Inhalt

Deutsche Gesellschaft für Zellbiologie

Titelbild: Photomontage basierend auf einem migrierenden Fibroblasten, dessen Aktin-Zytoskelett durch fluoreszierendes Phalloidin visualisiert wurde (Originalabbildung zur Verfügung gestellt von Kathrin Schloen, AG Stradal, Helmholtz Zentrum für Infektionsforschung, Braunschweig).

Wofür brauchen wir die Gesellschaft?

Der neue Vorstand

Mit dieser Ausgabe der Zellbiologie aktuell möchte sich der neue Vorstand vorstellen und allen, die uns gewählt haben, für ihr Vertrauen danken. Diejenigen, die zur Mitgliederversammlung auf der Jahrestagung in Regensburg gekommen waren, haben uns schon kennengelernt und einiges von dem, was wir verändern oder voranbringen wollen, von uns gehört. Dazu mehr im Folgenden. Dieses Mal handelt es sich wieder um einen kompletten Vorstandswechsel. Ob dies immer so sinnvoll für die Kontinuität der Aktivitäten der Gesellschaft ist, soll an andere Stelle aufgegriffen werden. Auf jeden Fall möchten wir dem scheidenden Vorstand – Reinhard Fässler, Jürgen Wehland, Dirk Breitkreutz und Michael Sixt – im Namen der Gesellschaft für ihren Einsatz und ihre erfolgreiche Arbeit danken. Ebenso den scheidenden Beirats-Mitgliedern, Elisabeth Knust, Christof Hauck und Dietmar Vestweber, sowie den langjährigen Mitgliedern des Gutachtergremiums für die Preisvergabe der Walther-Flemming-Medaille und des Binder-Innovationspreises, Carmen Birchmeier, Enno Hartmann und Mathias Montenarh.

Anja Bosserhoff, Vize-Präsidentin, und Eugen Kerkhoff, Sekretär, lehren beide an der Universität Regensburg und waren bisher noch nicht in einem Amt der DGZ tätig. Ralph Gräf, der neue Geschäftsführer, vertritt an der Universität Potsdam das Fach Zellbiologie und war schon einmal Sekretär der DGZ von 2002 bis 2004. Ich arbeite am Deutschen Krebsforschungszentrum, lehre im Fach Zellbiologie an der Universität Heidelberg und war von 2002 bis 2006 Geschäftsführer der Gesellschaft. Anja Bosserhoff hat zudem die diesjährige Tagung der DGZ in Regensburg organisiert, ich die Jubiläumstagung in Heidelberg im Jahr 2005. Das Regensburger Meeting war ausgezeichnet, und von der Verknüpfung mit dem Treffen des German Melanoma Research Networks (Melanomverbund) haben beide Gesellschaften profitiert. Diese Art der Verknüpfung mit aktuellen wissenschaftlichen Spezialgebieten, die für die Zellbiologie wichtig sind oder in denen entscheidend zellbiologisch gearbeitet wird, ist sicher sehr sinnvoll, und dieser Ansatz sollte auf jeden Fall in entsprechender Weise in Zukunft weiter verfolgt werden.

Evolution der wissenschaftlichen Organisation

Eine neue Aufgabe beginnend mag mancher geneigt sein, erst einmal alles in Frage zu stellen, und vielleicht erwartet man das auch teilweise von ihm. Eine Gesellschaft lebt andererseits auch von der Konstanz. Angesichts des natürlichen „Turnovers" in der Gesellschaft ist es um so wichtiger, dass junge Mitglieder einbezogen und in Verantwortung gebracht werden. Drei der vier Mitglieder des neuen Vorstandes sind Anfang/Mitte vierzig und damit in einer frühen Phase ihrer Karrieren. Sie garantieren frischen Wind und stellen eine ideale Ergänzung zu den „alten Füchsen" in Vorstand und Beirat dar. Letztere haben die Aufgabe, Bewährtes zu vertreten und dafür zu sorgen, dass es nicht leichtfertig entsorgt wird. Dies betrifft besonders die Ziele der DGZ. Kurz ausgedrückt ist dies die Förderung der Zellbiologie in Deutschland, sowohl im öffentlichen Diskurs als auch in der konkreten Förderung der jungen Wissenschaftler. Eine der wichtigsten Aufgaben ist dabei ihre Einbeziehung in und Vorbereitung auf die neueste Forschung.

Meet the Expert

Die Neuerungen, die das Internet als Kommunikationsmethode bietet, sind gewaltig und auch von erfahrenen Wissenschaftler kaum noch zu bewältigen. Viele von uns, die in der Vergangenheit Tage vor den Xerox-Maschinen verbracht haben, sitzen heute wohl immer noch ab und zu staunend vor dem Schirm ob der tausende von zugänglichen wissenschaftlichen Zeitschriften. Nun, heruntergeladen ist schnell, gelesen weniger schnell, verstanden noch langsamer. „Journal Clubs" sind ein Weg, um unsere Studenten und uns selbst neue Entwicklungen nahe zu bringen. Um wie viel wertvoller ist es dann, in „Plenary lectures" oder wie diesmal in Regensburg in Vorlesungen der Art „Meet the Expert", im Feld führende Wissenschaftler komprimiert und ideal kommentiert vortragen zu hören, was die neuen Entwicklungen sind, welche Methoden in deren Zusammenhang entwickelt wurden und was diese leisten können und was nicht. Dies sind einmalige Gelegenheiten Wissen aufzunehmen, wie man sie im Labor- und Computer-Alltag nicht geboten bekommt. Daraus folgt eigentlich, dass jeder Gruppenleiter größtes Interesse haben sollte, seine Mitarbeiter auf die Jahrestagungen der DGZ zu schicken – und selbst zu gehen. Ich habe öfter schon erlebt, dass etablierte Wissenschaftler auf ein Jahrestagungs-Programm schauen und dann sagen: Ist nichts für mich dabei, und außerdem gehe ich lieber auf Spezial-Tagungen. Falsch, würde ich sagen. Auf die Spezial-Tagungen müssen wir „Etablierte" sowieso. Und, in der heutigen Zellbiologie wird der Begriff „Allgemeinbildung" praktisch nicht verwendet. Dennoch ist dies ein absolut wichtiger Teil unserer Fähigkeit, diese Probleme zu erkennen. Was nützt es einem Wissenschaftler, wenn er zum Beispiel jedes Molekül eines Fokalkontaktes kennt sowie alle erhältlichen Reagenzien und die Labors, in denen sie verwendet werden, wenn er auf dem Gebiet der Stammzellen ein kompletter Ignorant ist. Mir hat Andreas Trumpp in seinem Vortrag in Regensburg jedenfalls demonstriert, was ich alles nicht wusste, konzeptionell falsch eingeschätzt hatte und auch in Bezug auf machbare Experimente und mögliche Kooperationen nicht wusste. Den meisten Biologen ist das „Young's modulus" oder Elastizitätsmodul sicher ein „Un-Begriff". Wer aber neueste Arbeiten von biophysikalisch orientierten Zellbiologen wie Dennis Discher liest wird sehen, dass es eine sich differenzierende Stammzelle durchaus interessiert, wie hart das Substrat ist auf dem sie wächst, und dass sie sich in Reaktion auf diese physikalischen Qualität entscheidet, den Weg zur Nerven- oder Muskelzelle zu gehen. Das heißt, eine kontinuierliche Teilnahme an den Jahrestagungen der DGZ zahlt sich aus, besonders wenn man in die Sessions geht, von denen man nichts versteht. Und man wird lernen, die Physiker interessieren sich für uns und kommen zu den Tagungen, wenn man relevante Symposien organisiert - eine ideale Gelegenheit miteinander zu reden, Kontakte zu knüpfen und neue Forschungskonzepte zu erarbeiten.

Was geht?

Damit komme ich zu den neuen Aufgaben, die wir uns vorgenommen haben. Zuallererst wollen wir die Jahrestagungen stärken und versuchen, wieder mehr Mitglieder zur regelmäßigen Teilnahme zu bewegen. Kleinere Experimente mit dem Tagungsformat sind geplant, wobei die Programme der Tagungen in der Vergangenheit ausgezeichnet waren und diese Qualität gilt es zu halten. Auch in Regensburg wurden wir von ausländischen Sprechern gefragt, wieso bei einem solch exzellenten Programm nicht mehr Teilnehmer kommen. Nun sind 600 Teilnehmer nicht schlecht und eine Maximierung der Zahl der Teilnehmer nicht oberstes Ziel, aber es könnten schon noch ein paar mehr sein, um die Möglichkeiten für Interaktionen zu erhöhen. Kurz, es wundert uns warum nicht mehr Mitglieder zur Tagung kommen. Liegt es am Geld? Vielleicht sind die alten Mitglieder auch saturiert, um nicht „bequem geworden" zu sagen. Wahr-

scheinlich sind sie auch zu sehr gefordert in ihrer jeweiligen Position und tauchen nur noch gelegentlich aus dem Treibsand, in dem sie stecken, auf. Die Aussage, ich gehe nur noch auf Spezial-Meetings lässt auf einen gewissen Grad von Verdruss und Resignation schließen, und eine umfassendere Neugier, die sie wohl in die Wissenschaften geführt hat, scheint abhanden gekommen zu sein. Diese Kollegen gilt es zu motivieren, auch indem wir ihnen die Gelegenheit geben, Symposien zu organisieren und damit vielleicht ihr Feld wieder in einem anderen Zusammenhang zu erfahren.

Was wir auf jeden Fall forcieren wollen ist, dass noch mehr Doktoranden kommen und ihre Arbeiten vorstellen. In diesem Zusammenhang möchte ich noch einmal alle Gruppenleiter darauf hinweisen, dass die DGZ Reisestipendien vergibt. Die Prozedur ist äußerst einfach: Es reicht ein eingereichtes Abstract, ein Lebenslauf des Antragstellers und ein kurzes Gutachters des Betreuers. Diese Möglichkeit ist in den letzten Jahren aus unerfindlichen Gründen relativ wenig genutzt worden. Einzige Bedingung ist, dass der Antragssteller Mitglied der DGZ ist oder wird. Der studentische Mitgliedsbeitrag ist absolut günstig und steht in keinem Verhältnis zu dem, was geboten wird.

Putting Cell Biology on the Map

Im Rahmen der Planung der jeweiligen Jahrestagungen stellten wir immer wieder fest: Da gibt es Leute vor Ort, die sehr interessante Arbeiten machen, selber aber nie zur Jahrestagung kommen, und die man bisher noch nicht gekannt hat. Ein Sinn der Forschungsprofile der Zellbiologie aktuell war es seit ihrer Gründung, die Breite der Expertise in der Zellbiologie in Deutschland aufzuzeigen und weiterhin jungen Gruppenleitern und Habilitierten die Möglichkeit zu geben, sich einer breiteren Öffentlichkeit vorzustellen. Das wollen wir weiter entwickeln und aus diesem Grund wird die ZAK in Zukunft vierteljährlich erscheinen. Zudem haben wir uns entschlossen, die Redaktion der ZAK auf breitere Schultern zu stellen und deshalb einen Redaktionsstab zusammengestellt. Er umfasst, neben Ralph Gräf (verantwortlich) und mir, Friedemann Kiefer, Ludwig Eichinger, Thomas Magin und Oliver Gruss. Dies also eine weitere Neuerung. Zudem wollen wir nach und nach neue Rubriken einführen, z.B. wie in diesem Heft die *Perspectives*. Hierunter stellen wir uns vor, dass ausgewiesene „Schwergewichte" der Forschung ihr Gebiet, die Entwicklung im Verlauf der letzten Jahre und deren möglichen Fortgang diskutieren, um dadurch unsere tägliche Forschungsperspektive zu erweitern. In dieser Ausgabe werden Ada und Don Olins, die in den letzten 10 Jahren häufig in Deutschland geforscht haben, vorstellen, was aus der Entdeckung der „Nucleosomen" (Science, 1974) hervorgegangen ist und wie sich das Konzept dieser Art der DNA-Organisation auf die heutigen Fragestellungen zur funktionellen Organisation des Chromatins ausgewirkt hat.

Etwas in der Richtung habe ich gerade gestern auf dem 75. Cold Spring Harbor Symposium on Quantitative Biology („Nuclear Organization and Function") erlebt: Steve Henikoff hat zum Abschluss der Tagung zusammengefasst, was Neues und Bemerkenswertes in den sechs Tagen berichtet worden war – und stellte es ins Verhältnis zu den Ergebnissen des 38. Symposiums (Chromosome Structure and Function) von 1973 und den dort gemachten Vorhersagen. Für ihn war es damals das erste große Meeting gewesen, an dem er teilnahm und wo er die „Großen" von nahem sehen und sie ansprechen konnte. Wie er abschließend sagte, dieses Erlebnis hat sein zukünftiges Forscherleben vollkommen bestimmt.

Eine weitere konkrete Frage wird in einem der nächsten Hefte sein: Wissen wir eigentlich was Actin ist. Die „Mikrofilament-Kollegen" häufen Daten auf Daten, aber wieso hat dieses Cytoskelettprotein Kern-Exportsignale und was macht es im Zellkern? Junge Studenten sind perplex wenn man ihnen sagt, dass eine kurze Behandlung von Zellen mit DMSO das zelluläre Actin komplett in den Zellkern treibt – dies Arbeiten von Yoshio Fukui und auch Klaus Weber von vor 30 Jahren.

Special Interest Meetings

Das Protein „Actin" bringt mich zu einer weiteren neuen Aktivität, die wir in diesem Jahr erstmals ausprobieren wollen. In Reaktion auf die Äußerung einiger Mitglieder, das Programm der Jahrestagungen sei ihnen zu „heterogen" (wir würden sagen, es versucht die Breite der Zellbiologie abzudecken), werden wir in diesem Jahr zum ersten Mal eine Extra-Tagung im September über „Actin dynamics" abhalten. Eugen Kerkhoff, Theresia Stradahl und Clemens Rottner haben dieses „Special interest meeting" organisiert. Da das neue Format erhebliche Aktivitäten innerhalb der Gesellschaft erfordert hat, werden wir in diesem Jahr keine Nachwuchswissenschaftler-Tagung abhalten. Im nächsten Jahr wird sie aber wieder wie gewohnt in Jena in Zusammenarbeit mit der Firma Carl Zeiss abgehalten werden. Da wir sehr zuversichtlich sind, dass dieses neue Tagungsformat erfolgreich sein wird, bereiten Ralph Gräf und Manfred Schliwa schon eine weitere Spezial-Tagung über die zellulären Aktivitäten von Mikrotubuli für den Juni 2011 in Potsdam vor. Für das Jahr 2012 erwarten wir Vorschläge von unseren Mitgliedern !!!

Im Sinn der oben gemachten Ermutigungen, sich auch allgemeiner zu bilden, möchten wir besonders alle Nicht-Spezialisten ermuntern, sich das weite Feld, das zellbiologisch auf Mikrofilamenten gründet und vielfältige Aspekte zellulärer Aktivitäten beeinflusst, durch Teilnahme zu erschließen und möglicherweise zu erfahren, wie unmittelbar es ihr eigenes Feld betrifft. Auch für diese Tagung werden Doktoranden die Möglichkeit haben, Reisestipendien zu beantragen. Dies ist, neben der für Studenten praktisch kostenlosen Nachwuchswissenschaftler-Tagung, eine weitere erhebliche finanzielle Unternehmung der DGZ zur Förderung der Zellbiologie in Deutschland. Diese Leistungen werden sowohl aus den Mitgliedsbeiträgen als auch, in durchaus erheblichem Maß, aus den Beiträgen der Industrie-Aussteller auf der Jahrestagung ermöglicht.

Internet-Auftritt

Dies führt mich nun zur letzten „Neuerung". Aus verschiedenen Gründen, auf die ich nicht im Detail eingehen möchte, mußte unsere „Homepage" umziehen. Ralph Gräf hat sich darum gekümmert und diesen Umzug gleich genutzt, die Seite neu zu gestalten. Das Ergebnis unserer Bemühungen können Sie in Kürze unter www.zellbiologie.de sehen.

Zum Abschluss: Zeitprobleme

Einigen der regelmäßigen ZAK-Lesern mag aufgefallen sein, dass dieses Vorwort etwas länger geworden ist als gewohnt. Also schon fast wieder nichts für jemanden ohne Zeit. Und wie üblich auf die letzte Minute (wegen der Aktualität), diesmal eben auf einer Tagungsreise. Ich hätte natürlich auch eine „sight-seeing tour" machen können, aber ich will damit sagen, wir alle, wenn uns etwas an der Entwicklung der Zellbiologie in Deutschland gelegen ist, müssen etwas von unserer Zeit investieren. Beiträge liefern, an den Tagungen teilnehmen. Es bleibt trotzdem Zeit für anderes. Auf dem Hinflug habe ich angefangen, den „Idiot" von Dostojewski in der neuen Übersetzung von Swetlana Geier zu lesen - ein zweites Mal, nach 40 Jahren. Ein interessanter Kontrast zu den heutigen Problemen der Zellbiologie. Wie wohl, Probleme der ethischen Grundlagen der Wissenschaft, wie im „Idiot" angesprochen, mögen ein zukünftiges Thema sein.

Ihr
Harald Herrmann-Lerdon
Cold Spring Harbor, 9. Juni, 2010

International Meeting of the German Society for Cell Biology on Actin Dynamics

September 15th – 18th, 2010
Fachhochschule Jena, Carl-Zeiss-Promenade

Organizers: Eugen Kerkhoff, Klemens Rottner, Theresia Stradal

Social Event
On Friday afternoon, we will have a guided tour through the historic city of Weimar. A bus transfer to Weimar will be provided. Please note when you register if you are going to attend the tour.

Registration
The number of participants is restricted to 250 people, including invited speakers. The registration fee will cover all expenses for food including coffee breaks and the trip to Weimar. Lodging will have to be covered by participants.

Speakers:
Art Alberts (USA), Alexander Bershadsky (Israel), Sven Bogdan (Germany), Laurent Blanchoin (France), Marie-France Carlier (France), Roberto Dominguez (USA), Michael Eck (USA), Jan Faix (Germany), Daniel Fletcher (USA), Alexis Gautreau (France), Matthias Geyer (Germany), Bruce Goode (USA), Nir Gov (Israel), Robert Grosse (Germany), Ari Helenius (Switzerland), Henry Higgs (USA), Alan Rick Horwitz (USA), David Kovar (USA), Pekka Lappalainen (Finnland), Peter Lenart (Germany), John Leong (USA), Stefan Linder (Germany), Laura Machesky (UK), Dyche Mullins (USA), James Nelson (USA), Michael Olson (UK), Ewa Paluch (Germany), Guido Posern (Germany), Britta Qualmann (Germany), Margot Quinlan (USA), Anne Ridley (UK), Giorgio Scita (Italy), Vic Small (Austria), Richard Treisman (UK), Dimitrios Vavylonis (USA), Michael Way (UK), Walter Witke (Germany)

Registration fee is EUR 120,00 and EUR 50,00 for DGZ members. The registration for students is free.

Information/Registration:
www.zellbiologie.de

Deadline: August 1st, 2010

SCIENTIFIC PROGRAMME

WEDNESDAY, September 15th

12:00	Arrival/Registration
14:00	Opening Reception
16:00 – 18:00	**Opening Session: Actin dynamics in eukaryotes and prokaryotes**
16:00 – 17:00	Keynote Lecture I Marie-France Carlier
17:00 – 18:00	Keynote Lecture II Dyche Mullins
18:00 – 18:30	Coffee break
18:30 – 20:15	**Session 1: Signalling and cancer**
18:30 – 19:05	Anne Ridley
19:05 – 19:40	Alexander Bershadsky
19:40 – 20:15	Michael Olson
20:15	Dinner and Poster Session

THURSDAY, September 16th

08:30 – 10:50	**Session 2: Molecular mechanisms of actin nucleation**
08:30 – 09:05	Laura Machesky
09:05 – 09:40	Art Alberts
09:40 – 10:15	Britta Qualmann
10:15 – 10:50	Margot Quinlan
10:50 – 11:20	Coffee break
11:20 – 13:05	**Session 3: From cells to tissues & organisms**
11:20 – 11:55	Peter Lenart
11:55 – 12:30	Pekka Lappalainen
12:30 – 13:05	Walter Witke
13:05 – 15:00	Lunch
15:00 – 17:20	**Session 4: Biochemistry of actin assembly & disassembly**
15:00 – 15:35	Bruce Goode
15:35 – 16:10	Henry Higgs
16:10 – 16:45	David Kovar
16:45 – 17:20	Laurent Blanchoin
17:20 – 17:50	Coffee break
17:50 – 19:35	**Session 5: Shaping membranes**
17:50 – 18:25	Giorgio Scita
18:25 – 19:00	Alexis Gautreau
19:00 – 19:35	Sven Bogdan
20:00	Dinner and Poster Session

FRIDAY, September 17th

09:00 – 10:45	**Session 6: Structural insights into nucleation & elongation factors**
09:00 – 09:35	Roberto Dominguez
09:35 – 10:10	Michael Eck
10:10 – 10:45	Matthias Geyer
10:45 – 11:15	Coffee break
11:15 – 13:00	**Session 7: Actin dynamics & transcription & back**
11:15 – 11:50	Richard Treisman
11:50 – 12:25	Guido Posern
12:25 – 13:00	Robert Grosse
13:00 – 14:00	Lunch
14:00 – 15:30	**Session 8: Future demands & challenges in microscopy**
14:00 – 14:30	Warming-up Introduction
14:30 – 15:30	Panel Discussion hosted by Carl Zeiss
15:30	Trip to Weimar
20:00	Dinner and Poster Session

SATURDAY, September 18th

09:00 – 10:45	**Session 9: Lamellipodia/Filopodia/Blebs**
09:00 – 09:35	Vic Small
09:35 – 10:10	Jan Faix
10:10 – 10:45	Ewa Paluch
10:45 – 11:15	Coffee break
11:15 – 13:00	**Session 10: Adhesion**
11:15 – 11:50	Alan Rick Horwitz
11:50 – 12:25	James W. Nelson
12:25 – 13:00	Stefan Linder
13:00 – 15:00	Lunch
15:00 – 16:45	**Session 11: Host-pathogen interaction**
15:00 – 15:35	Ari Helenius
15:35 – 16:10	Michael Way
16:10 – 16:45	John Leong
16:45 – 17:15	Coffee break
17:15 – 19:00	**Session 12: Modelling and Physics**
17:15 – 17:50	Dimitrios Vavylonis
17:50 – 18:25	Nir Gov
18:25 – 19:00	Daniel Fletcher
19:30	Dinner and Party

Protokoll der Mitgliederversammlung 2010 der Deutschen Gesellschaft für Zellbiologie e.V.

