of the German Society for Cell Biology (DGZ) entitled "RNA & Disease". Excellent speakers from around the world, graduate students, postdocs and young group leaders enjoyed a meeting in a familiar atmosphere to exchange inspiring new data and vibrant scientific discussions about the fascinating history and exciting future of non-coding RNA research including microRNA, piRNA and long non-coding RNA as well as their function in cancer, diabetes and neurodegenerative diseases.

Well-known friends: the fascinating world of miRNAs

Brian D. Brown (Mount Sinai Medical Center, New York) opened the meeting introducing a technology that enables stable and corrective gene transfer in immunocompetent mice using a lentiviral vector containing microRNA (miRNA) binding sites in its 3'UTR. Consequently, the vector can be posttranscriptionally regulated by endogenous miRNAs in a cell and thereby provides cell-specific expression, a major hurdle to successful gene therapy[1]. Using this elegant vector system, he reported studies correlating miRNA expression and target suppression, and showed the majority of detectable miRNAs in a cell have little suppressive activity[2]. He also showed work examining the stoichiometry between miRNA and target which demonstrated multiple turnover regulation in the non-slicing pathway[3].

Frank Slack (Yale University, New Haven) highlighted the strong potential of miRNAs to revolutionize targeted therapies. He gave an overview about the last years of groundbreaking research that opened up the way for let-7 and miR-34 as targeted therapies in lung cancer where the first clinical trials in humans are going to start prospectively next year specifically delivering the miRNAs into the lung of a patient. The potential of the tumor suppressor let-7 that is frequently downregulated in cancer patients and maps to fragile regions associated with various cancers, was studied in a Lox-Kras^{G12D} mouse model. Let-7 did not only reduce tumor growth in xenografts and reduced lung tumor formation[4], it also caused remission of preformed lung tumors[5], even upon systemic delivery[6]. These molecules are now being tested singly and in combination in Kras$^{G12D/+}$p53$^{R17/+}$ mice.

Dirk Grimm (Bioquant, Heidelberg), the co-organizer of the meeting, gave an insight into the design of adeno-associated vectors (AAV) with improved specificity, efficiency and safety with regard to biomedical applications. The initial observation that the high-level expression of shRNA from AAV/shRNA vector genes induced either dose-dependent liver-damage or caused liver failure and death in adult mice[7] led to a further screening of alternative shRNAs with less

*These authors contributed equally.

side effects yielding one very safe candidate. The high-level expression of shRNA in adult mouse liver correlated with a down-regulation of liver-derived miRNAs, implicating that a competition for rate-limiting factors of the RNAi machinery occurs. In fact, Exportin-5 and Ago2 were identified as rate-limiting factors by *in vivo* shRNA transfection studies[8]. To improve the specificity and safety for potential biomedical application of AAV/shRNA vectors, a controlled shRNA expression in the target tissues will be crucial.

Reuven Agami (Netherlands Cancer Institute, Amsterdam) presented exciting new data on the differential use of alternative polyadenylation signals and their impact on a mouse model of a degenerative muscular disorder.

Markus Landthaler's (Max-Delbrück-Center for Molecular Medicine, Berlin) research aims at elucidating the assembly and dissociation of mRNP (mRNA ribonucleoprotein particle) complexes as well as unraveling the influence of mRNPs on the stability, localization and translation of mRNAs. Using the PAR-CLIP (photoactivatable-ribonucleoside-enhanced crosslinking and immunoprecipitation) technique, RNA binding sites for the FET family of proteins (FUS, EWSR1 and TAF15) protein were identified to be mainly localized in intronic regions. Furthermore, Landthaler aims at the biochemical characterization of the human protein-RNA interactome using quantitative mass spectrometry-based identification of precipitated protein families such as (putative) RBPs, ribosomal proteins, helicases, translation factors and RNA modifying enzymes. RRM, helicase and KH domains were suggested to be RNA recognition motifs that bind RNA domains most frequently. However, this hypothesis was derived from structure term analyses and requires experimental validation that is now possible using Landthaler's technology. In the near future, the functional characterization of candidate RBPs and other novel RNA-interacting proteins will be performed.