Versammlungsort:
Universität Regensburg, Hörsaal H2

Versammlungstag: Donnerstag, 11. März 2010

Beginn: 12.30 Uhr, Ende: 13.20 Uhr

Anwesend sind insgesamt 38 Teilnehmer – 34 Mitglieder und der amtierende Vorstand der Gesellschaft, Prof. Dr. Reinhard Fässler (Präsident), Prof. Dr. Jürgen Wehland (Vizepräsident), Prof. Dr. Dirk Breitkreutz (Geschäftsführer) und Dr. Michael Sixt (Sekretär).

TOP 1: Begrüßung und Bestätigung des Protokolls der letzten Mitgliederversammlung

Der Präsident begrüßt die versammelten Mitglieder und stellt die Frage, ob es zum Protokoll der letzten Mitgliederversammlung in Konstanz, abgedruckt in „Zellbiologie aktuell", Ausgabe 2/2009, Änderungs- oder Ergänzungswünsche gibt. Es werden keine Änderungs- oder Ergänzungswünsche geäußert.

TOP 2: Jahresbericht des Präsidenten

Der Präsident fasst die Ereignisse des vergangenen Vereinsjahres zusammen und beginnt mit einem Resumee der Jahrestagung 2009 in Konstanz. Das Meeting war ein herausragender Erfolg sowohl hinsichtlich der Besucherzahlen als auch der wissenschaftlichen Qualität. Speziell erwähnt wird die Verleihung der Zeiss-Lecture an Rudolf Jaenisch, aber auch der dank der hervorragenden Organisation (durch Tagungspräsident Christoph Hauck) entstandene finanzielle Gewinn für die Gesellschaft (siehe Kassenbericht). Es wird bemerkt, dass auch die laufende Jahrestagung 2010 in Regensburg auf dem Wege ist, ein ähnlicher Erfolg zu werden. 510 Besucher und ein beeindruckendes wissenschaftliches Programm und eine makellose Organisation sind der Tagungspräsidentin Anja Bosserhoff zu verdanken. Der Präsident berichtet, dass Vorstand und Beirat in einem internen Treffen zu dem Schluss kamen, das Format der Jahrestagung auch künftig beizubehalten. Da der Versuch, die Tagung gemeinsam mit anderen europäischen zellbiologischen Gesellschaften abzuhalten, sich als wenig fruchtbar erwies, soll auf solche „joint meetings" nicht notwendigerweise hingearbeitet werden. Trotz der hohen Besucherzahlen, wird angemerkt, sei der Anteil der DGZ-Mitglieder an den Tagungsteilnehmern mit ca. 15% nach wie vor enttäuschend gering. Jeder sei aufgefordert mitzuwirken, mehr Mitglieder für die Jahrestagung zu mobilisieren. Die nächste Jahrestagung soll in Bonn stattfinden und wird von Prof. Dieter Fürst und Prof. Walter Witke als Tagungspräsidenten organisiert werden.

Der Präsident erwähnt im Anschluss das „Young Scientist Meeting", welches 2009 in Martinsried bei München unter der Organisation von Jochen Wittbrodt und Michael Sixt stattfand. Thema des Meetings war „Imaging Cell Migration" und die Veranstaltung war sowohl hinsichtlich der Besucherzahlen als auch der wissenschaftlichen Qualität ein voller Erfolg. Das nächste Meeting wird vom 15. bis 18. September in Jena stattfinden und die Organisatoren Eugen Kerkhoff, Klemens Rottner und Theresia Stradal haben unter dem Titel „Actin Dynamics" bereits eine extrem beeindruckende Reihe an bestätigten Sprechern gewinnen können. Ausnahmsweise wir für dieses Meeting das Format leicht geändert und es wird sich bei dem Kongress um ein „International Meeting" handeln, was bedeutet, dass das Format etwas grösser als gewöhnlich ausfallen wird.

Abgesehen von den Tagungen soll auch weiterhin daran gearbeitet werden, die Sichtbarkeit der DGZ vor allem für junge Wissenschaftler zu erhöhen. In einem internen Meeting von Vorstand und Beirat wurde vor allem angedacht, den Internetauftritt der Gesellschaft zu erneuern.

Der Präsident erwähnt dankend die Firma Zeiss, welche das Preisgeld für die Carl Zeiss Lecture von 2000 auf 7000 Euro erhöht hat und leitet damit auf den Kassenbericht über.

TOP 3: Geschäfts und Kassenbericht

Der Sekretär erläutert die Finanzlage der Gesellschaft und listet zunächst die von der DGZ gegebenen Spenden als auch die von der DGZ empfangenen Spenden auf. Die Gesamtsumme der gegebenen Spenden betrug EUR 14.942,90, welche sich aus den

Einnahmen/Ausgaben-Zusammenstellung 2009

Einnahmen	EUR	**Ausgaben**	EUR
Mitgliedsbeiträge	45.942,18	Bankkosten	932,77
Spenden *(u.a. Preisgelder)*	14.700,00	Retoure/Mitgliedsbeiträge	1.223,00
Zinsen	3.991,36	Reisekosten	14.537,87
DGZ-Zeitschrift ZAK, Firmen-Links	15.212,50	Spenden	14.942,90
Überträge	20.000,00	Bürokosten	293,04
Sonstige *(u.a. Abrechnung Jahrestagung 2009)*	63.392,03	DGZ-Zeitschrift ZAK	29.663,33
		Überträge	20.000,00
		Sonstige	13.826,85
Summe der Einnahmen	163.238,07	Summe der Ausgaben	95.419,76
Guthaben am 31.12.2008	190.140,74	**Guthaben am 31.12.2009**	**257.959,05**

DGZ: 186.776,13
Werner Risau Preis: 71.182,92

Die Einnahmen und Ausgaben wurden geprüft und für richtig befunden.

Heidelberg, 2. März 2010

M.-C. Dabauvalle
Kassenprüferin

H.-G. Mannherz
Kassenprüfer

Preisgeldern, Reisekostenunterstützungen zur Jahrestagung 2009 und finanziellen Unterstützungen für das STS-Meeting AK „Signaltransduktion" und den Workshop „Cell Biology of Viral Infections" der Gesellschaft für Virologie zusammensetzten. Die bei der DGZ eingegangenen Spenden beliefen sich auf insgesamt EUR 14.700,00, wobei den größten Teil (EUR 14.000,00) die für die Preisverleihungen empfangenen Preisgelder ausmachten und die restlichen EUR 700,00 Firmenspenden im Rahmen der Nachwuchswissenschaftler-Tagung 2009 waren.

Die Gesamtsumme der Einnahmen 2009 betrug EUR 163.238,07, wovon die eingenommenen Mitgliedsbeiträge in Höhe von EUR 45.942,18) und der Überschuss aus der Jahrestagung 2009 in Konstanz in Höhe von EUR 55.052,03 die wesentlichen Anteile ausmachten. Die Summe der Ausgaben betrug 95.419,76. Das Gesamtguthaben hat sich zum 31.12.2009 somit von EUR 190.140,74 (Ende 2008) auf EUR 257.959,05 vermehrt. Es wird jedoch darauf hingewiesen, dass von dieser Summe EUR 71.182,92 auf das Konto des Werner-Risau-Preises entfallen, welches ausschließlich zweckgebunden für die Vergabe des Werner-Risau-Preises zur Verfügung steht, und das DGZ-Guthaben betrug zum 31.12.2009 somit EUR 186.776,13. Weiter wird darüber informiert, dass in 2009 vom Deutschen Krebsforschungszentrum (DKFZ) keine Projektmittel – für das Gehalt der DGZ-Sekretärin, Bürobedarf etc. – angefordert worden waren und daher im Laufe der ersten Jahreshälfte 2010 ein Betrag in Höhe von EUR 70.000,00 an das DKFZ zu überweisen sein wird.

TOP 4: Bericht der Kassenprüfer

Der Sekretär berichtet, dass die Kassenprüfung für das Jahr 2009 durch Frau Marie-Christine Dabauvalle und Herrn Hans-Georg Mannherz am 2. März 2010 in Heidelberg durchgeführt wurde. Herr Mannherz wird um einen kurzen Bericht gebeten und bestätigt, dass die Prüfung der Bücher stichpunktartig durchgeführt wurde und zur vollen Zufriedenheit der Kassenprüfer verlief. Die Buchführung durch die Sekretärin Frau Reichel-Klingmann erfolgte akkurat und es gab keinerlei Beanstandungen.

TOP 5: Entlastung des Vorstandes

Das Mitglied Angela Otto bringt den Antrag zur Entlastung des Vorstandes ein, der ohne Gegenstimmen bei 4 Enthaltungen der Vorstandsmitglieder angenommen wird.

TOP 6: DGZ-Wahlen

Die anwesenden Mitglieder werden anhand einer detaillierten Übersicht mit den einzelnen Stimmenverteilungen über das Ergebnis der DGZ-Wahlen 2010 (Vorstand, Beirat, Kassenprüfer und Preisjury) informiert. Es waren 134 Stimmzettel eingegangen, wovon 133 gültig und 1 ungültig waren.

Harald Herrmann-Lerdon wurde mit 115 Ja-Stimmen zum neuen Präsidenten gewählt und Herr Herrmann-Lerdon nimmt die Wahl an. Anja Bosserhoff wurde mit 121 Ja-Stimmen zur neuen Vizepräsidentin gewählt und Frau Bosserhoff nimmt die Wahl an. Ralph Gräf wurde mit 128 Ja-Stimmen zum neuen Geschäftsführer gewählt und Herr Gräf nimmt die Wahl an. Eugen Kerkhoff wurde mit 121 Ja-Stimmen zum neuen Sekretär gewählt und Herr Kerkhoff nimmt die Wahl an.

Volker Gerke wurde mit 125 Ja-Stimmen als Beirat wiedergewählt und Herr Gerke nimmt die Wahl an. Elisabeth Knust hatte uns gebeten, wegen zahlreicher anderer Verpflichtungen künftig von ihrer Tätigkeit als Beiratsmitglied entbunden zu werden. Als neuer Beirat wurde Eckhard Lammert mit 113 Ja-Stimmen gewählt und Herr Lammert nimmt die Wahl an. Doris Wedlich wurde mit 118 Ja-Stimmen als Beirat wiedergewählt und Frau Wedlich nimmt die Wahl an.

Marie-Christine Dabauvalle wurde mit 126 Ja-Stimmen als Kassenprüferin wiedergewählt und Frau Dabauvalle nimmt die Wahl in Abwesenheit an. Hans-Georg Mannherz wurde mit 127 Ja-Stimmen als Kassenprüfer wiedergewählt und Herr Mannherz nimmt die Wahl an.

Zur Entlastung der bisherigen Preisjury, die länger als 10 Jahre erfolgreich tätig gewesen war, hatten sich drei neue Juroren zur Wahl gestellt:
Frank Schnorrer wurde mit 110 Ja-Stimmen in die Preisjury gewählt und Herr Schnorrer nimmt die Wahl an. Sabine Werner wurde mit 122 Ja-Stimmen in die Preisjury gewählt und Frau Werner nimmt die Wahl in Abwesenheit an. Walter Witke wurde mit 118 Ja-Stimmen in die Preisjury gewählt und Herr Witke nimmt die Wahl an.

TOP 7: Satzungsänderung

Der Präsident berichtet, dass beim letzten gemeinsamen Treffen von Vorstand und Beirat der Vorschlag ausgearbeitet wurde, den Beirat auf insgesamt 12 Mitglieder zu erweitern. Wie gehabt soll sich der Beirat aus drei gewählten Mitgliedern, dem jeweiligen Tagungspräsidenten und dem ausgeschiedenen Präsidenten zusammensetzen. Zusätzlich sollen vom Präsidenten weitere Mitglieder benannt werden, um auf eine Gesamtzahl von 12 Beiratsmitgliedern zu kommen. Dies habe den Effekt, dass bei Diskussionen eine entsprechende kritische Masse an Meinungen und Ideen zur Verfügung steht und dass das Fehlen einzelner Beiratsmitglieder bei Sitzungen abgepuffert werden kann. Als zusätzliche Beiratsmitglieder werden Suzanna Storchova, Klemens Rottner und Robert Grosse vorgeschlagen. Suzanna Storchova ist anwesend und bestätigt, dass sie zur Verfügung steht.
Einer entsprechenden Satzungsänderung wurde von allen 38 Anwesenden zugestimmt, es gab keine Enthaltungen und keine Gegenstimmen.

TOP 8: Verschiedenes

Der Präsident berichtet, dass bei der Gesellschaft immer wieder Anfragen eingehen, ob es für emeritierte/pensionierte Mitglieder einen reduzierten Mitgliedsbeitrag gibt. Bisher war dies nicht vorgesehen und der Präsident stellt daher zur Abstimmung, ob es künftig für emeritierte/pensionierte Mitglieder einen reduzierten Jahresbeitrag geben soll. Die anwesenden Mitglieder entscheiden einstimmig gegen einen reduzierten Jahresbeitrag für emeritierte/pensionierte Mitglieder.

Der Präsident fragt, ob es noch Fragen oder Anregungen seitens der Mitglieder gibt. Da es keine Meldungen gibt, schließt der Präsident die Mitgliederversammlung mit einem Dankwort an alle Mitglieder, den Vorstand und den Beirat in dem er die fruchtbare und angenehme Zusammenarbeit in den letzten beiden Jahren seiner Amtszeit herausstellt. Besonders erwähnt wird die unermüdliche und für die Gesellschaft essentielle Arbeit der DGZ Sekretärin Sabine Reichel Klingmann. Der Präsident wünscht dem neuen Vorstand eine erfolgreiche Amtsperiode.

Prof. Dr. Reinhard Fässler, Präsident
Dr. Michael Sixt, Sekretär

Walther Flemming Medaille: Florian Bassermann

Implications of SCF and APC/C ubiquitin ligases in cell cycle control, apoptosis, and DNA damage response

Background

The ubiquitin-proteasome system controls molecular networks that regulate fundamental cellular functions such as DNA replication, DNA repair, transcription, protein synthesis, cell differentiation and apoptosis. In this process, ubiquitin is covalently coupled to lysine residues either on itself or in other proteins through the action of an E1-E2-E3 ubiquitin transfer cascade. Substrate-linked lysine-48 (K48) poly-ubiquitin chains promote recognition by the proteasome, thereby promoting degradation of the ubiquitylated protein (1, 2).

E3 ubiquitin ligases are responsible for the specific recognition of a large number of target proteins. This requires specificity and versatility, which are provided by the existence of 500-1000 different ligases. The RING-finger-type E3s are thought to be the largest family and many of these are involved in the ubiquitinylation of cancer-related substrates (3). Two multi-subunit E3 ligases, the Skp1-Cul1-F-box-protein (SCF) ubiquitin ligases and the Anaphase Promoting Complex or Cyclosome (APC/C) are prominent among this family, as they are critically implicated in the control of cell cycle progression. Both ligases consist of invariable and variable components with the later being responsible for substrate binding and thus substrate specificity (Figure 1). In the case of SCF ligases, F-box proteins are the variable components. Currently, 69 different F-box proteins are known in mammals and only 8 have been matched to their respective substrate. The APC/C has 2 substrate binding determinants, Cdc20 and Cdh1. While Cdc20 is exclusively active in mitosis, Cdh1 exerts its activity from late mitosis throughout G1 (reviewed in (4).

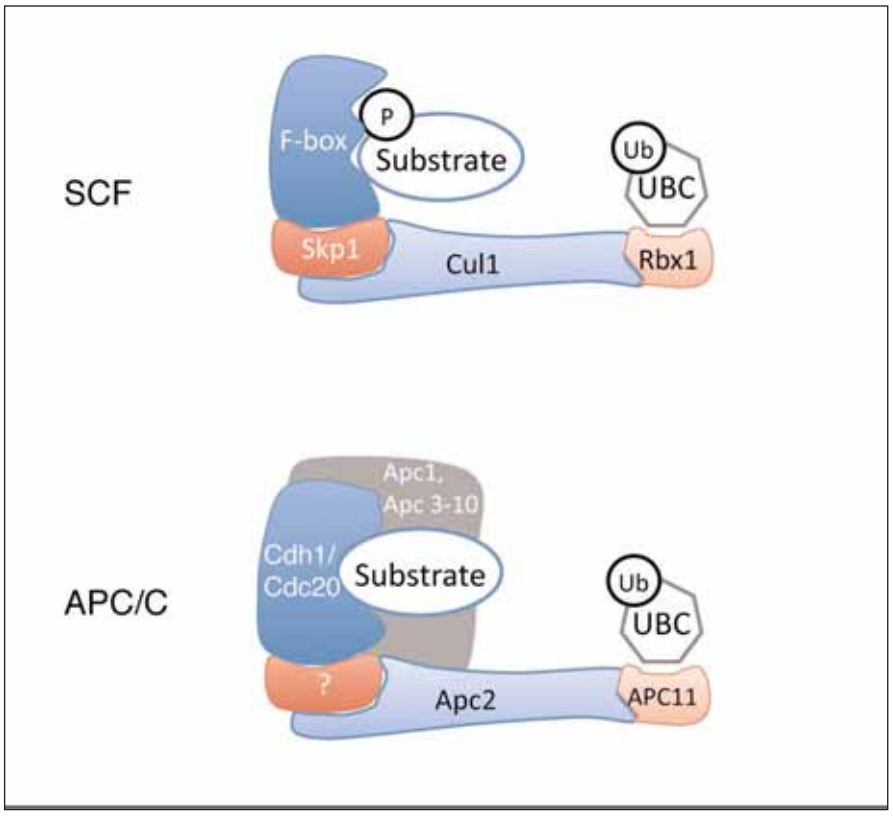


Figure 1: Structure of SCF and APC/C ubiquitin ligases. Both ubiquitin ligases share a Cullin (light blue) subunit and a RING finger (light red) protein, which mediates binding to the ubiquitin conjugating enzyme (UBC). The substrate-binding subunits are the variable components in each family of ligases (dark blue) and mediate substrate-binding specificity. Cdh1 and Cdc20 are the variable components of the APC/C, whereas SCF complexes are characterized by a large class of substrate-binding components, the F-box proteins (69 in humans). SCF ligases typically recognize phosphorylated substrates, whereas the APC/C targets specific motifs present in the primary sequence of the substrate. Figure adapted from (6).

Similar to other covalent modifications, such as phosphorylation or methylation, ubiquitylation is reversible. The human genome harbors approximately 80 deubiquitylating enzymes (DUBs), which cleave ubiquitin off substrates and thereby terminate ubiquitin-dependent signaling. DUBs have only recently been appreciated as essential and specific components of the ubiquitin pathway, and accordingly only few have been assigned functions or substrates. Present evidence suggests that DUBs are highly regulated and function with a high level of specificity, making them comparable to ubiquitin ligases, thus underscoring a model in which ubiquitylation and deubiquitylation are controlled by the balance between two opposing enzymes. Notably, several members of the USP family have already been implicated in DNA damage response, including USP1, USP7 (also known as HAUSP), USP10, and USP28, and function as tumor suppressors (5-7).

Aberrant functions for components of the ubiquitin-proteasome system (particularly for ubiquitin ligases and deubiquitylases that provide substrate specificity) have been implicated in the pathogenesis of many human cancers (8). Mutational inactivation of the machinery involved in linking ubiquitin to specific substrates can occur in ubiquitin ligases involved in restraining cell growth and proliferation (e.g.

the tumor suppressor F-box protein Fbxw7) or in ubiquitin ligases implicated in DNA repair and genome integrity [for example, breast cancer type 1 susceptibility protein (Brca1)]. Furthermore, overexpression of genes encoding for ubiquitin ligases/deubiquitylases is linked to tumorigenesis, like in the case of Mdm2 and the F-box protein Skp2, which mediate degradation of the tumor suppressors p53 and p27, respectively, or USP9x, which stabilizes the oncogene MCL1. In addition, targets of the ubiquitin-proteasome system (e.g. c-Myc and β-catenin) are mutated in human tumors. The target protein, normally programmed for degradation, becomes mutated in residues that are required for recognition by the ubiquitin ligase, making this protein resistant to the action of the degradation machinery.

Our previous work has identified specific cellular functions of distinct SCF complexes (SCF^{NIPA}, $SCF^{\beta TrCP}$, SCF^{Fbxl3}) and the APC^{Cdh1} complex through the identification of novel specific substrates. These mechanisms and their biological implications are briefly summarized below.

Cell cycle regulation and DNA damage checkpoint response by SCF^{NIPA} and APC/C^{Cdh1} ubiquitin ligases

The F-box like protein NIPA (Nuclear Interaction Partner of ALK) was originally cloned in complex with the oncogenic tyrosine kinase NPM-ALK, the later resulting from a t(2;5) translocation leading to the fusion of N-terminal NPM (Nucleophosmin) and C-terminal ALK (Anaplastic Lymphoma Kinase) sequences. The product of the translocation, NPM-ALK, is a chimeric, constitutively active tyrosine kinase which is considered to be the causative molecular aberration in anaplastic large cell lymphomas (ALCL) (9-11). Subsequently we characterized NIPA as an F-box like protein which forms a functional SCF complex with strong associated ubiquitin ligase activity (SCF^{NIPA}) (12). The nuclear fraction of cyclin B was identified as a substrate which is specifically targeted by the SCF^{NIPA} for proteasomal degradation in interphase only (12, 13). Mechanistically, the oscillating function of NIPA is procured by cell cycle specific inhibitory phosphorylation. This phosphorylation event is initiated in late G2 phase at serine residues 354 and 359, thereby mediating the initial dissociation of the SCF^{NIPA} complex. Subsequently, NIPA is phosphorylated at serine residue 395 by the then activated cyclin B1-Cdk1 complex in a feedback-like manner, thus further inactivating the complex. This process of processive phosphorylation of NIPA ensures timely and effective accumulation of cyclin B in the nucleus, which, when complexed to Cdk1, initiates mitosis (12, 14).

This mechanism is of particular interest with regard to NPM-ALK and the molecular pathogenesis of anaplastic large cell lymphomas. Expression of NPM-ALK associates with phosphorylation of NIPA at serine residue 354 which is therefore functionally inactivated. The resulting untimely expression of nuclear cyclin B puts the cell at risk of early mitotic entry, checkpoint malfunction and acquisition of potentially deleterious mutations. The functional association with NIPA therefore links NPM-ALK to the ubiquitin-proteasome system, thus generating a framework for future therapeutic approaches for ALCL involving modulations of the UPS.

Upon DNA damage in G2 phase, the cell must arrest the cell cycle to enable DNA damage repair, or, if damage is to excessive, induce apoptosis. During the initiation of the G2 checkpoint, numerous proteins, including the phosphoinositide 3-kinase-related kinases ATM, ATR, and DNA-PK, collaborate to activate the DNA damage response signaling network (15-17). In terms of checkpoint activation, these interwoven signaling cascades eventually focus on the master regulator of mitotic entry, Cdk1, whose activity must be inhibited to arrest the cell cycle (18). Briefly, ATM/ATR activate the checkpoint kinases Chk1 and Chk2 in response to double strand breaks (DSB), which in return induce proteasomal degradation of the Cdk1 activatory phosphatase Cdc25A and stabilization of p53, the later resulting in induction of the CDK inhibitor p21. As a consequence of Cdc25A elimination and p21 induction, Cdk1 is inactivated, thus establishing G2 arrest (2, 18, 19). We identified two novel components of this Network, Cdc14B and Cdh1, which define an activatory G2 checkpoint pathway in parallel to ATM (5). In more detail, we found that the nucleolar phosphatase Cdc14B translocates to the nucleus in response to double strand breaks to activate the APC/C^{Cdh1}-complex via dephosphorylation of Cdh1 (during the normal cell cycle, the APC/C^{Cdh1}-complex is kept inactive in S- and G2 phases by CDK dependent inactivation of Cdh1). This event results in proteasomal degradation of the promitotic kinase Plk1 (Polo like kinase 1), thereby stabilizing the crucial checkpoint molecules Wee1 and Claspin which results in G2 checkpoint activation and maintenance (5). Mitotic entry is subsequently inhibited, thus enabling the cell to repair DNA damage or induce apoptosis. Other substrates of the APC/C^{Cdh1}-complex are protected from proteasomal degradation by the deubiquitylating enzyme (DUB) USP28, thereby procuring specificity of the APC/C^{Cdh1}-ligase.

This new pathway is activated in parallel to ATM with potential important implications in tumorigenesis. It is well established, that mutations of the ATM gene result in genomic instability of affected cells. In particular, hematologic malignancies have been significantly associated with ATM mutations/deletions. This connection has been well documented in ATM -/- mouse models as well as in patients with Ataxia Teleangiectasia (20). Interestingly, alterations of components of the Cdc14B-Cdh1-Plk1 can be found in a multitude of human malignancies (Oncomine database, www.oncomine.com) (5). Thus, further studies on this signaling pathway appear promising to further understand the origin of genomic instability in tumor cells and the identification of potential new target structures of specific molecular tumor therapy.

Regulation of apoptosis via $SCF^{\beta TrCP}$- and Rsk1/2-dependent proteasomal degradation of BimEL

Bim (Bcl-2 Interacting Mediator of cell death) is a potent proapoptotic member of the Bcl-2 protein family, which is predominantly expressed in hematopoetic, epithelial, and germinal cells as well as in neurons (21). Alternative splicing results in three isoforms: short (BimS), long (BimL), and extra long (BimEL), with BimEL being the predominant isoform in most tissues (22). Bim plays an essential role in linking stress-induced signals with the intrinsic (mitochondrial) apoptotic pathway. In response to cellular stress, Bim activates the proapoptotic molecules Bak and Bax, which in return permeabilize the mitochondrial membrane. This event results in the release of cytochrome C with subsequent caspase activation and subsequent apoptotic cell death (23). Numerous tumor entities demonstrate low expression of BimEL, particularly in the presence of activated Erk1/2 signaling. For example, constitutive activation of Erk1/2 is present in tumors with activating mutants of the EGFR or upon expression of oncogenic cyctoplasmic tyrosine kinases such as Bcr-Abl (e.g. NSCLC, colon cancer, CML). Of notice,

inhibition of the EGFR or Bcr-Abl by specific inhibitors like Gefitinb or Imatinib associate with an accumulation of BimEL and subsequent apoptosis (24-27). Against this background, we uncovered a mechanism of proteasomal BimEL degradation, and unraveled the function of Erk1/2 signaling therein (28). We identified the $SCF^{\beta TrCP}$ complex to be the ubiquitin ligase of BimEL, which ubiquitylates BimEL and induces its proteasomal degradation in response to mitogenic signaling. This process was dependent on prior phosphorylation of BimEL in the specific βTrCP-recognition motif (degron) by the kinases Rsk1/2, which themselves become directly activated by Erk1/2 (28). By this means, Rsk1/2-βTrCP-dependent degradation of BimEL is integrated into the Erk1/2 signaling cascade, thus providing a mechanism of how Erk1/2 regulates stability of BimEL.

NSCLC cells frequently develop resistances against Gefitinib (a clinically relevant tyrosine-kinase inhibitor which induces apoptosis via BimEL). Downregulation of βTrCP or Rsk1/2 led to BimEL-dependent apotptosis both in Gefitinib-sensitive and Gefitinib-resistant cells (28). These results therefore suggest, that inhibition of the Rsk1/2-βTrCP-BimEL axis marks a valid strategy to induce apoptosis in NSCLC both in the Gefitinib sensitive and resistant setting. Moreover, this strategy may also

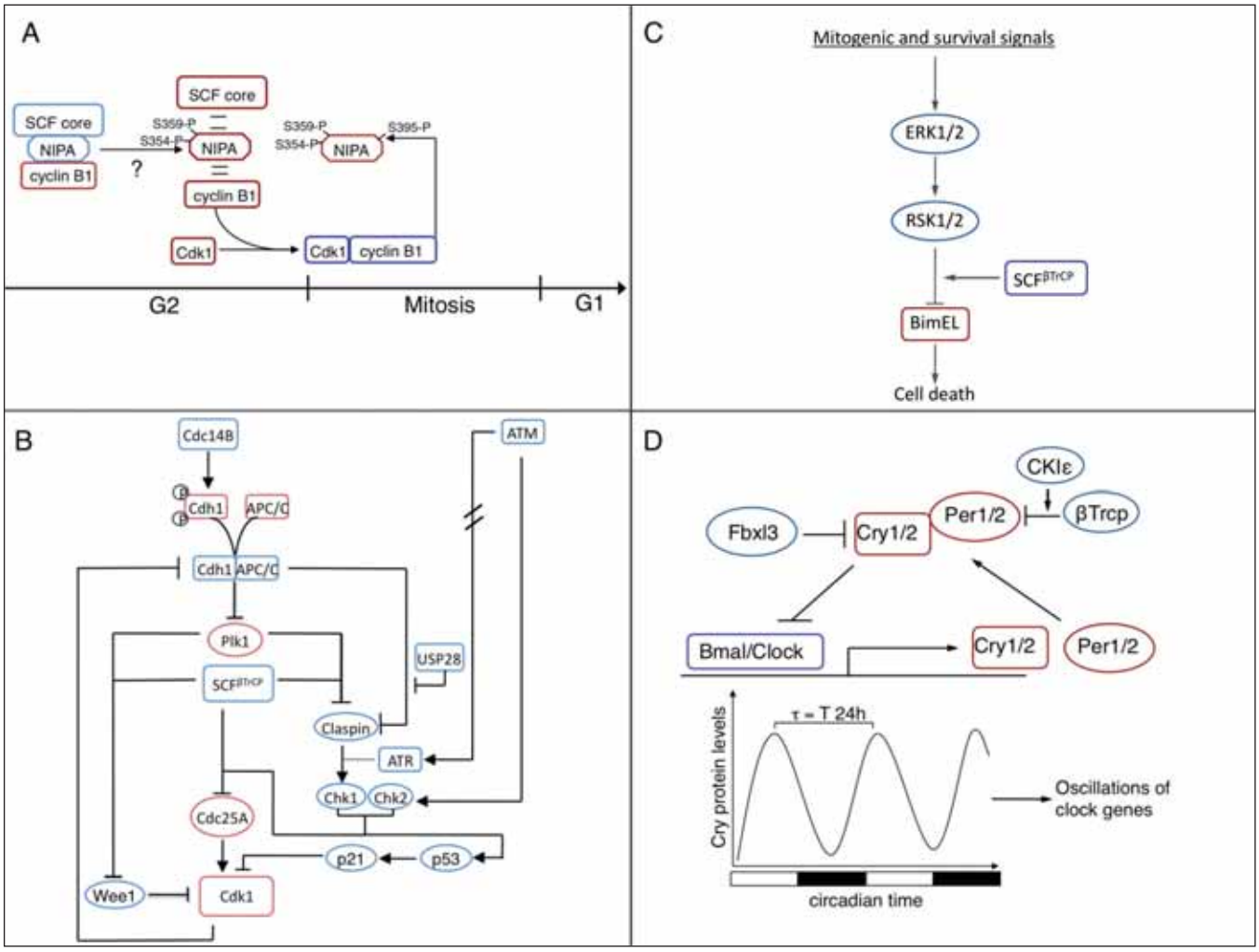


Figure 2: Distinct mammalian cellular functions are regulated by SCF and APC/C ubiquitin ligases *(blue denotes active, red denotes inactive/degraded forms of the respective proteins).*

(A) Processive phosphorylation of NIPA inactivates the SCF^{NIPA} complex at the G2/M transition and during mitosis. This event procures timely accumulation of nuclear cyclin B1 (so far the only known substrate of the SCF^{NIPA} complex) and subsequent mitotic entry. [Figure adapted from (9)].

(B) The Cdc14B-Cdh1-Plk1 axis is implicated in the regulation of the G2 checkpoint. After induction of double strand breaks (DSB) in G2, ATM directly activates Chk2. In addition, ATM activates exonucleases to initiate DSB resection, which contributes to the recruitment of ATR by generating RPA coated, single-stranded DNA. In parallel, Cdc14B translocates from the nucleolus to the nucleoplasm to activate APC/C^{Cdh1}, which subsequently targets Plk1 for proteasomal degradation. As a consequence of low Plk1 levels, phosphorylation of Claspin and Wee1 is reduced, thus preventing βTrCP-mediated degradation. Claspin is protected from APC/C^{Cdh1}-mediated degradation by Usp28. Stable Claspin promotes the ATR-mediated activation of Chk1, which, together with Chk2, targets Cdc25A for degradation and activates the p53-p21 axis. As a result, Cdk1 activity is attenuated, and cells arrest in G2. Stable Wee1 contributes to this inhibition by directly phosphorylating Cdk1.

(C) ,TrCP and RSK mediated degradation of BimEL inhibits apoptosis. Upon mitogenic stimuli, the Erk1/2-RSK1/2 axis becomes activated, and phosphorylates the potent pro-apoptotic BH3-only protein BimEL. This event specifically marks BimEL for subsequent ubiquitin mediated proteasomal degradation by the $SCF^{\beta TrCP}$ complex.

(D) SCF^{Fbxl3} regulates the circadian clock by oscillatory degradation of cryptochrome proteins. Per and Cry proteins accumulate during the night and repress the CLOCK/BMAL1 transcriptional complex that controls Per and Cry transcription. Repression is resolved by degradation of Per and Cry proteins. Per degradation requires phosphorylation mediated by casein kinase CKIε and subsequent recruitment of the SCF complex $SCF^{\beta TrCP}$. Degradation of Cry proteins is mediated by the SCF^{FBXL3} complex. Whether Cry proteins are modified posttranslationally prior to Fbxl3 binding is unknown. The model denotes the constellation at the beginning of the Cry/Per degradation cycle.

apply to other tumors characterized by an activation of the Erk1/2 pathway and low BimEL levels (e.g. CML).

Regulation of the circadian clock by SCF^{Fbxl3}-dependent proteasomal degradation of cryptochrome proteins – implications for linking cell cycle and circadian rhythms

The clock machinery is positively driven by two proteins, Clock and Bmal1, which heterodimerize to form an active transcription complex. Among several clock genes, the Clock:Bmal1 heterodimer drives the transcription of the *Cry* and *Per* genes. In turn, Cry1 and Cry2 inhibit Clock:Bmal1-dependent transcription, thereby creating a negative feedback loop that is central to the oscillation of the clock (reviewed in (29-31).

To identify novel, biologically significant substrates of the orphan F-box protein Fbxl3, we combined immunopurification strategies with analysis by mass spectrometry. Using this approach, we have identified the cryptochrome proteins *Cry*1 and *Cry*2 as novel interactors of Fbxl3. We found that Fbxl3-mediated ubiquitylation and degradation of Cry1 and Cry2 is a prerequisite for the efficient and timely reactivation of Clock:Bmal1 and the consequent transcription of Per1 and Per2. Significantly, knockdown of Fbxl3 produces no effect in Cry1-/-;Cry2-/- cells, demonstrating that Fbxl3 controls the circadian clock by mediating the degradation of CRY proteins. Our findings demonstrate that Fbxl3 is a novel regulatory component of the circadian rhythm that controls both the amplitude and the frequency of the clock oscillations.

These data have interesting implications for the regulation of cell proliferation and tumorigenesis in that the circadian clock is interconnected with the cell cycle regulatory machinery (30). This link is particularly visible in target genes of Clock:Bmal1, among which essential cell cycle proteins like Wee1 (Cdk inhibitor and important checkpoint protein at the G2/M transition), cyclin D1 and the oncogene c-Myc can be found. Moreover, both Per1 and Per2 have been implicated in DNA damage response signaling. This role is further supported by their binding to the central checkpoint-proteins ATM and ATR and their role as tumor suppressors, which has recently been demonstrated in murine Per2 knock out models (30, 32). While further studies are necessary to dissect the intricate link between these two central cellular rhythms, the impact of this network on tumorigenesis and circadian timing of therapeutic approaches is readily awaited.

Selected Publications

1. Hershko A, Ciechanover A. The ubiquitin system. *Annu Rev Biochem.* 1998;67:425-79.

2. Harper JW, Elledge SJ. The DNA damage response: ten years after. *Mol Cell.* 2007;28(5):739-45.

3. Petroski MD, Deshaies RJ. Function and regulation of cullin-RING ubiquitin ligases. *Nat Rev Mol Cell Biol.* 2005;6(1):9-20.

4. Guardavaccaro D, Pagano M. Stabilizers and destabilizers controlling cell cycle oscillators. *Mol Cell.* 2006;22(1):1-4.

5. Bassermann F, Frescas D, Guardavaccaro D, Busino L, Peschiaroli A, Pagano M. The Cdc14B-Cdh1-Plk1 axis controls the G2 DNA-damage-response checkpoint. *Cell.* 2008;134(2):256-67.

6. Bassermann F, Pagano, M. Dissecting the role of ubiquitylation in the DNA damage response checkpoint in G2. *Cell Death Differ.* 2010;17(1):78-85.

7. Komander D, Clague MJ, Urbe S. Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol.* 2009;10(8):550-63.

8. Nakayama KI, Nakayama K. Ubiquitin ligases: cell-cycle control and cancer. *Nat Rev Cancer.* 2006;6(5):369-81.

9. Ouyang T, Bai RY, Bassermann F, Von Klitzing C, Klumpen S, Miething C, et al. Identification and characterization of a nuclear interacting partner of anaplastic lymphoma kinase (NIPA). *J Biol Chem.* 2003;278(32):30028-36.

10. Chiarle R, Voena C, Ambrogio C, Piva R, Inghirami G. The anaplastic lymphoma kinase in the pathogenesis of cancer. *Nat Rev Cancer.* 2008;8(1):11-23.

11. Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science.* 1994;263 (5151):1281-4.

12. Bassermann F, von Klitzing C, Munch S, Bai RY, Kawaguchi H, Morris SW, et al. NIPA defines an SCF-type mammalian E3 ligase that regulates mitotic entry. *Cell.* 2005;122(1):45-57.

13. Bassermann F, Peschel C, Duyster J. Mitotic entry: a matter of oscillating destruction. *Cell Cycle.* 2005;4(11):1515-7.

14. Bassermann F, von Klitzing C, Illert AL, Munch S, Morris SW, Pagano M, et al. Multisite phosphorylation of nuclear interaction partner of ALK (NIPA) at G2/M involves cyclin B1/Cdk1. *J Biol Chem.* 2007;282(22):15965-72.

15. Lee JH, Paull TT. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. *Science.* 2005;308(5721):551-4.

16. Falck J, Coates J, Jackson SP. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature.* 2005;434(7033):605-11.

17. Jazayeri A, Falck J, Lukas C, Bartek J, Smith GC, Lukas J, et al. ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. *Nat Cell Biol.* 2006;8(1):37-45.

18. Bartek J, Lukas J. DNA damage checkpoints: from initiation to recovery or adaptation. *Curr Opin Cell Biol.* 2007;19(2):238-45.

19. Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. *Nature.* 2004;432(7015):316-23.

20. Lavin MF. Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer. *Nat Rev Mol Cell Biol.* 2008;9(10):759-69.

21. O'Reilly LA, Cullen L, Visvader J, Lindeman GJ, Print C, Bath ML, et al. The proapoptotic BH3-only protein bim is expressed in hematopoietic, epithelial, neuronal, and germ cells. *Am J Pathol.* 2000;157(2):449-61

22. O'Connor L, Strasser A, O'Reilly LA, Hausmann G, Adams JM, Cory S, et al. Bim: a novel member of the Bcl-2 family that promotes apoptosis. *EMBO J.* 1998;17(2):384-95. PMCID: 1170389.

23. Fletcher JI, Huang DC. Controlling the cell death mediators Bax and Bak: puzzles and conundrums. *Cell Cycle.* 2008;7(1):39-44.

24. Cragg MS, Kuroda J, Puthalakath H, Huang DC, Strasser A. Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. *PLoS Med.* 2007;4(10):1681-89

25. Costa DB, Halmos B, Kumar A, Schumer ST, Huberman MS, Boggon TJ, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. *PLoS Med.* 2007;4(10):1669-79

26. Kuroda J, Puthalakath H, Cragg MS, Kelly PN, Bouillet P, Huang DC, et al. Bim and Bad mediate imatinib-induced killing of Bcr/Abl+ leukemic cells, and resistance due to their loss is overcome by a BH3 mimetic. *Proc Natl Acad Sci U S A.* 2006;103(40):14907-12.

27. Belloc F, Moreau-Gaudry F, Uhalde M, Cazalis L, Jeanneteau M, Lacombe F, et al. Imatinib and nilotinib induce apoptosis of chronic myeloid leukemia cells through a Bim-dependant pathway modulated by cytokines. *Cancer Biol Ther.* 2007;6(6):912-9.

28. Dehan E, Bassermann F, Guardavaccaro D, Vasiliver-Shamis G, Cohen M, Lowes KN, et al. betaTrCP- and Rsk1/2-mediated degradation of BimEL inhibits apoptosis. *Mol Cell.* 2009;33(1):109-16.

29. Schibler U, Sassone-Corsi P. A web of circadian pacemakers. *Cell.* 2002;111(7):919-22.

30. Hunt T, Sassone-Corsi P. Riding tandem: circadian clocks and the cell cycle. *Cell.* 2007;129(3):461-4.

31. Gallego M, Virshup DM. Post-translational modifications regulate the ticking of the circadian clock. *Nat Rev Mol Cell Biol.* 2007;8(2):139-48.