Amaia Lujambio (Memorial Sloan-Kettering Cancer Center, New York) discussed the role of epigenetic regulation of miRNAs in cancer. Using methods of genetic and pharmacological unmasking, she identified methylated CpG islands of genomic miRNA loci[9, 10]. MiRNA expression profiles of the parental colon cancer cell line HCT-116 and the respective double knockout cells (DKO) defective in the DNA methyltransferases 1 and 3b revealed the association between DNA hypermethylation and the loss of miR-124a. Moreover, a correlation between the loss of the tumor suppressor miR-124a with the activation of the oncogene CDK6 and phosphorylation of the tumor suppressor Rb protein was established[11]. Thus, the epigenetic silencing of miR-124a is linked to the process of tumorigenesis. Using pharmacological unmasking, five miRNAs with a cancer-specific CpG hypermethylation were identified in lymph node metastasis cell lines, leading to the suggestion that miRNAs control the dissemination of tumor cells[12]. Additional results reveal a lack of expression also for transcribed ultraconserved regions (T-UCRs) in neuroblastoma due to hypermethylation of respective genomic regions.

Gunter Meister (University of Regensburg) presented data on the identification of NF90 as a novel factor in the human miRNA pathway whose depletion leads to silencing defects of reporter constructs and affects miRNP-mRNA interaction[13]. NF90 might potentially act as a so-called "quality control protein" checking each step of miRNA processing since it is interacting with all main players of the biogenesis pathway including pre-miRNAs and a small pool of mature miRNAs. Meister also introduced a SILAC-based approach to identify novel components of Ago2 protein interactions using WT (wildtype) and Dicer$^{-/-}$ MEFs (mouse embryonic fibroblasts) stably expressing FLAG/HA-tagged Ago2. This elegant study allows the identification of known as well as novel Ago2-interacting proteins that are either dependent on mature miRNAs, preferentially interact in the presence of miRNAs or that are independent of mature miRNAs such as Edc4 (enhancer of mRNA decapping 4), a component of the RNA-induced silencing complex (RISC) that might potentially stimulate decapping of mRNAs upon miRNA-target silencing. In concordance with the data of a recent publication, he also showed that an Ago2 Y529E mutant that is defective in binding miRNAs can still be associated with larger mRNAs.

Daniel Mertens (University of Ulm) highlighted the complex regulation at the 13q14 locus, a very critical region that is most commonly found to be defective in CLL (chronic lymphocytic leukemia) patients[14]. This region contains the microRNA cluster of miR-15/miR-16. After discovering that the genes of this particular region do not contain point mutations and that defective Drosha processing contributes to the well-known down-regulation of mature miR-15a/16-1, he focused on the interplay of methylation and the respective transcripts. Mertens showed that DNA demethylation at 13q14.3 impacts on the expression of non-coding and protein-coding transcripts of this particular region. Furthermore, he also introduced NF-κB as a central player in the pathomechanism of this disease.

Not all eggs in one basket: short AND long non-coding RNAs

George Calin (University of Texas / MD Anderson Cancer Center, Houston) presented an enthusiastic and comprehensive walk through the last decade of non-coding RNA research and encouraged the the young scientists to take part in this "non-coding RNA revolution" and to also go for risky projects aiming for novel insights. Calin discussed

latest findings on many aspects of miRNA research such as miRNAs targeting proteins (e.g. miR-382 controlling RNA-binding proteins)[15] and highlighted the direct and indirect interaction of miRNAs at the example of miR-15a and miR-16-1[16]. He assigned miR-21 as a potentially universal marker for cancerous tissues[17], discussed the high potential of plasma miRNAs for the prediction of treatment outcome of cancer patients[18] and introduced the usage of small molecules targeting miRNAs in therapy. Revealing data about long non-coding RNAs, Calin focused on the interaction between long ncRNAs and miRNAs. MiRNAs directly bind and regulate ultra-conserved long ncRNAs, but in turn also these UCRs possess the potential to regulate miRNA expression. A search for human-specific, non-conserved ncRNAs, that are characterized by intragenomic conservation ("short repetitive ncRNAs"), revealed tissue-specific profiles that correlate with clinical parameters. One short repetitive ncRNA is regulated by TP53 inducing epithelial-to-mesenchymal transition (EMT).