32. Fu L, Pelicano H, Liu J, Huang P, Lee C. The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. *Cell.* 2002;111(1):41-50.

PD Dr. Florian Bassermann

Department of Medicine III (Hematology/Oncology)
Klinikum rechts der Isar
Technische Universität München
Ismaninger Strasse 22
81675 München
E-mail: florian.bassermann@lrz.tum.de

Reinhard Fässler und Florian Bassermann (rechts)

Since January 2009	Groupleader of the Emmy Noether-research group "Cell cycle control and DNA damage response", Dept. of Medicine III (Hematology and Oncology), Technical University of Munich
January 2010	Habilitation on the function of SCF and APC/C ubiquitin ligases Technical University of Munich
May 2006 – January 2009	Postdoctoral fellow in the group of Dr. Michele Pagano Dept. of Pathology and NYU Cancer Institue, Howard Hughes Medical Institute, New York University School of Medicine
September 2002 – May 2006	Medical Resident and Postdoctoral Fellow Dept. of Medicine III (Hematology and Oncology) Technical University of Munich
March 2001 – September 2002	Internship in Internal Medicine Dept. of Medicine III, Technical University of Munich
01/1998-01/2002	Dissertation, Laboratory of Leukemogenesis (group of Dr. Justus Duyster), Technical University of Munich
10/1993-11/2000	Medical School, University of Ulm, Technical University of Munich, Memorial Sloan Kettering Cancer Center (NY)

Zur Information für unsere Mitglieder:

Lehrende Dozenten können jederzeit (solange der Vorrat reicht) eine begrenzte Stückzahl von ***Zellbiologie aktuell***-Exemplaren **kostenlos** anfordern, um sie an interessierte Studenten weiterzugeben.

Bei Interesse wenden Sie sich bitte an das Sekretariat der DGZ (dgz@dkfz.de)

Binder Innovationspreis: Anne Spang

A role for intracellular traffic in the asymmetric distribution of proteins and mRNA

Most cells are intrinsically asymmetric. This asymmetry manifests itself either through the shape of the cell, physical barriers in the plasma membrane, appearance and maintenance of membrane domains, asymmetric distribution of protein, mRNA or entire organelles, or forces acting differentially at distinct places. Mostly a combination of the different hallmarks is found in a particular cell, generating a tremendous versatility in cell types dedicated to a specific function. Examples for asymmetry in shape is perhaps most obvious in neurons, which can roughly be divided into the cell body, axons and dendrites, the latter two be more precisely divided into pre- and post-synaptic space, respectively, and the protrusions. Physical barriers are observed in all epithelial cells, in which the basolateral membrane is separated from the apical membrane by tight junctions, and only this diffusion barrier allows the epithelial cells to fulfill their functions. It is important to note that over 90% of all cancers occur in epithelial cells, and that a hallmark of metastasis is the epithelial mesenchymal transition, which is characterized by a loss of tight junctions and of apical and basolateral identity. It is important to note that membrane domains can also assemble without tight junctions. Those domains are usually more dynamic and proteins and lipids segregate into domain compartmentalizing membranes, which at least in some cases act as specialized functional domains. These membrane domains or confinements can differ greatly in size, but stay in the nanometer range. Examples of such membrane micro domains are lipid rafts, ER exit sites and sites of polarized secretion.

Even migrating cells are asymmetric, they have a distinct front and rear and they position their centrosome and Golgi apparatus specifically to either the rear or the front. If the cell decides to change direction, the Golgi apparatus and the centrosome are repositioned. So entire organelles can be asymmetrically localized, and although in the case of the centrosome and the Golgi it remains unclear why they would have to be strictly positioned, the cell very much cares about this, and thus the positioning must serve an important function. Cellular asymmetry is also important in development. Stem cells divide asymmetrically to generate an exact copy of itself and a daughter cell that it is less potent in respect to cell fate decisions than its sibling or the mother, and may go on to differentiate. It is commonly accepted that 'cell fate determinants' are asymmetrically distributed in the mother cell prior to cell division, and that these factors then initiate a transcriptional program that drives differentiation. These cell fate determinants can either be segregated as mRNA and only be translated after cell division is completed or as proteins. In either case the mRNAs and the proteins are anchored somewhere mostly at or close to the cortex in the prospective differentiating part, of the mother cell. Other factors are specifically retained in the mother cell and may help to maintain the toti- or pluripotency of the stem cell.

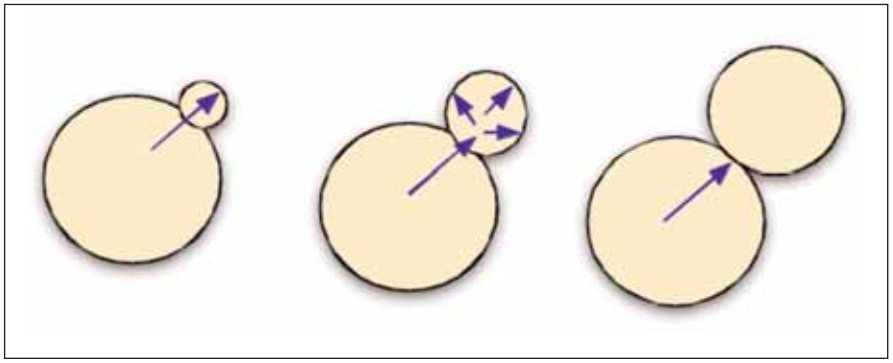


Figure 1: Schematic depiction of the major sites of secretion during the yeast cell cycle.

How does studying intracellular transport in the budding yeast Saccharomyces cerevisiae help to understand asymmetry establishment and maintenance? A yeast cell is intrinsically asymmetric as the yeast cell forms a protrusion, the bud, which determines the site of polarized growth. Interestingly, the plasma membrane of the growing bud is physically separated by a barrier, the bud neck, from the plasma membrane of the mother cell. Thus, in a very simplified model, the yeast cell has much in common with an epithelial cell, namely two different and distinct plasma membrane domains that are separated by a barrier. Moreover, even the cortical endoplasmic reticulum underlying the plasma membrane has very restricted diffusion of membrane proteins from the mother into the bud and vice versa (Luedeke et al., 2005). The main site of secretion in a yeast cell is in the bud, which later after cytokinesis becomes the daughter cell (Fig. 1). Early in the cell-cycle, secretion is even more locally restricted, to the tip of the growing bud. Later secretion can happen all over the bud membrane surface, before becoming restricted again to the bud neck prior to cytokinesis. Although it seems as though the sites of endocytosis do not change so much over the cell cycle, the delicate balance of regulated endocytosis and exocytosis significantly contributes to the protein repertoire at the plasma membrane and its compartmentalization.

Not only proteins can be asymmetrically segregated in yeast cells, mRNA is also asymmetrically distributed. The ASH1 mRNA, which encodes for a transcriptional repressor of the mating-type switch program, is specifically transported into the bud and anchored at the bud tip (Long et al., 1997; Shepard et al., 2003; Takizawa et al., 2000). While the transport machinery, the SHE complex, is well studied (Bohl et al., 2000; Long et al., 2000; Takizawa and Vale, 2000), much less is known about the anchoring and the translational repression, as the translation of ASH1 mRNA is prevented until after cytokinesis.

Still, once translated Ash1p migrates into the nucleus performing its function. This mechanism ensures that the mating-type switch program can only be initiated in the mother cell, but not in the daughter. Given that yeast has two different mating types, a and α, the probability of a yeast cell in the wild becoming diploid is highly increased by such a mechanism. The diploid life style is evolutionary preferred over the haploid state because DNA damage is better compensated. In fact by now over 20 mRNAs have been identified that are enriched in the bud over the mother cell (Shepard et al., 2003). But there is no reason to believe that mRNAs are only segregated to one or the other pole in yeast cells. In fact, mRNAs can also be enriched at particular organelles, such as mitochondria, the endoplasmic reticulum or peroxisomes (Aragon et al., 2009; Garcia et al.; Zipor et al., 2009). It is well established that localization of membrane proteins to the different intracellular organelles including the plasma membrane requires vesicular transport, which is the mean of communication between most membrane-bounded cellular organelles. Interestingly, in recent years evidence is accumulating that the same machinery that is involved in intracellular protein transport plays a pivotal role in mRNA localization.

We are interested in the regulation of intracellular traffic and how it impacts on asymmetric distribution of proteins and mRNA. Small GTPases are key regulators of intracellular traffic, and we focus on particular on small GTPases of the Ras superfamily, ARF/SAR, and the Rab family. The small GTPases of the ARF/SAR family are involved in numerous cellular processes, the best-characterized one is the function in coated-vesicle biogenesis. ARF/SAR GTPases are required to recruit coat proteins to specific sites on membranes, so-called exit sites (Spang, 2008) from which vesicles will form. Actually, we could show that coated vesicles form from synthetic liposomes just in the presence of coat proteins and the activated form of the small GTPase (Matsuoka et al., 1998; Spang et al., 1998). Small GTPases have practically no intrinsic GTPase activity and need the interaction with an effector, GTPase activating protein (ArfGAP), to hydrolyze GTP. In the GDP-bound state the GTPases are inactive, but guanine nucleotide exchange factors (GEFs) activate the GTPases by exchanging GDP for GTP. The GDP- and GTP- state of small GTPases represent distinct conformation. Thus small GTPases are molecular switches that depend on interaction with effector molecules to perform their action. We study ArfGEFs and ArfGAPs and their regulation and concentrate for this purpose in particular on machinery involved the retrograde transport from the Golgi to the ER because we can recapitulate and manipulate this transport step in an *in vitro* assay system (Spang and Schekman, 1998). We showed that two ArfGEFs Gea1p and Gea2p have overlapping function in retrograde transport from the Golgi to the ER (Spang et al., 2001). Both proteins can substitute in Golgi to ER transport for each other, but they have also evolved specific function in intracellular transport. Interestingly ArfGAPs also work in an overlapping manner. The two ArfGAPs Gcs1p and Glo3p both act in COPI vesicle generation at the cis side of the Golgi (Lewis et al., 2004; Poon et al., 1999). Importantly, Glo3p, but not Gcs1p, is an intrinsic component of the COPI vesicles (Lewis et al., 2004). While Gcs1p contains a domain with which it can sense membrane curvature and links this curvature sensing to activation of the GTPase activity through the GAP domain (Antonny et al., 2005; Bigay et al., 2005), Glo3p possesses a region that interacts with the major constituents of the COPI coat, coatomer, cargo proteins and SNAREs (Schindler et al., 2009; Spang et al., 2010). SNAREs are essential for the correct targeting of transport vesicles in the cell. The vesicles contain v-SNAREs that interact in a key-lock recognition with t-SNAREs on the target membrane. The v- and t-SNAREs zipper up bringing the vesicle membrane in close proximity to the target membrane and promote thereby fusion of both membranes. The coatomer, cargo and SNARE binding domain (BoCCS) in Glo3p communicates with the ArfGAP domain via Glo3p regulatory motif and couples thereby stimulation of GTPase activity to cargo and SNARE recruitment (Schindler et al., 2009). The BoCCS domain in Glo3p is also most likely responsible for the conformational change in SNARE proteins that can be induced by Glo3p (Rein et al., 2002; Schindler and Spang, 2007), which is necessary to promote the interaction with Arf1p and thus may provide the mechanism by which SNARE proteins are efficiently included into transport vesicles, not only at the Golgi-ER interface but also at other exit sites.

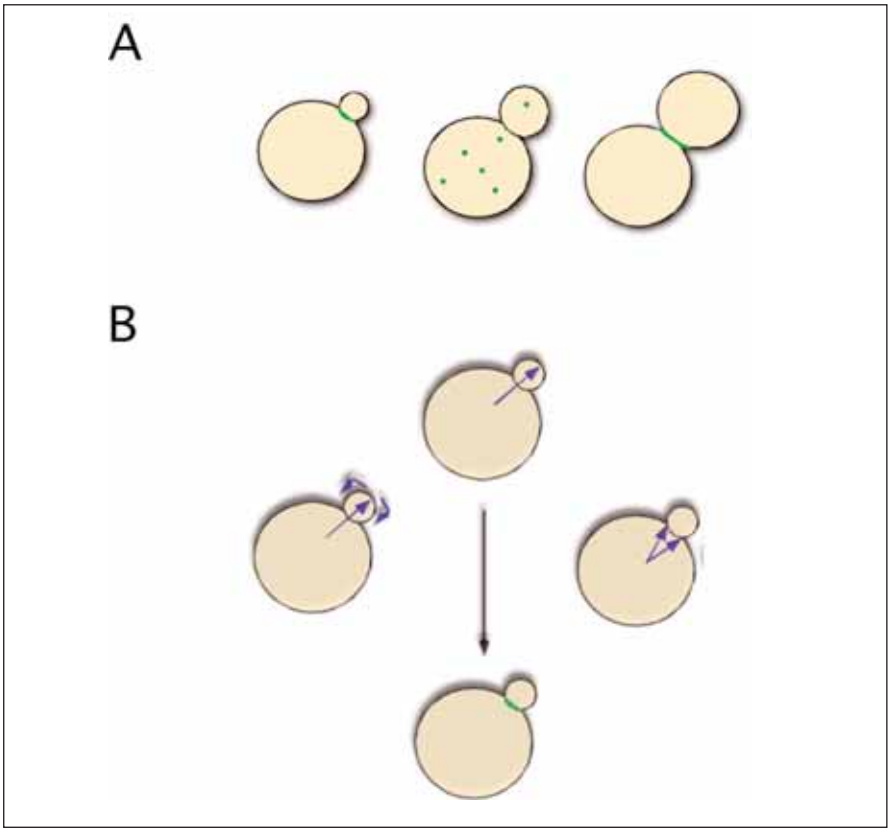


Figure 2: *Chs3p localization. (A) Chs3p localization over the cell cycle. Early (small budded cells) and late (large budded cells) in the cell cycle Chs3p is localized to the bud neck, while in late S-Phase, and G2 (medium budded cells), Chs3p is exclusively found in internal stores. (B) Chs3p localization could be achieved by two different mechanisms: One would operate by directly targeting transport vesicles to the bud neck region. The other would use a diffusion and anchoring mechanism. In (A) and (B) Chs3p is depicted in green. The arrows in (B) indicate the possible Chs3p trafficking pathways.*

In order to better understand the molecular function of the Arf1p in yeast, we took advantage of the distinct conformations dependent on the activation state of Arf1p and performed a differential affinity chromatography approach using either the active or inactive form of Arf1p as a bait and yeast cytosol as prey (Trautwein et al., 2004). Through this biochemical screen, we identified Chs5p and a novel protein family, the ChAPs (Chs5p and Arf1p binding Proteins) (Trautwein et al., 2006). Chs5p and the ChAPs are also collectively referred to as exomer complex (Wang et al., 2006). The exomer complex is required for plasma membrane localization of a set of specialized cargoes. In this process, Chs5p and Arf1p are supposed to recruit ChAP proteins to the trans-Golgi network (TGN), where the ChAPs are retained through interaction with cargo, and thereby act as cargo receptors or adaptors for inclusion of cargo into special transport containers formed at the TGN. Whether exomer represents a sorting complex or a novel coat remains unclear to date. In particular, the chitin synthase III, Chs3p depends on exomer for export form the TGN and for bud neck localization early –during and just after bud emergence- and late –just prior and during cytokinesis- in the cell cycle. In between these time points, Chs3p is located in internal stores, the chitosomes, which are most likely endosomes (Fig. 2A). From these chitosomes, Chs3p is recycled back to the TGN and exported back to the bud neck. Mounting evidence suggests that multiple (at least 3) different pathways exists that yield plasma membrane localization; the route that Chs3p takes is only one of them. In principle it is conceivable that nature provides sheer redundancy with these different pathways. However, this is interpretation is rather unlikely because although there is some possible redundancy, the pathways do transport different cargoes and only a part of them can be re-routed into another alternative pathway. Chs3p is confined at the plasma membrane to the bud neck, also at a time when the exocytic machinery is still restricted to the bud tip region in small-budded cells. Thus the question arises how Chs3p is localized to bud neck region? In principle two distinct possibilities are plausible: either vesicles are fusing directly at the bud neck region or vesicles fuse at the bud tip and the released Chs3p would diffuse and then anchored at the bud neck (Fig. 2B). We are currently investigating these two possibilities and are hopeful to know the answer to this question soon. The implication for higher systems is immense, as the results of the research performed in other and our labs on this issue will provide essential insights into trafficking of components to tight junctions in epithelial cells, and hence the maintenance of functional epithelial cell layers.

The second major interactor with the activated form of Arf1p that we identified in the biochemical screen was Pab1p, which is the major polyA binding protein in yeast (Trautwein et al., 2004). Binding of Pab1p to the polyA tail impacts on mRNA stability and efficiency of mRNA translation. We found that Pab1p associates with COPI-coated vesicles in an mRNA-dependent manner, indicating that COPI vesicles could help bringing mRNAs to the ER, where they might be translated or anchored. If this were the case, we should find mRNAs that are mislocalized in mutants of the COPI machinery, notably *arf1*. We first looked at the asymmetrically localized mRNA ASH1, because in this case the localization/mislocalization can be scored unambiguously. We found that *ARF1* mutants and some secretory transport mutants show a defect in correctly localizing ASH1 mRNA (Trautwein et al., 2004). Interestingly, at least in some cases we could exclude that the mutants caused secondary defects in the SHE machinery or the actin cytoskeleton, both of which are essential for proper ASH1 mRNA localization. Again the involvement of secretory pathway components in mRNA localization is not something, which is restricted to yeast but this phenomenon is also observed in mammalian cells (Bi et al., 2007) underscoring the usefulness of S. cerevisiae in studying basic cell biological processes.

Exciting times lay ahead of us because understanding the basic principles of how cellular asymmetry is generated and maintained will have an immense impact on cell biology, most notably stem cell biology and cancer biology, and also for medicine, and drug development and delivery.

References

Antonny, B., J. Bigay, J.F. Casella, G. Drin, B. Mesmin, and P. Gounon. 2005. Membrane curvature and the control of GTP hydrolysis in Arf1 during COPI vesicle formation. *Biochem Soc Trans.* 33:619-22.

Aragon, T., E. van Anken, D. Pincus, I.M. Serafimova, A.V. Korennykh, C.A. Rubio, and P. Walter. 2009. Messenger RNA targeting to endoplasmic reticulum stress signalling sites. *Nature.* 457:736-40.

Bi, J., N.P. Tsai, H.Y. Lu, H.H. Loh, and L.N. Wei. 2007. Copb1-facilitated axonal transport and translation of kappa opioid-receptor mRNA. *Proc Natl Acad Sci U S A.* 104:13810-5.

Bigay, J., J.F. Casella, G. Drin, B. Mesmin, and B. Antonny. 2005. ArfGAP1 responds to membrane curvature through the folding of a lipid packing sensor motif. *Embo J.* 24:2244-53.

Bohl, F., C. Kruse, A. Frank, D. Ferring, and R.P. Jansen. 2000. She2p, a novel RNA-binding protein tethers ASH1 mRNA to the Myo4p myosin motor via She3p. *Embo J.* 19:5514-24.

Garcia, M., T. Delaveau, S. Goussard, and C. Jacq. Mitochondrial presequence and open reading frame mediate asymmetric localization of messenger RNA. *EMBO Rep.* 11:285-91.

Lewis, S.M., P.P. Poon, R.A. Singer, G.C. Johnston, and A. Spang. 2004. The ArfGAP Glo3 Is Required for the Generation of COPI Vesicles. *Mol Biol Cell.* 14:14.

Long, R.M., W. Gu, E. Lorimer, R.H. Singer, and P. Chartrand. 2000. She2p is a novel RNA-binding protein that recruits the Myo4p-She3p complex to ASH1 mRNA. *Embo J.* 19:6592-601.

Long, R.M., R.H. Singer, X. Meng, I. Gonzalez, K. Nasmyth, and R.P. Jansen. 1997. Mating type switching in yeast controlled by asymmetric localization of ASH1 mRNA. *Science.* 277:383-7.

Luedeke, C., S.B. Frei, I. Sbalzarini, H. Schwarz, A. Spang, and Y. Barral. 2005. Septin-dependent compartmentalization of the endoplasmic reticulum during yeast polarized growth. *J Cell Biol.* 169:897-908.

Matsuoka, K., L. Orci, M. Amherdt, S.Y. Bednarek, S. Hamamoto, R. Schekman, and T. Yeung. 1998. COPII-coated vesicle formation reconstituted with purified coat proteins and chemically defined liposomes. *Cell.* 93:263-75.

Poon, P.P., D. Cassel, A. Spang, M. Rotman, E. Pick, R.A. Singer, and G.C. Johnston. 1999. Retrograde transport from the yeast Golgi is mediated by two ARF GAP proteins with overlapping function. *Embo J.* 18:555-64.

Rein, U., U. Andag, R. Duden, H.D. Schmitt, and A. Spang. 2002. ARF-GAP-mediated interaction between the ER-Golgi v-SNAREs and the COPI coat. *J Cell Biol.* 157:395-404.

Schindler, C., F. Rodriguez, P.P. Poon, R.A. Singer, G.C. Johnston, and A. Spang. 2009. The GAP Domain and the SNARE, Coatomer and Cargo Interaction Region of the ArfGAP2/3 Glo3 are Sufficient for Glo3 Function. *Traffic* (Copenhagen, Denmark). 10:1362-1375.

Schindler, C., and A. Spang. 2007. Interaction of SNAREs with ArfGAPs precedes recruitment of Sec18p/NSF. *Mol Biol Cell.* 18:2852-63.

Shepard, K.A., A.P. Gerber, A. Jambhekar, P.A. Takizawa, P.O. Brown, D. Herschlag, J.L. DeRisi, and R.D. Vale. 2003. Widespread cytoplasmic mRNA transport in yeast: identification of 22 bud-localized transcripts using DNA microarray analysis. *Proc Natl Acad Sci U S A.* 100:11429-34. Epub 2003 Sep 17.

Spang, A. 2008. The life cycle of a transport vesicle. *Cell Mol Life Sci.* 65:2781-9.

Spang, A., J.M. Herrmann, S. Hamamoto, and R. Schekman. 2001. The ADP ribosylation factor-nucleotide exchange factors Gea1p and Gea2p have overlapping, but not redundant functions in retrograde transport from the Golgi to the endoplasmic reticulum. *Mol Biol Cell.* 12:1035-45.

Spang, A., K. Matsuoka, S. Hamamoto, R. Schekman,

and L. Orci. 1998. Coatomer, Arf1p, and nucleotide are required to bud coat protein complex I-coated vesicles from large synthetic liposomes. *Proc Natl Acad Sci U S A.* 95:11199-204.

Spang, A., and R. Schekman. 1998. Reconstitution of retrograde transport from the Golgi to the ER in vitro. *J Cell Biol.* 143:589-99.

Spang, A., Y. Shiba, and P.A. Randazzo. 2010. Arf GAPs: Gatekeepers of vesicle generation. *FEBS Lett.*

Takizawa, P.A., J.L. DeRisi, J.E. Wilhelm, and R.D. Vale. 2000. Plasma membrane compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier. *Science.* 290:341-4.

Takizawa, P.A., and R.D. Vale. 2000. The myosin motor, Myo4p, binds Ash1 mRNA via the adapter protein, She3p. *Proc Natl Acad Sci U S A.* 97:5273-8.

Trautwein, M., J. Dengjel, M. Schirle, and A. Spang. 2004. Arf1p provides an unexpected link between COPI vesicles and mRNA in Saccharomyces cerevisiae. *Mol Biol Cell.* 15:5021-37. Epub 2004 Sep 8.

Trautwein, M., C. Schindler, R. Gauss, J. Dengjel, E. Hartmann, and A. Spang. 2006. Arf1p, Chs5p and the ChAPs are required for export of specialized cargo from the Golgi. *Embo J.* 25:943-54.

Wang, C.W., S. Hamamoto, L. Orci, and R. Schekman. 2006. Exomer: A coat complex for transport of select membrane proteins from the trans-Golgi network to the plasma membrane in yeast. *J Cell Biol.* 174:973-83.

Zipor, G., L. Haim-Vilmovsky, R. Gelin-Licht, N. Gadir, C. Brocard, and J.E. Gerst. 2009. Localization of mRNAs coding for peroxisomal proteins in the yeast, Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A.* 106:19848-53.

Prof. Dr. Anne Spang

Professor of Biochemistry
Biozentrum, University of Basel
Klingelbergstrasse 50/70
CH-4056 Basel
Switzerland
E-mail: anne.spang@unibas.ch

Reinhard Fässler (links), Wolfgang Preter (Product Manager Science, Binder GmbH) und Anne Spang

Since 2005	Professor of Biochemistry at the University of Basel, Biozentrum, Basel (Switzerland)
1999-2006	Independent group leader at the Friedrich Miescher Laboratory of the Max Planck Society in Tübingen (Germany)
1996-1999	Postdoc with Randy Schekman at the University of California, Berkeley, Howard Hughes Medical Institute, Berkeley (USA) Work on Golgi-ER retrograde transport
1992-1996	PhD in Biochemistry with Elmar Schiebel at the Max Planck Institute for Biochemistry, Genecenter, Martinsried (Germany) Work on characterization of spindle pole body components in S. cerevisiae
1986-1991	Studied Chemical Engineering at the University for Applied Sciences in Darmstadt (Germany) and Biochemistry at the University Pierre et Marie Curie, Paris VI, Paris (France)

Werner Risau Preis: Boris Strilic

The molecular mechanism of vascular lumen formation

The cardiovascular circulatory system is pivotal for embryonic development, homeostatic regulation in the adult and is involved in various pathological situations such as atherosclerosis and cancer. In mammals, the circulatory system is composed of endothelial cells (ECs) that form multicellular tubes with a central vascular lumen. The process of vascular lumen formation in the developing aorta is called vasculogenesis, since this blood vessel forms *de novo* from ECs. In contrast, the formation of secondary blood vessels, for example the intersomitic vessels, is called angiogenesis, because these blood vessels form from pre-existing vessels (i.e. the aorta). Interestingly, lumen formation during both vasculogenesis and angiogenesis involves three distinct steps: (i) formation of a multicellular EC cord (Fig. 1A), (ii) formation of a central vascular lumen in this cord (Fig. 1B), and (iii) initiation of blood flow through the vascular lumen (Fig. 1C). In larger blood vessels, the latter step is subsequently followed by further expansion and remodeling of the blood vessel (Fig. 1D).

Several proteins have been identified as important for blood vessel lumen formation. In particular, deletion of one allele of the vascular endothelial growth factor VEGF-A results in formation of blood vessels with no vascular lumen (Carmeliet et al., 1996; Ferrara et al., 1996). Besides VEGF-A, the vascular endothelial-cadherin (VE-cadherin) is also required for blood vessel lumen formation (Carmeliet et al., 1999). This cadherin is exclusively expressed on ECs and deletion, similar to the deletion of VEGF-A, results in embryonic death.

The mechanism by which EC cords form a vascular lumen has been debated (Blum et al., 2008; Kamei et al., 2006; Nelson and Beitel, 2009). For many decades, lumen formation was believed to result from intracellular *vacuole coalescence* (Folkman and Haudenschild, 1980; Kamei et al., 2006; Sabin, 1920). According to this model, each EC within an EC cord forms a large intracellular vacuole through fluid up-take, called pinocytosis, and the vacuoles from adjacent ECs subsequently coalesce to form a continuous vascular lumen (Kamei et al., 2006). This model received some support from *in vivo* imaging of intersomitic vessels in zebrafish embryos (Kamei et al., 2006), since vacuole-like structures were observed at the onset of lumen formation in these vessels. However, two years later, Blum and colleagues re-investigated lumen formation in these blood vessels and found that the vascular lumen does not develop from intracellular vacuoles, but instead forms extracellularly between adjacent ECs (Blum et al., 2008). Taken together, so far neither model (*vacuole coalescence* versus *extracellular vascular lumen formation*) explained the molecular mechanism underlying blood vessel lumen formation and thus did not explain the roles of VEGF-A and VE-cadherin in this important process.

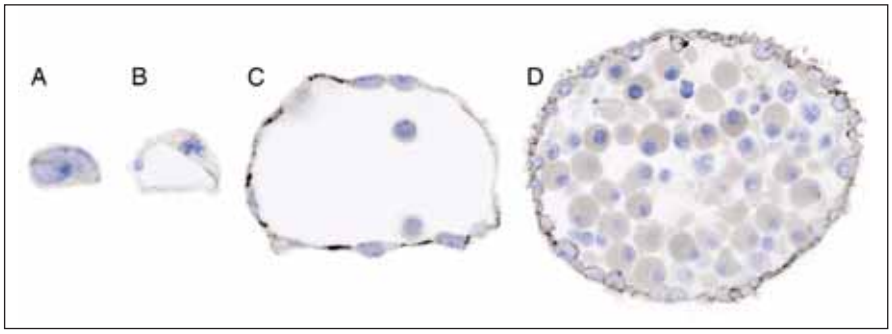


Figure 1: *Transition from non-lumenized vascular cord to lumenized blood vessel*
Confocal images of a series of sections through the developing mouse aorta are shown. From the left to the right, the aorta starts its development as an endothelial cell cord (A) that develops a central vascular lumen (B), which is slowly filled with red blood cells (C) and expands further (D). Cell nuclei are shown in blue, and endothelial cells are shown in black.

In the present study, we used the developing mouse (dorsal) aorta as a model system to investigate the molecular mechanism of vascular lumen formation. The aorta is the first and largest arterial blood vessel to develop in all mammals and allows precise temporal and spatial staging of the transition from a non-lumenized EC cord to a vascular lumen (see Fig. 1) (Strilic et al., 2009). In addition, embryos isolated prior to lumen formation of the aorta and those injected with intervening substances, can be used to study *in vivo* the process of vascular lumen formation (Strilic et al., 2009). These whole embryo cultured (WEC) embryos develop a functional circulatory system, fully lumenized aortae and a beating heart under control conditions. The aorta thus offers several advantages over *in vitro* systems, which are known to be prone to artifacts and often do not represent the mammalian *in vivo* situation (Kucera et al., 2009).
Using this model system, we found that at embryonic day (E) 8.0 at the 1-2 somite (S) stage of mouse development, the aortic EC cords have not yet formed a vascular lumen, whereas at the 6-8S stage all cords have formed a vascular lumen larger than 5 μm in diameter (Strilic et al., 2009). This shows that in the aorta the transition from a non-lumenized cord to a lumenized blood vessel only takes several hours and can be staged, depending on the number of somites (Strilic et al., 2009). More importantly, by careful analyses using single plane illumination microscopy (SPIM), confocal light microscopy and electron microscopy we found that lumen formation in the aorta occurs in the absence of intracellular vacuoles. Instead, our data suggested that the lumen develops extracellularly at the cell-cell contact between adjacent ECs (Strilic et al., 2009).
Since the formation of specialized luminal plasma membranes is thought to be a key step during lumen formation in epithelial cells (reviewed by (Bryant and Mostov, 2008; Lubarsky and Krasnow, 2003)), we checked

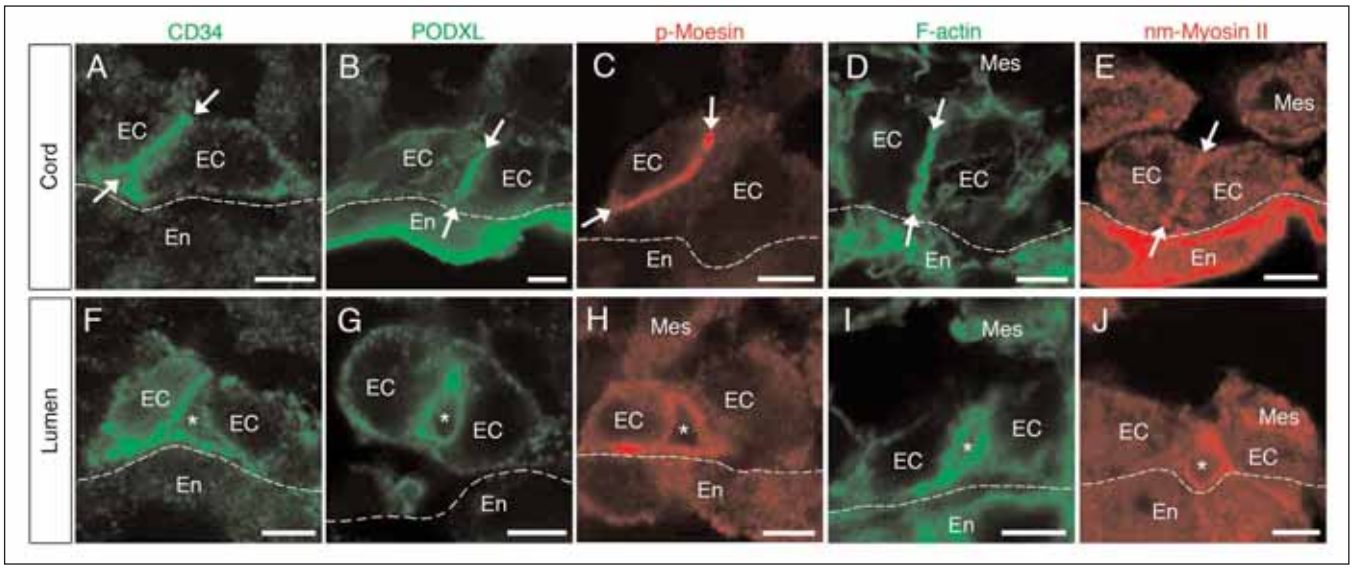


Figure 2: *Localization of CD34-sialomucins, phospho-moesin, F-actin and nm-myosin II at the endothelial cell-cell contact at the onset of vascular lumen formation*

Confocal images of transverse sections through the developing aortae are shown. Arrows point to the endothelial cell-cell contact. Asterisks mark the developing lumen. En, endoderm; Mes, mesenchyme.

At the cord stage, during onset of vascular lumen formation, the molecules CD34 (A), PODXL (B), phospho (p)-moesin (C), F-actin (D) and some nm-myosin II (E) localize at the endothelial cell-cell contact. All molecules remain enriched at the plasma membrane facing the developing lumen (F-J). Scale bars, 5 µm. Image adopted from (Strilic et al., 2009).

for CD34-sialomucins at the endothelial cell-cell contact at the onset of vascular lumen formation. CD34-sialomucins are transmembrane proteins with a highly glycosylated and negatively charged extracellular domain, and are commonly used as an apical/luminal cell surface marker in epithelial cells (Meder et al., 2005). In line with the notion that the vascular lumen develops at the endothelial cell-cell contact, we observed that the CD34-sialomucins CD34 and podocalyxin (PODXL) localized at the endothelial cell-cell contact at the cord stage (Fig. 2A and B) and remained localized at the luminal plasma membrane thereafter (Fig. 2F and G). CD34-sialomucins can furthermore link the luminal plasma membrane to the underlying F-actin cytoskeleton via adapter molecules (for review see (Bretscher et al., 2002)), and these adapter molecules were shown to be required for epithelial tubulogenesis (Gobel et al., 2004; Van Furden et al., 2004). In addition, we found the activated adapter molecule phospho (p)-moesin together with F-actin localized at the apical pole interface between adjacent ECs (Fig. 2C and D) and at the luminal plasma membrane (Fig. 2H and I). Furthermore, F-actin is known to interact with non-muscle (nm) myosin II to induce cell shape changes in different cell types, including ECs (Furman et al., 2007). Similarly, nm-myosin II was enriched at the endothelial cell-cell contact before (Fig. 2E) and during initial vascular lumen formation (Fig. 2J). Together, our data suggested that negatively charged CD34-sialomucins initially separate the ECs from each other and subsequently, an apical actomyosin complex consisting of F-actin and nm-myosin II exerts the physical force to further separate the ECs from each other, giving rise to a vascular lumen.

Despite the detailed morphological data on vascular lumen formation in the aorta, we still did not understand the molecular mechanism and in particular we did not know when and how VE-cadherin and VEGF-A are involved in this process. To address these questions, we used several genetic and pharmacologic approaches. We first investigated the role of VE-cadherin, since cadherins are known to induce cell polarity in epithelial cells (Nejsum and Nelson, 2007), and establishment of polarity was important in vascular lumen formation (see Fig. 2). Interestingly, mice deficient for VE-cadherin failed to form a proper lumen in the aorta, and this defect was not due to a decreased number of ECs or an increased rate of apoptosis (Strilic et al., 2009). Instead, these embryos failed to localize the above-mentioned molecules (CD34-sialomucins, p-moesin, F-actin and nm-myosin II) at the endothelial cell-cell contact (Strilic et al., 2009). Therefore, we could show that VE-cadherin, possibly via the phosphatase and tensin homolog (PTEN), is required for establishing endothelial cell polarity and vascular lumen formation. This data is also in line with other data showing that cadherins associate with PTEN (Vogelmann et al., 2005) and that PTEN is necessary for establishing apical cell polarity and lumen formation in epithelial cells (Martin-Belmonte et al., 2007).

Since PODXL and moesin were the first molecules to localize at the endothelial cell-cell contact, we asked whether these molecules were required for vascular lumen formation. To this end, we analyzed mice that were deficient for either PODXL or moesin. Interestingly, analysis of each individual knockout mouse revealed that lumen formation in the aorta was delayed and that lumens were significantly smaller (Strilic et al., 2009). Importantly, in both cases, F-actin was not enriched at the endothelial cell-cell contact, providing genetic evidence that the CD34-sialomucin PODXL and the adapter molecule moesin are both required for linking the luminal plasma membrane to the F-actin cytoskeleton and for vascular lumen formation.

Next, we asked how the activation of moesin is regulated, since only phosphorylated moesin links CD34-sialomucins to the F-actin cytoskeleton (for review see (Bretscher et al., 2002)). Using several inhibitors against pan-PKC- or ROCK I/II-mediated signaling, we found *in vitro* that inhibition of PKC prevented phosphorylation of moesin, but did not affect phosphorylation of myosin light chain (MLC), another known target downstream of

PKC signaling. In contrast, inhibition of ROCK I/II prevented phosphorylation of MLC, but did not affect phosphorylation of moesin. This data was confirmed *in vivo* using WEC. ECs of developing aortae from mice injected with either PKC- or ROCK-inhibitors were normally polarized (i.e. PODXL was localized at the endothelial cell-cell contact). However, in mouse embryos injected with the PKC inhibitor, p-moesin, F-actin and nm-myosin were not enriched at the endothelial cell-cell contact (Strilic et al., 2009). In contrast, embryos injected with the inhibitor against ROCK, showed normal phosphorylation of moesin and F-actin localization at the endothelial cell-cell contact, but failed to position nm-myosin II to F-actin (Strilic et al., 2009). In both cases, vascular lumens formed to a lesser extent. This data showed that PKC-signaling is required for phosphorylation of moesin, recruitment of F-actin to the cell-cell contact and vascular lumen formation, while ROCK-mediated signaling is required for phosphorylation of MLC, nm-myosin II recruitment to the F-actin cytoskeleton at the cell-cell contact and vascular lumen formation.

VEGF-A is known to regulate ROCK activity and is required for embryonic blood vessel formation (Carmeliet et al., 1996; Ferrara et al., 1996). Similar to the results that we obtained after injection of ROCK-inhibitors into mouse embryos, mice that were haploinsufficient for VEGF-A showed correct polarization, phosphorylation of moesin and localization of F-actin at the endothelial cell-cell contact but failed to position nm-myosin II to the latter complex (Strilic et al., 2009). Importantly, this defect was not due to decreased endothelial cell proliferation or an increased rate of apoptosis.
Since the analyzed mouse embryos still harbored half of the endogenous VEGF-A, it was likely that this reduced amount of VEGF-A was sufficient for moesin phosphorylation and F-actin recruitment. For this reason, we tested two differentially acting inhibitors against VEGF-A-mediated signaling both *in vitro* and *in vivo* in WEC (Flt1-Fc and SU5416). The data confirmed that in the absence of VEGF-mediated signaling polarity establishment and moesin phosphorylation were normal, but MLC phosphorylation was reduced, nm-myosin II was absent from the endothelial cell-cell contact and lumen formation was impaired.