Sven Diederichs (German Cancer Research Center (DKFZ) & University Hospital Heidelberg), the organizer of the meeting, presented a successful example on how to develop four different scientific projects from carefully dissecting one single Northern blot on the role of Argonaute (Ago) proteins in miRNA biogenesis: Human Ago1-4 increase miRNA abundance by stabilizing them[19, 20] and Ago2 does not only generate the ac-pre-miRNA during miRNA processing[19] but also increases the efficacy and specificity of RNAi[21]. After describing the discovery of the well-known and intensively studied long ncRNA MALAT-1, Diederichs reported recent progress in revealing novel functional long non-coding RNAs in cancer. One very promising candidate in lung cancer is termed LuCaiR1 (lung cancer intergenic RNA 1), that is lost upon the induction of DNA damage by the chemotherapeutic drug Cisplatin and affects the viability of lung tumor cells. After the identification of novel tumor-associated long ncRNAs in lung and liver cancer, the comprehensive functional analysis includes RNA affinity purifications to uncover the ncRNA-protein-networks as well as the creation of human knockout cancer cell lines using zinc finger nuclease methodology.

There is more out there: seminal research on other non-coding RNA species

Utz Fischer (University of Würzburg) emphasized the role of mRNA metabolism defects as a cause for three genetic diseases with tissue-specific phenotypes and presented zebrafish as a suitable model system for *in vivo* studies. The first disease, spinal muscular atrophy (SMA), causes severe muscular atrophy and is characterized by the loss of functional SMN1 (survival motor neuron 1). SMN1 is an essential component of a multiprotein complex that triggers the assembly of spliceosomal snRNPs. Knockdown studies of SMN in zebrafish induced a human disease-like pathological effect which did not emerge when exogenic snRNP was added[22]. This links SMN1 deficiency to defects in mRNA splicing and thus altering the expression profile of the cell. This is also the case for Retinitis pigmentosa (RP), an eye disease that is characterized by the degeneration of rod photoreceptor cells due to mutations in splicing factors such as Prp31[23]. Distal SMA type 1 (DSMA1) is caused by mutations in the ribosome-associated helicase IGHMBP2 (mmunoglobulin mu-binding protein 2). This helicase associates with ribosomes and is functionally linked to mRNA translation promoting the expression of not yet identified target mRNAs[24].

Ingrid Grummt (German Cancer Research Center (DKFZ), Heidelberg) presented comprehensive data on a new mechanism of transcription regulation by promoter-associated ncRNA transcripts. Questioning how chromatin modifying enzymes are guided to their target sequence, ncRNA transcripts in sense direction, termed pRNA (promoter-associated RNA), and in antisense direction were identified upstream of the pre-rRNA transcription initiation site and within the rDNA genomic region[25]. Transfection and knockdown studies revealed a role for pRNA in rDNA promoter regulation at the epigenetic level. A pRNA stretch of 20 nt was identified as the essential element triggering the *de novo* CpG methylation of rRNA genes by recruiting the DNA methyltransferase DNMT3b and thereby inducing transcriptional silencing[26]. Interestingly, *in vitro* binding studies suggested the formation of a triplex structure between the T_0 element of the rDNA promotor and the pRNA and furthermore, revealed a selective binding of DNMT3b to DNA:RNA triplex structures. For the antisense ncRNA, a direct interaction with the histone methyltransferase Suv4-20 was demonstrated using RNA pull-down assays. This finding explains the increased rate of histone methylation correlating with upregulation of the antisense transcripts.