Conclusively, this study for the first time proposes a model that explains how ECs form a vascular lumen on a molecular level and integrates key players such as VE-cadherin and VEGF-A. We could show that the aortic lumen develops extracellularly at the endothelial cell-cell contacts between ECs, rather than through coalescence of intracellular vacuoles. Furthermore, we identified a molecular pathway comprising CD34-sialomucins, (p)-moesin, F-actin and nm-myosin II. These proteins are localized to the endothelial cell-cell contacts to define the luminal cell surface and to trigger cell shape changes required for aortic lumen formation. More precisely, CD34-sialomucins localize to EC contacts in a VE-cadherin dependent-manner, and the repellent activity of these CD34-sialomucins initiates lumen formation (Fig. 3A to B). The CD34-sialomucins are also required for the recruitment of moesin, a step that is driven by the activation and phosphorylation by PKC (Fig. 3B to C). Moesin helps to stabilize the F-actin network at the cell-cell contact. Importantly, VEGF-A and ROCK are not involved in the early steps of vascular lumen formation (i.e. polarization and moesin phosphorylation), but recruit nm-myosin II to the cortical F-actin network by phosphorylating MLC, and furthermore regulate cell-shape changes required for vascular lumen formation (Fig. 3C to D). Finally, components of this pathway are also present during blood vessel formation in other organ systems and tumors (Strilic et

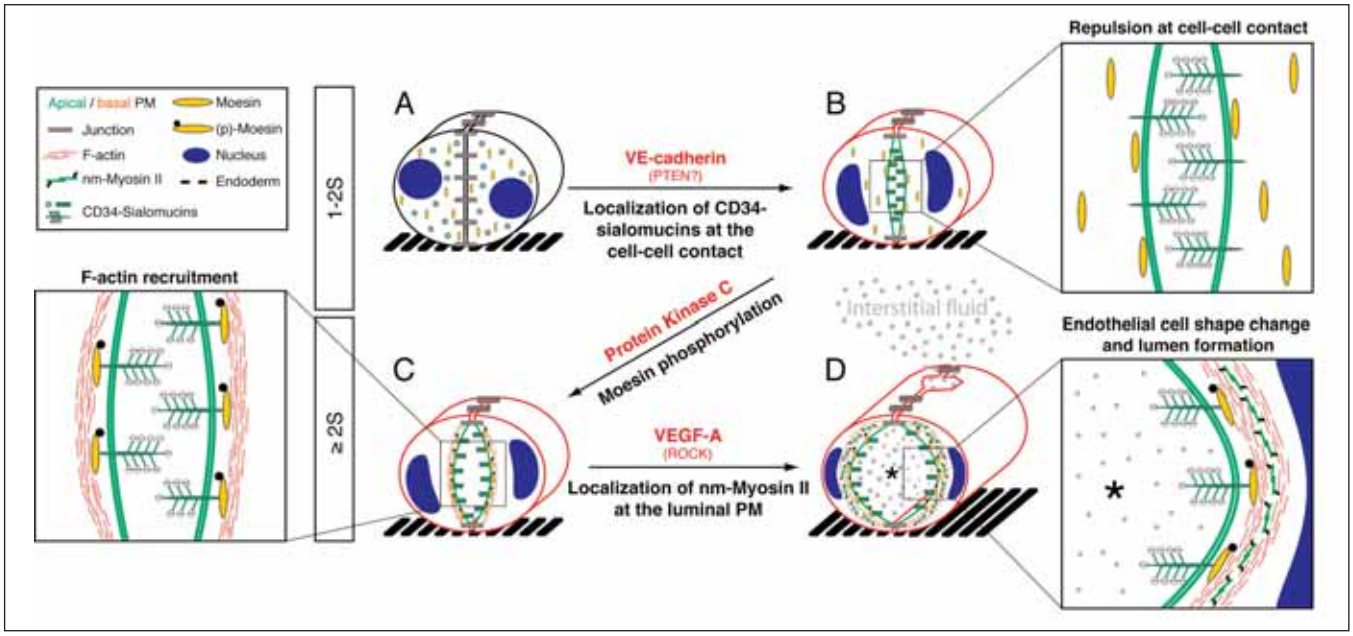


Figure 3: *Molecular mechanism of in vivo vascular lumen formation in the developing aorta*
(A) Adjacent endothelial cells (ECs) adhere to each other via junctions at multiple positions along the endothelial cell-cell contact.
(B) VE-cadherin is required for localizing of CD34-sialomucins to the endothelial cell-cell contact, possibly via its interaction with PTEN. The anti-adhesive CD34-sialomucins are involved in separating apical endothelial cell surfaces from each other.
(C) PKC activity is required for phosphorylating moesin, which is involved in recruiting F-actin to the endothelial cell-cell contact.
(D) VEGF-A activates ROCK, which is required for myosin light chain (MLC) phosphorylation and recruitment of nm-myosin II to the apically enriched F-actin. VEGF-A and ROCK are required for fully separating apical endothelial cell surfaces from each other, for EC shape changes and for vascular lumen formation. Since the developing lumen (asterisk) is leaky, extravascular interstitial fluid (Fig. 3D, grey dots) passively enters via paracellular openings. Image from (Strilic et al., 2009).

al., 2009), suggesting that this model may represent a general mechanism for vascular lumen formation.

Acknowledgements

My sincere thanks go to Dr. Eckhard Lammert for his guidance, support and encouragement and the entire Lammert-lab (in particular Jan Eglinger) for support and friendship. In particular I want to thank my lovely wife for her everlasting support.

References

Blum, Y., Belting, H.G., Ellertsdottir, E., Herwig, L., Luders, F., and Affolter, M. (2008). Complex cell rearrangements during intersegmental vessel sprouting and vessel fusion in the zebrafish embryo. *Dev Biol* 316, 312-322.

Bretscher, A., Edwards, K., and Fehon, R.G. (2002). ERM proteins and merlin: integrators at the cell cortex. *Nat Rev Mol Cell Biol* 3, 586-599.

Bryant, D.M., and Mostov, K.E. (2008). From cells to organs: building polarized tissue. *Nat Rev Mol Cell Biol* 9, 887-901.

Carmeliet, P., Ferreira, V., Breier, G., Pollefeyt, S., Kieckens, L., Gertsenstein, M., Fahrig, M., Vandenhoeck, A., Harpal, K., Eberhardt, C., et al. (1996). Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* 380, 435-439.

Carmeliet, P., Lampugnani, M.G., Moons, L., Breviario, F., Compernolle, V., Bono, F., Balconi, G., Spagnuolo, R., Oostuyse, B., Dewerchin, M., et al. (1999). Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis. *Cell* 98, 147-157.

Ferrara, N., Carver-Moore, K., Chen, H., Dowd, M., Lu, L., O'Shea, K.S., Powell-Braxton, L., Hillan, K.J., and Moore, M.W. (1996). Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 380, 439-442.

Folkman, J., and Haudenschild, C. (1980). Angiogenesis in vitro. *Nature* 288, 551-556.

Furman, C., Sieminski, A.L., Kwiatkowski, A.V., Rubinson, D.A., Vasile, E., Bronson, R.T., Fassler, R., and Gertler, F.B. (2007). Ena/VASP is required for endothelial barrier function in vivo. *J Cell Biol* 179, 761-775.

Gobel, V., Barrett, P.L., Hall, D.H., and Fleming, J.T. (2004). Lumen morphogenesis in C. elegans requires the membrane-cytoskeleton linker erm-1. *Dev Cell* 6, 865-873.

Kamei, M., Saunders, W.B., Bayless, K.J., Dye, L., Davis, G.E., and Weinstein, B.M. (2006). Endothelial tubes assemble from intracellular vacuoles in vivo. *Nature* 442, 453-456.

Kucera, T., Strilic, B., Regener, K., Schubert, M., Laudet, V., and Lammert, E. (2009). Ancestral vascular lumen formation via basal cell surfaces. *PLoS ONE* 4, e4132.

Lubarsky, B., and Krasnow, M.A. (2003). Tube morphogenesis: making and shaping biological tubes. *Cell* 112, 19-28.

Martin-Belmonte, F., Gassama, A., Datta, A., Yu, W., Rescher, U., Gerke, V., and Mostov, K. (2007). PTEN-mediated apical segregation of phosphoinositides controls epithelial morphogenesis through Cdc42. *Cell* 128, 383-397.

Meder, D., Shevchenko, A., Simons, K., and Fullekrug, J. (2005). Gp135/podocalyxin and NHERF-2 participate in the formation of a preapical domain during polarization of MDCK cells. *J Cell Biol* 168, 303-313.

Nejsum, L.N., and Nelson, W.J. (2007). A molecular mechanism directly linking E-cadherin adhesion to initiation of epithelial cell surface polarity. *J Cell Biol* 178, 323-335.

Nelson, K.S., and Beitel, G.J. (2009). More than a pipe dream: uncovering mechanisms of vascular lumen formation. *Dev Cell* 17, 435-437.

Sabin, F.R. (1920). Studies on the origin of blood-vessels and of red blood-corpuscles as seen in the living blastoderm of chicks during the second day of incubation. *Contr Embryol* 9, 215.

Strilic, B., Kucera, T., Eglinger, J., Hughes, M.R., McNagny, K.M., Tsukita, S., Dejana, E., Ferrara, N., and Lammert, E. (2009). The molecular basis of vascular lumen formation in the developing mouse aorta. *Dev Cell* 17, 505-515.

Van Furden, D., Johnson, K., Segbert, C., and Bossinger, O. (2004). The C. elegans ezrin-radixin-moesin protein ERM-1 is necessary for apical junction remodelling and tubulogenesis in the intestine. *Dev Biol* 272, 262-276.

Vogelmann, R., Nguyen-Tat, M.D., Giehl, K., Adler, G., Wedlich, D., and Menke, A. (2005). TGFbeta-induced downregulation of E-cadherin-based cell-cell adhesion depends on PI3-kinase and PTEN. *J Cell Sci* 118, 4901-4912.

Dr. Boris Strilic

Max Planck Institute for Heart and Lung Research,
W.G. Kerckhoff-Institute
Department of Pharmacology
Ludwigstr. 43
D-61231 Bad Nauheim, Germany
E-mail: boris.strilic@mpi-bn.mpg.de

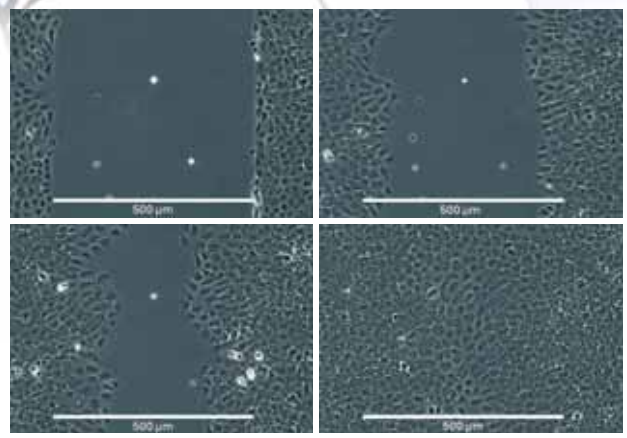
Britta Engelhardt (Vorsitzende des Werner-Risau-Preiskomitees) und Boris Strilic

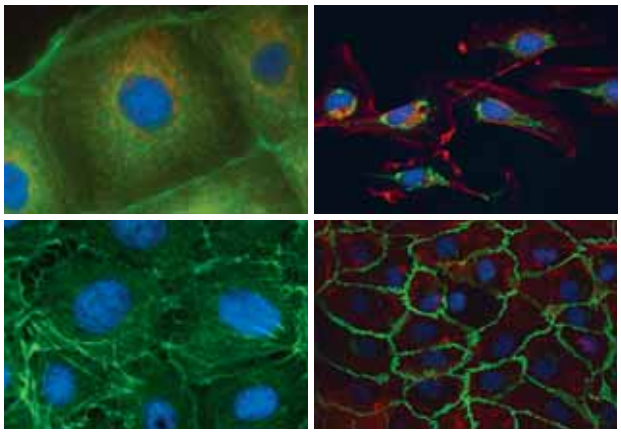
Since 2010	Postdoctoral Research Fellow at the Max Planck Institute for Heart and Lung Research, W.G. Kerckhoff-Institute in Bad Nauheim, Department of Pharmacology in the group of Prof. Dr. med. Stefan Offermanns
2009 – 2010	Postdoctoral Research Fellow at the Heinrich-Heine-University of Düsseldorf, Institute of Metabolic Physiology in the group of Prof. Dr. Eckhard Lammert
2005 – 2009	Doctoral Thesis at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden in the group of Prof. Dr. Eckhard Lammert
2004	Diploma Thesis at the Lombardi Cancer Center of the Georgetown University Hospital in Washington, DC (USA) in the group of Prof. Dr. med. Anton Wellstein

in Kooperation mit

AMG
ADVANCED MICROSCOPY GROUP

ibidi®
cells in focus





Live cell imaging mit time lapse

Immunfluoreszenz

... mit dem EVOS Mikroskop und ibidi µ-Slides

EVOS xl
(Phasenkontrast)
€ 9.750
Nr. 73022

EVOS fl
(Fluoreszenz)
€ 22.500
Nr. 73102

ibidi GmbH
Tel.: +49 89 / 520 46 17 - 0
Fax: +49 89 / 520 46 17 - 59
E-Mail: info@ibidi.de

www.ibidi.de

2010 / 05

Holger Bastians

Mechanisms of chromosomal instability in human cancer

Introduction

The majority of cancer cells exhibit an abnormal karyotype associated with a high grade of aneuploidy. In fact, this major cancer phenotype was described already more than 100 years ago by the cell biology pioneers David Hansemann and Theodor Boveri, who observed that cancer cells exhibit abnormal cell divisions leading to an unequal distribution of chromosomes onto the two daughter cells[1, 2]. Today, the perpetual gain or loss of whole chromosomes is referred to as chromosomal instability (CIN)[3] or, more explicitly, as whole chromosome instability (W-CIN). CIN describes a process that results in aneuploidy defined as a chromosome content that deviates from the modal chromosome number. In addition to W-CIN, cancer cells often exhibit another form of chromosomal instability termed segmental CIN (S-CIN), which includes chromosome loci amplifications, deletion and translocations[3, 4].

In this overview I will focus on the molecular mechanisms of W-CIN (from hereon simply termed CIN), which are still poorly understood.

The proper progression of mitosis ensures chromosomal stability

Chromosome segregation is executed during mitosis, which is associated with the most dramatic morphological changes of the cell during the cell cycle. Mitosis is initiated with the condensation of chromosomes and the breakdown of the nuclear envelope followed by the movement of the two duplicated centrosomes to opposite poles of the cell and the generation of a bipolar mitotic spindle mainly originating from the pole centrosomes. Subsequently, highly dynamic spindle microtubules attach to chromosomes by a stochastic search-and-capture mechanism finally leading to the alignment of chromosomes on a so-called metaphase plate. Once full alignment is achieved, sister chromatids separate during anaphase and are pulled towards to poles of the cell. It is conceivable that these dramatic and highly dynamic processes must be controlled very tightly to ensure proper chromosome segregation and thus, chromosomal stability and euploidy. Defects in either one of the different steps in mitosis is expected to cause chromosome missegregation, which is obviously a frequent event in cancer cells leading to the CIN phenotype. Various mitotic defects can underlie CIN including defects in centrosome integrity and function, aberrant spindle formation or microtubule dynamics, faulty chromosome attachments, loss of chromatid cohesion or loss of coordination between the various processes[5].

The basic principles of mitotic progression and many important proteins that directly or indirectly regulating mitosis and chromosome segregation have been identified. For instance, several mitotic kinases including cyclin dependent kinase 1 (CDK1), polo-like kinase 1 (PLK1) and Aurora kinases (Aurora-A and Aurora-B) as well as a large family of mitotic kinesins have been identified as key regulators of mitosis[6, 7]. Perhaps most intriguingly, the controlled elimination of mitotic regulators by ubiquitin dependent protein proteolysis has been shown to be a central theme of mitotic regulation. In particular, the metaphase to anaphase transition requires the ubiquitin dependent proteolysis of securin, an inhibitor of anaphase onset. Moreover, the degradation of cyclin B at the end of mitosis results in inactivation of the CDK1 kinase and is required for the exit from mitosis. The ubiquitin dependent proteolysis of securin and cyclin B requires a large ubiquitin ligase complex known as the anaphase promoting complex or cyclosome (APC/C), which is essential for the onset of anaphase and the subsequent exit from mitosis[8].

The mitotic spindle assembly checkpoint

During the early phases of mitosis, the attachment of chromosomes to the mitotic spindle is a stochastic process, which results in the fact that some chromosomes complete alignment before others. Therefore, anaphase onset has to be prevented until every single chromosome has achieved bi-orientation and full alignment. This regulation is mediated by a mitotic surveillance pathway known as the mitotic spindle assembly checkpoint (SAC). The SAC comprises a number of evolutionary conserved checkpoint proteins including Mad1, Mad2, Bub1, BubR1, Bub3 among others that are specifically recruited to kinetochores that are not attached to microtubules. In addition, the SAC is also activated by the loss of tension, which is generated across sister kinetochores when they are correctly attached to microtubules. Although the exact mechanism of checkpoint activation is not completely understood, the recruitment of the various checkpoint proteins to kinetochores appears to be required for the formation of a mitotic checkpoint complex that binds to and inhibits the ubiquitin ligase activity of the APC/C[9]. Hence, the metaphase to anaphase transition is under tight control of the SAC

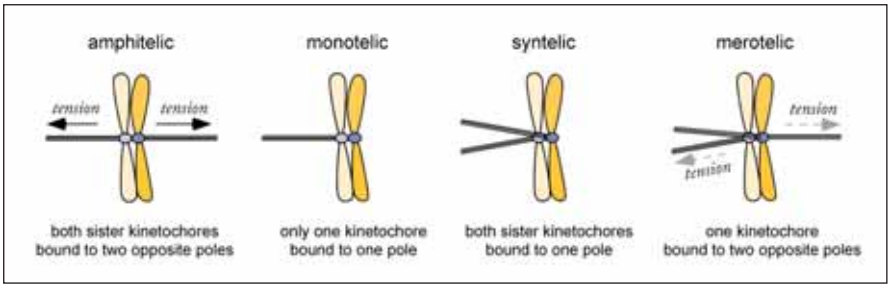


Figure 1: Schematic depiction of correct and incorrect microtubule-kinetochore attachments during mitosis. A normal bipolar mitotic spindle provides a bias for correct amphitelic chromosome attachments where both sister kinetochores are bound to the opposite poles and tension can be generated across sister kinetochores. Incorrect attachments include mono-, syn- and merotelic attachments. The latter might be in particular harmful, because it can partially generate kinetochore tension and is therefore not recognized by the mitotic spindle assembly checkpoint.

that inhibits the mitotic protein proteolysis of securin and cyclin B.

The role of the spindle assembly checkpoint in human cancer

Inhibition of the SAC function can result in premature activation of the APC/C and leads to the untimely separation of sister chromatids that is associated with chromosome missegregation and the induction of aneuploidy. Indeed, various SAC knockout models in mice have demonstrated that partial inhibition by e.g. loss of one allele of *MAD2* results in chromosome missegregation and CIN. Most importantly, the induction of CIN in most of these knockout mice causes a late onset of tumorigenesis and supports tumor progression after treatment with carcinogens[4, 10]. These studies have fuelled the long-standing debate whether CIN causes tumorigenesis or rather represents a bystanding effect and strongly support the notion that CIN is indeed a source of tumor development. Interestingly, while a partial loss of SAC causes CIN and tumorigenesis, a complete loss of SAC function, e.g. after deletion of both *MAD2* alleles in mice or after severe repression of SAC genes in tissue culture cells, results in severe chromosome missegregation that no longer allows cell survival[11-14]. Hence, in contrast to other cell cycle checkpoints, the SAC is essential for cell viability. In line with these observations it has been demonstrated that elevating the rate of chromosome missegregation during mitosis by inhibiting the SAC can be – under certain circumstances – even tumor suppressive[15]. This interesting observation opens up an avenue to exploit the SAC as a possible cancer therapy target - a concept that is intensively investigated in our lab[16, 17].

According to the observations in mice and in tissue culture systems, partial malfunction of the SAC mediated by reduced expression or by mutations of SAC genes has been detected in human cancer. In particular, some cancer cell lines show decreased levels of Mad2 and dominant mutations in the *BUB1* gene have been identified in colon cancer[18-20]. However, due to the essential nature of the SAC, a complete loss of SAC function (e.g. after homozygous SAC gene deletions) has not been detected in human cancer and SAC gene alterations are found at rather low frequencies. In fact, most cancer cells exhibiting CIN have a robust SAC[21] indicating that although the SAC is clearly important for the maintenance of chromosomal stability its inactivation can hardly account for CIN in most human cancer cells.

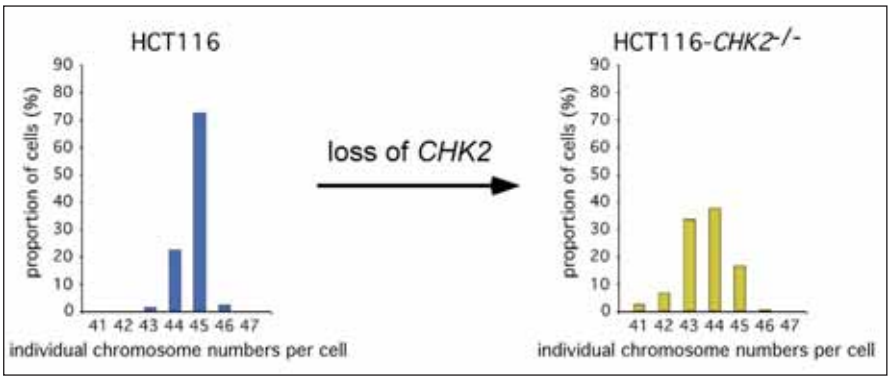


Figure 2: Loss of the tumor suppressor gene CHK2 induces CIN in chromosomal stable human somatic cells. The karyotype of parental HCT116 and CHK2 deficient derivative cells were determined and the number of chromosomes per metaphase was determined. After a defined time span CHK2 knockout cells exhibit aneuploidy whereas the parental cells retain their stable karyotype. (Data from Stolz et al., Nature Cell Biology 2010)

Mal-oriented kinetochore attachments are a major cause of CIN in human cancer

Faithful chromosome alignment and segregation requires the bipolar attachment of microtubules to both sister kinetochores, generating so-called amphitelic kinetochore attachments (Figure 1). Obviously, a normal bipolar mitotic spindle provides a bias for those bi-oriented kinetochore attachments, but due to the stochastic interaction between microtubules and kinetochores incorrect attachments can be generated[22, 23]. For instance, both sister kinetochores can transiently bind to microtubules from the same pole via syntelic attachments leading to mono-orientation of chromosomes. Alternatively, kinetochores can concomitantly attach to both poles generating merotelic attachments (Figure 1). The latter might be in particular harmful since chromosomes that are attached to both poles can generate at least some tension across sister kinetochores and can evade recognition by the mitotic spindle checkpoint[24]. When progressing through mitosis merotelic chromosome attachments can subsequently result in the generation of so-called "lagging" chromosomes that cannot be segregated properly onto a daughter cell (Figure 4). Significantly, the appearance of "lagging" chromosomes in anaphase is a common feature in cancer cells exhibiting CIN[25] indicating that chromosome mal-attachments might significantly contribute to chromosome missegregation and CIN.

It is conceivable that chromosome mal-attachments must be corrected in order to allow proper chromosome alignment and segregation. In fact, the correction of mal-oriented chromosomes requires high dynamics of kinetochore-microtubule binding and theses dynamics are particularly high in the early phases of mitosis ($t_{1/2}$=2-3 minutes) while it is suppressed when cells progress into anaphase ($t_{1/2}$= ~ 50 minutes)[26]. Thus, chromosome segregation fidelity depends on highly regulated chromosome attachment dynamics, which molecular basis is only poorly understood. Notably, hyperstable kinetochore-microtubule attachments have been detected in cancer cells exhibiting CIN, suggesting that not only the generation, but also the reduced ability to correct faulty kinetochore-microtubule attachments might be an important source of CIN in human cancer[27]. However, it is still largely unknown which alterations in human cancer support chromosome mal-attachments and the generation of hyper-stable chromosome attachments.

Abnormal spindle structures cause CIN by facilitating the generation of mal-oriented chromosomes

A major source of merotelic attachments during mitosis might be abnormal spindle morphology that can be associated with supernumerary centrosomes. It has been assumed that amplified centrosomes cause multipolar mitotic spindles associated with multipolar chromosome segregation producing three or more highly aneuploid daughter cells. However, recent studies have surprisingly revealed that multipolar cell divisions are very rare and - when experimentally induced - typically produce inviable daughter cells. Instead, multiple centrosomes cause the formation of multipolar spindle intermediates restricted to the early phases of mitosis that facilitate the generation of merotelic chromosome attachments and lagging chromosomes. Importantly, the abnormal spindle intermediates are resolved

before anaphase by clustering of the supernumerary centrosomes at the spindle poles. This allows the formation of a bipolar anaphase spindle and bipolar chromosome segregation, although unresolved merotelic chromosome attachments contribute to reduced segregation fidelity[28, 29]. Thus, these studies demonstrate that abnormal spindle intermediates rather than centrosome amplification per se can significantly contribute to CIN by facilitating the generation of mal-oriented chromosomes.

Subtle alterations in mitotic progression rather than severe ablation of mitotic regulation are a source for CIN

The possible mechanisms of CIN described so far indicate that subtle alterations of the normal mitotic progression rather than severe mitotic abrogation might be the major source of CIN in human cancer. In fact, while a partial impairment of the mitotic spindle checkpoint clearly supports premature sister chromatid segregation and CIN significant ablation of the checkpoint results in lethality. Similarly, little changes in microtubule dynamics can interfere with the normal kinetochore-microtubule attachments and thus, with normal chromosome segregation, whereas severe inhibition of microtubule function inevitably result in cell death. Especially the latter is exploited for therapeutic purpose during chemotherapy for years: anti-mitotic drugs including various microtubule inhibitors (e.g. Taxanes, Epothilones or *Vinca* alkaloids) that are used routinely in the clinic significantly inhibit microtubule dynamics leading to a severe ablation of mitotic progression and subsequently, resulting in cell death[30]. From this point of view it is conceivable that alterations in cancer leading to CIN are not expected to result in severe mitotic abnormalities. Thus, it is a major challenge to define those subtle mitotic alterations and to identify the underlying gene alterations in human cancer.

CHK2 is a major tumor suppressor in human lung adenocarcinomas that ensures chromosomal stability in human somatic cells

Several mouse knockout studies have shown that the experimental induction of CIN can initiate and facilitate tumor growth *in vivo*, strongly supporting the notion that CIN is the cause rather than a consequence of tumorigenesis. Intriguingly, the induction of aneuploidy in mice is commonly associated with the induction of lung adenocarcinomas suggesting that lung epithelial cells might possess a particular high propensity for transformation in response to CIN[4, 5]. Although the reason for this tissue selectivity is currently unclear, it nevertheless implicates that CIN might play a key role in lung tumorigenesis.

Therefore, our laboratory aims to identify those tumor associated CIN genes that are on one hand involved in the maintenance of chromosomal stability and on the other hand altered at high frequency in human cancer, in particular in lung adenocarcinomas. Most recently, we identified *CHK2* as such a key CIN gene in human lung cancer[31]. Chk2 has been previously identified to be involved in the DNA damage response pathway in human and murine cells and it has been proposed that Chk2 represents the kinase responsible for the phosphorylation of the tumor suppressor and transcription factor p53 as well as for the cell cycle regulatory phosphatase cdc25A[32]. However, this view has been challenged, at least in human somatic cells, where Chk2 is neither required for p53 function nor for cdc25A inactivation upon DNA damage and consequently, Chk2 seems also to be dispensable for a cell cycle arrest after DNA strand breaks[33-35]. Chk2 has further been implicated in the phosphorylation and regulation of the breast cancer associated tumor suppressor protein Brca1 and its DNA repair activity in response to DNA double strand breaks might be under control of Chk2[36, 37]. The *CHK2* gene has been analyzed previously in human cancer and infrequent mutations were found in various tumor entities, which led to the notion that *CHK2* might be a low penetrance tumor susceptibility gene[38].

In contrast to these previous studies, we investigated *CHK2* specifically in highly aneuploid human lung cancer cells. Surprisingly, by analyzing lung cancer specimens from 108 patients we found that *CHK2* expression is severely repressed or even completely absent in about 53% of the cases and, even more impressive, we observed a loss of *CHK2* in about 68% of lung adenocarcinoma samples[31]. Thus, *CHK2* can be ranked among the top tumor associated alterations in human lung cancer together with such prominent examples as *TP53*, *EGFR* and *KRAS*. These results suggest that *CHK2* might be a major tumor suppressor gene in human lung adenocarcinomas, which prompted us to investigate the role of the Chk2 kinase for the maintenance of chromosomal stability.

When analyzing the role of *CHK2* in CIN, our

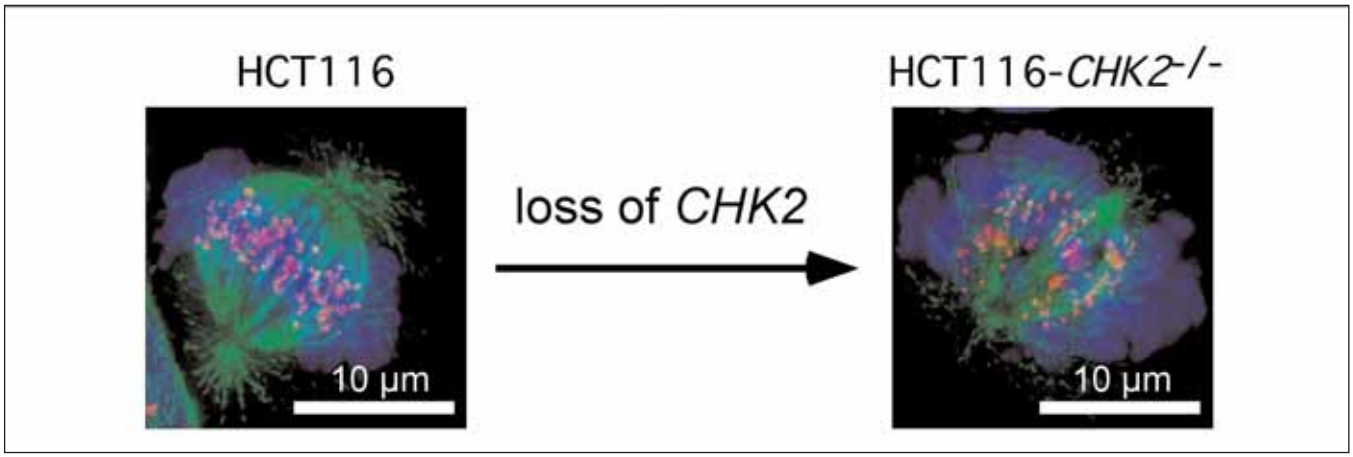


Figure 3: *Loss of CHK2 causes the formation of abnormal mitotic spindles. CHK2 proficient and deficient HCT116 cells were subjected to immunofluorescence studies and the mitotic spindle (α-tubulin, green), kinetochores (CREST, red) and mitotic chromosomes (DAPI, blue) were detected. Examples of merged deconvolved images of mitotic cells are given. CHK2 deficient cells show highly abnormal spindle morphologies associated with incomplete chromosome alignment. This phenotype is observed transiently and is resolved in later stages of mitosis. (Data from Stolz et al., Nature Cell Biology 2010)"*

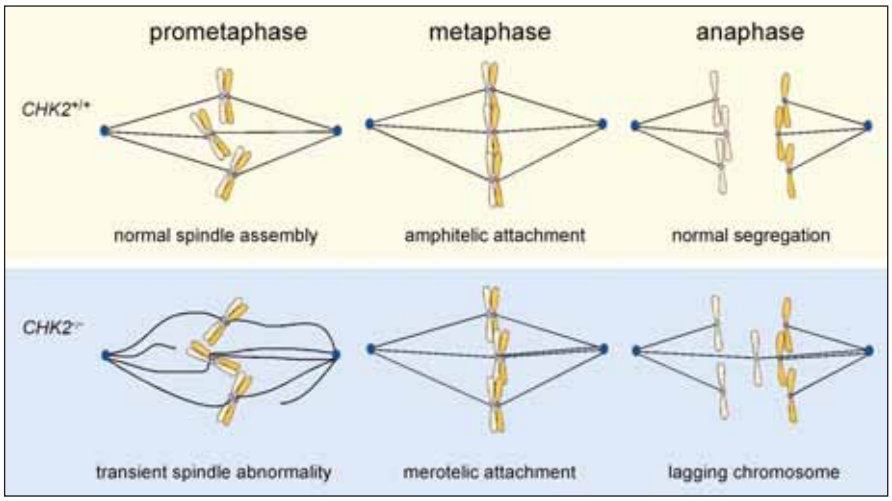


Figure 4: Schematic depiction of the mitotic progression in cells in the presence of absence of CHK2. CHK2 deficient cells exhibit a delay in early mitosis due to transient spindle formation defects, which facilitates the formation of merotelic chromosome attachments and subsequently results in the generation of lagging chromosomes during anaphase.

karyotype analyses revealed that the loss of *CHK2* is sufficient to induce aneuploidy in otherwise chromosomal stable human colon carcinoma cells (Figure 2). Moreover, aneuploidy is further evolving with time and even a partial repression of *CHK2* generates aneuploidy in chromosomal stable cancer cells and also in non-transformed human fibroblasts[31]. Thus, *CHK2* fulfils the key requirements for a *bona fide* CIN gene in human cancer: Its loss results in the induction of CIN in otherwise chromosomal stable cells and it is frequently lost in human cancer tissues.

Loss of *CHK2* causes transient defects in mitotic spindle assembly, which support the generation of merotelic chromosome attachments and lagging chromosomes

To uncover the mitotic abnormalities that underlie CIN in response to the loss of *CHK2* tumor suppressor, we analyzed the progression of mitosis in detail. Intriguingly, we found that synchronized *CHK2* deficient cells exhibit highly aberrant spindle morphologies that are associated with incomplete and abnormal chromosome alignment (Figure 3). Interestingly, these aberrant spindle structures were no longer observed in the later stages of mitosis suggesting that the spindle abnormalities are resolved when cells progress through mitosis. This was also verified in live-cell analyses where a transient prometaphase delay became apparent that was followed by a timely initiation and progression through anaphase. These studies also revealed that chromosome missegregation is often associated with lagging chromosomes appearing in anaphase, which strongly suggested that the transient spindle abnormalities might facilitate the generation of merotelic chromosome attachments. Indeed, we could show that the number of lagging chromosomes as well as CIN can be suppressed by the kinetochore based kinesin MCAK that has previously been implicated in the correction process of mal-oriented chromosome attachments[31]. Thus, our results demonstrate that the loss of the tumor suppressor gene *CHK2* results in transient abnormal spindle assembly in the early phases of mitosis, which supports the generation of merotelic chromosome attachments leading to lagging chromosomes and missegregation during anaphase (Figure 4). Importantly, these findings strongly support the previous suggestions postulating that merotelic chromosome attachments associated with lagging chromosomes are a major source of CIN in human cancer[24, 25, 39] and we are providing direct evidence for a tumor associated gene that causes this common cancer phenotype.

Regulation of the tumor suppressor protein Brca1 by Chk2 is required for proper spindle assembly and chromosomal stability

It has previously been shown that the Chk2 kinase is activated in response to DNA damage, which classified Chk2 as a DNA damage response kinase[32]. In addition, we found that Chk2 is also activated in mitosis in the absence of DNA damage, which further corroborates its new role for the maintenance of chromosomal stability. Moreover, we demonstrated in various reconstitution experiments that the Chk2 kinase activity is indeed required for normal mitotic spindle assembly and for proper chromosome segregation[31]. Clearly, these results enforced the question for a relevant mitotic target of Chk2 that mediates its role in spindle assembly and chromosome segregation and our studies revealed the tumor suppressor protein Brca1 as such a candidate.

Brca1 (BReast CAncer 1) is a 220 kDa protein with multiple, but not well defined functions. However, its role in DNA repair might be best understood so far. The *BRCA1* gene represents an established tumor suppressor gene that is frequently inactivated in breast and ovarian carcinomas[40]. Interestingly, a frequent loss of *BRCA1* has also been described in human lung cancer[41]. After DNA

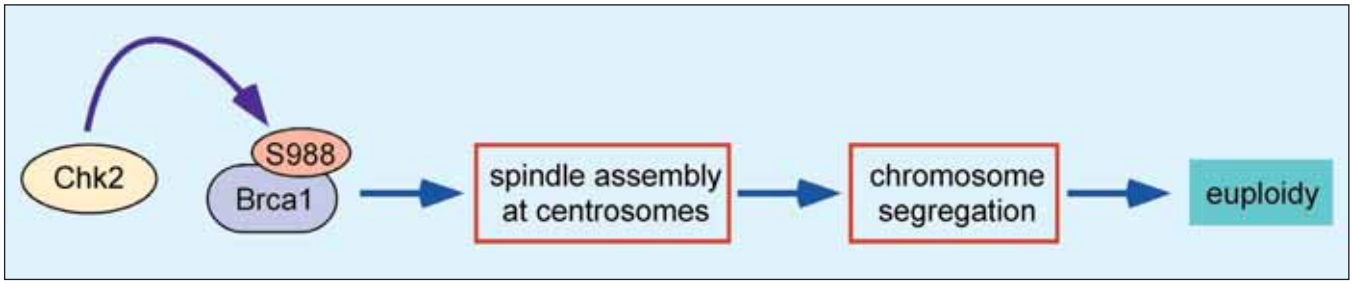


Figure 5: Model for the role of the CHK2-BRCA1 tumor suppressor pathway for the progression of mitosis. The Chk2 kinase phosphorylates Brca1 on serine-988, which is required for proper mitotic spindle assembly and correct chromosome attachment and alignment. Thus, both, CHK2 and BRCA1 are required for the maintenance of chromosomal stability.

damage the Chk2 kinase can phosphorylate Brca1 on serine-988 and this phosphorylation contributes to its activity as a DNA repair factor[36, 37]. Surprisingly, when analyzing mitotic cells we found that activated Chk2 can phosphorylate Brca1 on the same residue in the absence of DNA damage. Moreover, exactly this Chk2 mediated phosphorylation is required for normal mitotic spindle assembly and for timely chromosome alignment (Figure 5). Accordingly, the loss of *BRCA1* fully mimics the mitotic defects observed in *CHK2* deficient cells and, importantly, loss of *BRCA1* also leads to the generation of lagging chromosomes, chromosome missegregation and CIN[31]. Thus, these results clearly show that Brca1 is a relevant downstream target of Chk2 during mitosis required for faithful chromosome segregation. Since both, *CHK2* and *BRCA1* are inactivated at high frequency in human cancer, this tumor suppressor pathway might represent a key mitotic pathway that is required for chromosomal stability in human somatic cells.

Open questions and future directions

The underlying mechanisms of CIN involve subtle, sometimes transient changes in the mitotic progression rather than global inhibition of mitotic regulation and the generation of mal-attached chromosomes might represent a major source of CIN in human cancer. As shown for cells with supernumerary centrosomes[29] and also after loss of *CHK2* or *BRCA1*[31] abnormal spindle intermediates can significantly contribute to elevated rates of merotely and this mechanism might indeed be the starting point for CIN. However, the detailed functions of Chk2 and Brca1 for spindle assembly are still unknown and under intensive investigation in our laboratory. Clearly, it will be crucial to identify and to characterize the mitotic pathways associated with the *CHK2-BRCA1* tumor suppressor pathway that ensure proper and timely spindle assembly. Those pathways are likely candidates for further alterations in human cancer leading to elevated rates of mal-oriented chromosomes that are frequently found in cancer.

On the other hand, it is an important unanswered question why those chromosome mal-attachments – once generated - are not properly resolved. Too little is known about the correction machinery that is required to resolve those insufficiencies. However, a first picture of the attachment correction machinery is beginning to emerge. Several kinetochore and centromere localized proteins have been identified as regulators required for resolving mal-attached chromosomes. Among them is the Aurora-B kinase, which phosphorylates and regulates the activity of several kinetochore- and centromere-based proteins that are involved in destabilizing microtubule-kinetochore attachments[42]. These include members of the kinesin-13 family, Kif2b and MCAK, which possess microtubule depolymerizing activity[39, 43]. According to an elegant recent study, Aurora-B can only phosphorylate substrates at the outer kinetochore when no tension is generated across the two sister kinetochores[44]. In this scenario, the lack of kinetochore tension, which might serve as an indicator of mal-oriented chromosomes, allows the efficient phosphorylation of the adjacent targets resulting in destabilization of the microtubule-kinetochore attachment. In contrast, amphitelic attachments that generate tension pull the substrates away from the centromere-bound kinase leading to a stabilization of the existing attachments[44]. Thus, Aurora-B might represent a key player regulating microtubule regulators at kinetochores and centromeres, but the details on its regulation still remain elusive. Interestingly, Aurora-B is found to be overexpressed in many tumors and it has been implicated as a candidate oncogene[45]. Direct evidence for the latter has not been provided yet and the consequences of Aurora-B overexpression have not been investigated thoroughly so far.

Conclusion

Based on the observations made in human cancer cells the generation of unresolved chromosome mal-attachments might be a major mechanism of CIN. In particular, merotelic microtubule-kinetochore attachments are generated in response to transient spindle formation defects induced by supernumerary centrosomes or after inactivation of the *CHK2-BRCA1* tumor suppressor pathway. Those mal-attachments are the main source of lagging chromosomes that can give directly rise to CIN.

Since CIN might directly contribute to tumorigenesis future work will show whether defects in the pathways responsible for the generation of mal-attached chromosomes and defects in the correction machinery also cooperate in tumorigenesis.

Acknowledgements

I thank Heike Krebber and the members of my lab for critically reading the manuscript and I am especially grateful to the Deutsche Forschungsgemeinschaft for supporting me with a Heisenberg fellowship and grant support.

References

1. Boveri, T. Zur Frage der Entstehung maligner Tumoren. Gustav Fischer Verlag, Jena, 1914.

2. Hansemann, D. Ueber asymetrische Zelltheilung in Epithelkrebsen und deren biologische Bedeutung. *Virchows Arch Pathol Anat* **119**, 299-326 (1890).

3. Lengauer, C., Kinzler, K.W. & Vogelstein, B. Genetic instability in colorectal cancers. *Nature* **386**, 623-7 (1997).

4. Ricke, R.M., van Ree, J.H. & van Deursen, J.M. Whole chromosome instability and cancer: a complex relationship. *Trends Genet* **24**, 457-66 (2008).

5. Holland, A.J. & Cleveland, D.W. Boveri revisited: chromosomal instability, aneuploidy and tumorigenesis. *Nat Rev Mol Cell Biol* **10**, 478-87 (2009).

6. Malumbres, M. & Barbacid, M. Cell cycle kinases in cancer. *Curr Opin Genet Dev* **17**, 60-5 (2007).

7. Miki, H., Okada, Y. & Hirokawa, N. Analysis of the kinesin superfamily: insights into structure and function. *Trends Cell Biol* **15**, 467-76 (2005).

8. Peters, J.M. The anaphase promoting complex/cyclosome: a machine designed to destroy. *Nat Rev Mol Cell Biol* **7**, 644-56 (2006).

9. Musacchio, A. & Salmon, E.D. The spindle-assembly checkpoint in space and time. *Nat Rev Mol Cell Biol* **8**, 379-93 (2007).

10. Schvartzman, J.M., Sotillo, R. & Benezra, R. Mitotic chromosomal instability and cancer: mouse modelling of the human disease. *Nat Rev Cancer* **10**, 102-15 (2010).

11. Dobles, M., et al. Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2. *Cell* **101**, 635-45 (2000).

12. Michel, L., et al. Complete loss of the tumor suppressor MAD2 causes premature cyclin B degradation and mitotic failure in human somatic cells. *Proc Natl Acad Sci* U S A **101**, 4459-64 (2004).

13. Michel, L.S., et al. MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. *Nature* **409**, 355-9 (2001).

14. Kops, G.J., Foltz, D.R. & Cleveland, D.W. Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint. *Proc Natl Acad Sci* U S A **101**, 8699-704 (2004).

15. Weaver, B.A., et al. Aneuploidy acts both oncogenically and as a tumor suppressor. *Cancer Cell* **11**, 25-36 (2007).

16. Kaestner, P., Stolz, A. & Bastians, H. Determinants for the efficiency of anticancer drugs targeting either Aurora-A or Aurora-B kinases in human colon carcinoma cells. *Mol Cancer Ther* **8**, 2046-56 (2009).

17. Stolz, A., et al. Pharmacologic abrogation of the mitotic spindle checkpoint by an indolocarbazole discovered by cellular screening efficiently kills cancer cells. *Cancer Res* **69**, 3874-83 (2009).