Ute Kothe (University of Lethbridge) gave an insight into an elegant pre-steady state kinetic analysis of pseudouridine synthases, enzymes that catalyze the most abundant RNA modification in a cell which is present in all RNAs. Stopped-flow absorbance assays revealed that substrate tRNA binding by TruB takes place in two steps and chemical quench-flow studies to measure single turnover of pseudouridyine formation identified catalysis as the rate-limiting step. Further investigations using two other *E.coli* enzymes, RluA and TruA, revealed slow catalysis to be a rather general feature of pseudouridine synthases, proposing that these enzymes that share a conserved catalytic domain, supposedly use the same catalytic mechanism[27]. These studies were further compared to studies of the archaeal pseudouridine synthase Cbf5 which acts as part of the H/ACA

small ribonucloeprotein complex.

Kannanganattu Prasanth (University of Illinois Urbana-Champaign) presented new data on the nuclear-retained (nr) long non-coding RNA (MALAT1) as a multifaceted regulator of gene expression[28]. Using RNA-FISH (RNA fluorescent in situ hybridization) approach, MALAT1 was identified as a nuclear speckle component. MALAT1 was originally identified by Diederichs and colleagues as an RNA that is up-regulated in metastatic lung cancer patient samples[29]. A role for MALAT1 in the modulation of synaptogenesis has been proposed recently[30]. MALAT1 interacts with several pre-mRNA processing factors including SR (serine/arginine) splicing factors, regulates the distribution of splicing factors to nuclear speckles and sites of transcription and finally modulates SR splicing factor phosphorylation and alternative splicing of pre-mRNAs in cancerous human cells[31-34]. In his talk, Prasanth showed evidence for the involvement of MALAT1-associated SR proteins in the organization of nuclear speckles and discussed the potential role of nrRNAs in the organization of sub nuclear domains. To stress the importance of MALAT1, Prasanth outlined the effect of MALAT1 knock down in several cell lines (primary and transformed), showing cell type-specific phenotypes including cell cycle arrest and mitotic defects.

The 12th meeting of the German Society for Cell Biology was completed by excellent short talks and poster presentations of young scientists that were rewarded with five prizes sponsored by RNA Biology. For his work on zinc finger nuclease-mediated silencing of non-protein-coding RNAs[35], Tony Gutschner was awarded the first prize. Danilo Allegra ("Defective Drosha processing contributes to downregulation of miR-15/16 in chronic lymphocytic leukemia"), Jens Bohne ("A 5' splice site created by a point mutation in the 3' UTR of P14 is the molecular trigger of a primary immunodeficiency"), Ahmet Ucar ("miR-212/132 family dependent regulation of the epithelial-stromal interactions is necessary for the pubertal mammary gland development") and Carla Winterling ("Induction of long non-coding transcripts by influenza A: regulators of virus infections?") shared the second place.

As highlighted above, this meeting provided new insights and prompted further questions and new - sometimes collaborative - projects. The powerful research on all kinds of non-coding RNAs will undoubtedly maintain a great future for these molecules, maybe even as main players of targeted therapies in different kinds of diseases including cancer. The discussions held at this meeting stimulated further progress in each of these directions and meeting attendees left Jena-Paradies with multiple new ideas and dreams about their future research projects.

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This meeting report has already been published in RNA Biology[36].

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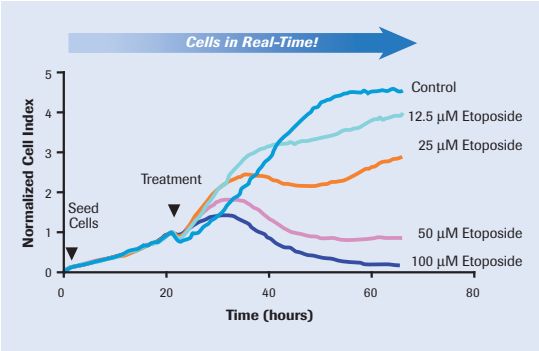

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