18. Cahill, D.P., et al. Mutations of mitotic checkpoint genes in human cancers. Nature 392, 300-3 (1998).

19. Wang, X., et al. Significance of MAD2 expression to mitotic checkpoint control in ovarian cancer cells. *Cancer Res* **62**, 1662-8 (2002).

20. Wang, X., et al. Correlation of defective mitotic checkpoint with aberrantly reduced expression of MAD2 protein in nasopharyngeal carcinoma cells. *Carcinogenesis* **21**, 2293-7 (2000).

21. Tighe, A., Johnson, V.L., Albertella, M. & Taylor, S.S. Aneuploid colon cancer cells have a robust spindle checkpoint. *EMBO Rep* **2**, 609-14 (2001).

22. Cimini, D. Merotelic kinetochore orientation, aneuploidy, and cancer. *Biochim Biophys Acta* **1786**, 32-40 (2008).

23. Salmon, E.D., Cimini, D., Cameron, L.A. & DeLuca, J.G. Merotelic kinetochores in mammalian tissue cells. *Philos Trans R Soc Lond B Biol Sci* **360**, 553-68 (2005).

24. Cimini, D., et al. Merotelic kinetochore orientation is a major mechanism of aneuploidy in mitotic mammalian tissue cells. *J Cell Biol* **153**, 517-27 (2001).

25. Thompson, S.L. & Compton, D.A. Examining the link between chromosomal instability and aneuploidy in human cells. *J Cell Biol* **180**, 665-72 (2008).

26. Zhai, Y., Kronebusch, P.J. & Borisy, G.G. Kinetochore microtubule dynamics and the metaphase-anaphase transition. *J Cell Biol* **131**, 721-34 (1995).

27. Bakhoum, S.F., Genovese, G. & Compton, D.A. Deviant kinetochore microtubule dynamics underlie chromosomal instability. *Curr Biol* **19**, 1937-42 (2009).

28. Silkworth, W.T., Nardi, I.K., Scholl, L.M. & Cimini, D. Multipolar spindle pole coalescence is a major source of kinetochore mis-attachment and chromosome mis-segregation in cancer cells. *PloS one* **4**, e6564 (2009).

29. Ganem, N.J., Godinho, S.A. & Pellman, D. A mechanism linking extra centrosomes to chromosomal instability. *Nature* **460**, 278-82 (2009).

30. Jordan, M.A. & Wilson, L. Microtubules as a target for anticancer drugs. *Nat Rev Cancer* **4**, 253-65 (2004).

31. Stolz, A., et al. The CHK2-BRCA1 tumour suppressor pathway ensures chromosomal stability in human somatic cells. *Nat Cell Biol*, (2010).

32. Ahn, J., Urist, M. & Prives, C. The Chk2 protein kinase. *DNA Repair* (Amst) **3**, 1039-47 (2004).

33. Ahn, J., Urist, M. & Prives, C. Questioning the role of checkpoint kinase 2 in the p53 DNA damage response. *J Biol Chem* **278**, 20480-9 (2003).

34. Jallepalli, P.V., Lengauer, C., Vogelstein, B. & Bunz, F. The Chk2 tumor suppressor is not required for p53 responses in human cancer cells. *J Biol Chem* **278**, 20475-9 (2003).

35. Jin, J., et al. Differential roles for checkpoint kinases in DNA damage-dependent degradation of the Cdc25A protein phosphatase. *J Biol Chem* **283**, 19322-8 (2008).

36. Lee, J.S., et al. hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. *Nature* **404**, 201-4 (2000).

37. Zhang, J., et al. Chk2 phosphorylation of BRCA1 regulates DNA double-strand break repair. *Mol Cell Biol* **24**, 708-18 (2004).

38. Antoni, L., Sodha, N., Collins, I. & Garrett, M.D. CHK2 kinase: cancer susceptibility and cancer therapy - two sides of the same coin? *Nat Rev Cancer* **7**, 925-36 (2007).

39. Bakhoum, S.F., Thompson, S.L., Manning, A.L. & Compton, D.A. Genome stability is ensured by temporal control of kinetochore-microtubule dynamics. *Nat Cell Biol* **11**, 27-35 (2009).

40. Deng, C.X. BRCA1: cell cycle checkpoint, genetic instability, DNA damage response and cancer evolution. *Nucleic Acids Res* **34**, 1416-26 (2006).

41. Lee, M.N., et al. Epigenetic inactivation of the chromosomal stability control genes BRCA1, BRCA2, and XRCC5 in non-small cell lung cancer. *Clin Cancer Res* **13**, 832-8 (2007).

42. Cimini, D., Wan, X., Hirel, C.B. & Salmon, E.D. Aurora kinase promotes turnover of kinetochore microtubules to reduce chromosome segregation errors. *Curr Biol* **16**, 1711-8 (2006).

43. Thompson, S.L., Bakhoum, S.F. & Compton, D.A. Mechanisms of chromosomal instability. *Curr Biol* **20**, R285-95 (2010).

44. Liu, D., et al. Sensing chromosome bi-orientation by spatial separation of aurora B kinase from kinetochore substrates. *Science* **323**, 1350-3 (2009).

45. Keen, N. & Taylor, S.S. Aurora-kinase inhibitors as anticancer agents. *Nat Rev Cancer* **4**, 927-36 (2004).

PD Dr. rer.nat. Holger Bastians

Philipps Universität Marburg
Institut für Molekularbiologie und Tumorforschung (IMT)
Emil-Mannkopff-Strasse 2
35037 Marburg
Email: bastians@imt.uni-marburg.de

1999 – today	Group leader at the Institute for Molecular Biology and Tumor Research, Philipps-University Marburg
since 2008	Heisenberg Fellow of the DFG
2007	Foundation and direction of the "TransMIT-Center for Cell Cycle associated Targets for Chemotherapy" and collaboration with the Oncology Research Department of Altana Pharma AG
2006	Habilitation in "Molecular Biology" Medical School, Philipps-University Marburg
1996 – 1999	Postdoctoral Fellow in the laboratory of Prof. Joan Ruderman, Harvard Medical School, Department for Cell Biology, Boston, USA
1993 – 1999	PhD thesis in the laboratory of Prof. Herwig Ponstingl, German Cancer Research Center (DKFZ), Division for "Molecular Biology of Mitosis", Heidelberg

Ada L. Olins and Donald E. Olins

Wanted: A Nuclear Architect

Candidate Qualifications: The candidate must have a detailed understanding of eukaryotic nuclear structure, including the various levels of chromatin folding, mechanisms for generating hetero- and euchromatin, mitotic chromosomes and chromosome territories, nuclear envelope structure, disassembly, post-mitotic reassembly, transport and signaling, and control of replication and transcription. The candidate should be knowledgeable of the evolutionary diversity of nuclear structures. **Job Description**: The candidate is expected to create new forms of the eukaryotic cell nucleus.

The "want ad", shown above, is not so far-fetched today as it was when we began our scientific careers in the chromatin field in 1964. In the 60's and early 70's, histones were regarded as repressors of transcription; i.e., histones blocked *in vitro* transcription on naked DNA templates with added E. coli RNA polymerase. There was no clear conception of the structure of the nucleohistone complex. Fanciful model drawings, at the time, showed highly α-helical histones bridging between parallel DNA molecules; the histones running within the grooves of the double helix. The prevalent model[1], derived from X-ray scattering, envisaged a single nucleohistone molecule twisted into a helical structure with a 12 nm pitch and 13 nm diameter ("Pardon and Wilkins supercoil"). When visualized by electron microscopy[2], chromatin fibers were somewhat thicker (~ 20-30 nm). The difference between transcriptionally-inactive (hetero-) and transcriptionally-active euchromatin was presumed to be due to the presence or absence of histones in a particular region of DNA.

Using electron microscopy, in 1973 (published in 1974[3]) we demonstrated that spread chromatin molecules bursting from a nucleus resembled "particles on a string", rather than the expected fibers with large gaps of naked DNA alternating with thicker chromatin regions (Figure 1A). This observation was a major surprise to us, as it soon would be to the entire chromatin field. Our electron microscope pictures revealed that these beads (called by us," υ bodies", and eventually "nucleosomes") were everywhere and all looked alike (~ 7-10 nm diameter). Because this chromatin was spilling out of chicken erythrocyte nuclei, which are transcriptionally-inactive, we speculated that we might be visualizing a fundamental nucleohistone repeating subunit, rather than RNA polymerases. Additionally, we demonstrated υ bodies in bursting nuclei from rat liver and calf thymus cells. We speculated that each υ body contained dimers of all the histones arranged around a postulated dyad axis. Further, we suggested that coiling or folding of the nucleosomal chain could explain the microscopic visualization of ~30 nm chromatin fibers. The estimated linear DNA compaction ratio per nucleosome was ~ 6:1, not enough compaction to stuff ~ 2 meters of DNA into the nucleus. A brief review of the history and timeline surrounding the discovery of the chromatin subunit has been published[4]. At the time of the discovery of υ bodies, most chromatin scientists believed that there were four highly conserved histones (H4, H3, H2A and H2B) with very few isoforms. We now know that the story is much more complicated; e.g., H2A exists in ~20 variant isoforms. The X-ray crystal structure[5, 6] has verified that the "core" of a nucleosome is rich in α-helicies (forming histone-histone interaction faces). The short basic amino-terminal histone tails emanate between the two turns of DNA wrapped around the histone octamer. These basic histone tails contain most of the sites for post-translational modification. But, why does the nucleosome possess a dyad axis? Is there internal cooperativity (an allosteric effect?) within mononucleosomes? If so, what function does it play? How do the histone variants influence nucleosome (or higher order) conformations? What role might nucleosome conformations play in chromatin higher order organization and functions? These types of questions are being actively investigated in many laboratories.

Histones and nucleosomes are very highly conserved in evolution; they are seen in the

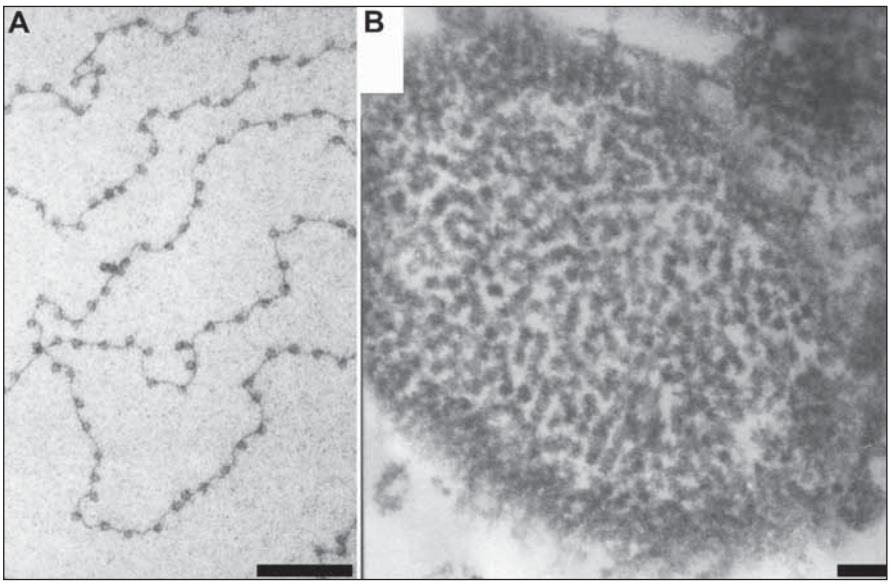


Figure 1: Chicken erythrocyte chromatin: the ultrastructure of transcriptionally-inactive chromatin. *A. Strands of chromatin spread on an electron microscope grid, revealing the repeat structure of nucleosomes (υ bodies)[3]. B. Section (0.1 μm thick) of isolated erythrocyte nucleus swollen in 0.02 M KCl buffer, prior to fixation, embedding and staining. Note the parallel alignment of ~ 30 nm chromatin fibers at the nuclear envelope. Magnification Bars: A and B, 100 nm.*

most primitive eukaryotes. This certainly argues that histones and nucleosomes are essential for nuclear functions (e.g., transcription, replication, and packaging). If they are so important, what evolutionary adaptation permitted their loss in the dinoflagellate[7, 8] algae? Instead, these organisms possess nuclei with condensed DNA "liquid crystals". In a typical eukaryotic nucleus, DNA replication of the condensed peripheral heterochromatin usually occurs during the latter part of S phase; whereas, the more internal euchromatin replicates earlier during S. Do the dinoflagellates regulate the timing and location of their DNA replication?

We readily accept the idea that DNA is chromosomal in length; i.e., each chromatid containing a single unbroken large DNA molecule stretching the entire length from telomere to telomere. So, why do the hypotrichous ciliated protozoa have short linear "gene-size" DNA molecules in their macronuclei? Furthermore, each gene is highly endoreplicated in the macronucleus. For example, in *Euplotes eurystomus* (a hypotrichous ciliate) the single macronucleus contains ~10^6 copies of the ribosomal 5S RNA gene[9], present as a single coding region on a short DNA (930 bp) minichromosome, containing four positioned nucleosomes and flanked by two telomeres. The hypotrich macronucleus divides by "amitosis", without forming condensed mitotic chromosomes, partitioning the gene-sized chromosomes in a statistical manner. These remarkable organisms possess an additional "eye-popping" nuclear structure, the replication band (RB)[10, 11] of the macronucleus. In *Euplotes eurystomus*, where the macronucleus is wurst-shaped (~ 150 µm long x ~10 µm wide), RBs begin at the tips of the macronuclei, migrating 1/2 the nuclear length, meeting and fusing in the middle. The internal ultrastructure of RBs is stratified, with the gene-sized chromatin molecules weaving into regular ~ 40-50 nm thick chromatin fibers, just prior to the zone of DNA synthesis. Ciliated protozoa also have micronuclei (with "chromosomal-size" DNA and mitotic chromosomes) which function as the germline for the species. Creatures like dinoflagellates and hypotrichous ciliates should make us pause and contemplate: "How do we define a eukaryotic nucleus?"

Now 36 years since the discovery of the nucleosome (which is universally accepted), the structure and *in vivo* existence of the ~30 nm higher order folding of nucleosomes within a nucleus remains an issue of controversy. The question of its existence is intimately tied up with the validity of various "fixation procedures", prior to sectioning the embedded nuclei for transmission electron microscopy visualization. In brief, Jacques Dubochet and co-workers[12] employ rapid high pressure freezing of cellular materials, combined with cryosectioning and cryotransfer of the section on a grid, and visualization of the cells in a cryostage. By never allowing the specimen to warm above liquid Nitrogen, the macromolecules of the cell undergo minimal movement. It is believed by proponents of this method that the macromolecules are visualized at conditions very close to the native *in vivo* state. The problem is that employing this procedure and focusing upon condensed heterochromatin, one does not visualize a ~ 30 nm chromatin fiber, but rather a "sea of nucleosomes". In contrast, employing a standard chemical fixation protocol with glutaraldehyde +/- OsO_4 postfixation, followed by dehydration and embedding in plastic, thin sectioning and staining, the ~ 30 nm chromatin fibers can be readily observed, especially adjacent to the nuclear envelope (Figure 1B). See, in addition, the magnificent images by Howard Davies and co-workers[2] of cross-sectional or tangential views of the nuclear envelope and underlying heterochromatin in the chicken erythrocyte nucleus.

In recent years, we have been studying an intriguing nuclear envelope structure, an extension of the most peripheral heterochromatin layer into large sheets of chromatin (originally described by Howard Davies and co-workers[13]), named "nuclear envelope-limited chromatin sheets", abbreviated by us as "ELCS"[14, 15]. These structures (Figure 2) resemble a sandwich with the central ~ 30nm chromatin layer bounded on both sides by nuclear envelope membranes, generating an overall thickness of ~ 40 nm and extending out into the cytoplasm. In this controversy about the *in vivo* reality of the ~ 30 nm chromatin fiber, we consider the existence of ELCS to be a persuasive argument that the heterochromatin

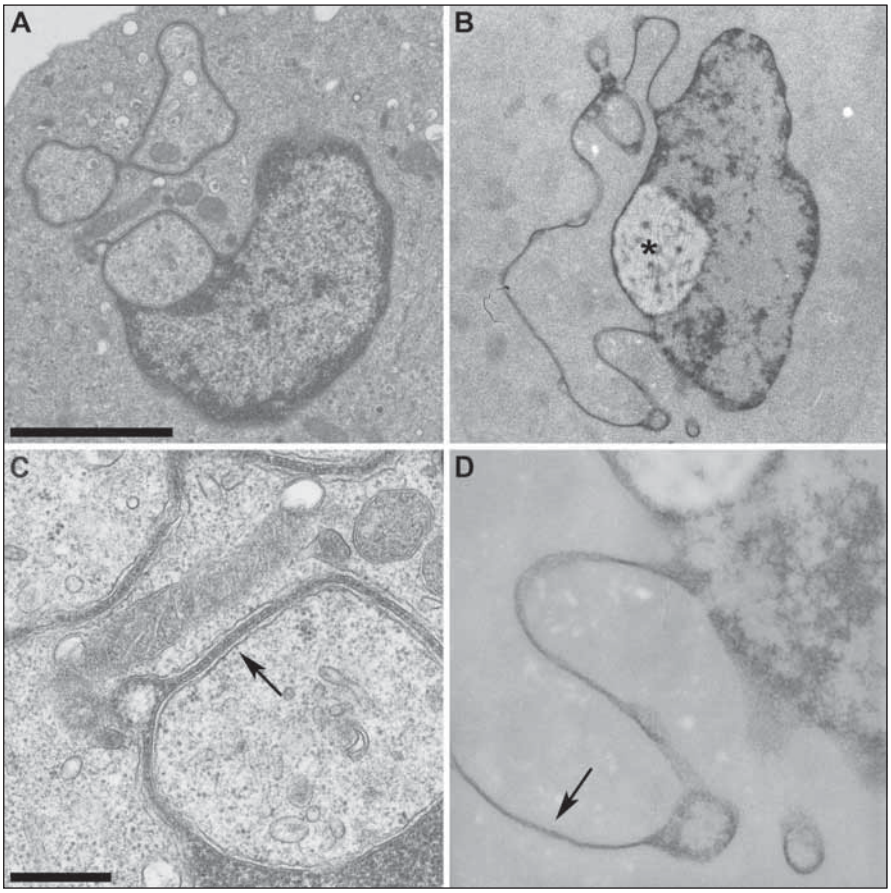


Figure 2. Granulocytic forms of HL-60/S4 cells: the ultrastructure of nuclear envelope-limited chromatin sheets (ELCS)[15]. *Panels A and C: different magnifications of the same nucleus, with the section stained by uranyl acetate and lead citrate. Panels B and D: different magnifications of the same nucleus, with the section stained using the DNA specific Osmium ammine (OAB) stain. Magnification Bars: A and B, 1 µm; C and D, 100 nm. The arrows in panels C and D point to a single sheet of ELCS. The asterisk in panel B denotes the nucleolar region, which is characteristically pale following the OAB stain.*

near the nuclear envelope is composed of closely packed ~30 nm fibers arranged in layers, as originally described by Howard Davies[2]. We argue that ELCS structures could not form in the millisecond time period during the rapid freezing of cells and do exist after high pressure freeze substitution (unpublished observations). If the ELCS do exist *in vivo*, it implies that heterochromatin has cleavage planes spaced ~ 30 nm apart. Furthermore, tangential electron microscopic images of ELCS clearly show regions with parallel ~ 30 nm chromatin fibers.

In all the years of our careers, we have studied aspects of nuclear structure in a wide variety of eukaryotic organisms, including amoeba, hypotrichous ciliates, slime mold, Drosophila, Chironomus, fish and avian nucleated red cells, mouse and human granulocytes, human myeloid leukemic cells and numerous human, mouse and plant tissue culture cell lines. Why? Because we, like many other cell biologists, are opportunists. Evolution has presented us with a great diversity of nuclear structures and phenomena. It is important to find the optimum cell system for a particular biological question, in order to comprehend the underlying nuclear mechanisms. This is part of a venerable tradition in important biological studies. See for example, the beautiful classical studies of chromosome behavior by Theodor Boveri (1862-1915), employing the eggs from the horse parasitic threadworm *Ascaris megalocephala* ("Bivalens race" with four chromosomes; "Univalens race" with two chromosomes)[16]. We fervently hope that nuclear architects of the near-future will design cellular systems, where various nuclear structural features are optimized for additional in depth studies, as evolution has done for us in the past.

References

1. Pardon JF, Richards BM, Cotter RI. X-ray diffraction studies on oriented nucleohistone gels. *Cold Spring Harb Symp Quant Biol* 1974; 38:75-81.

2. Everid AC, Small JV, Davies HG. Electron-microscope observations on the structure of condensed chromatin: evidence for orderly arrays of unit threads on the surface of chicken erythrocyte nuclei. *J Cell Sci* 1970; 7:35-48.

3. Olins AL, Olins DE. Spheroid chromatin units (v bodies). *Science* 1974; 183:330-2.

4. Olins DE, Olins AL. Chromatin history: our view from the bridge. *Nat Rev Mol Cell Biol* 2003; 4:809-14.

5. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* 1997; 389:251-60.

6. Harp JM, Hanson BL, Timm DE, Bunick GJ. Asymmetries in the nucleosome core particle at 2.5 A resolution. *Acta Crystallogr D Biol Crystallogr* 2000; 56:1513-34.

7. Moreno Diaz de la Espina S, Alverca E, Cuadrado A, Franca S. Organization of the genome and gene expression in a nuclear environment lacking histones and nucleosomes: the amazing dinoflagellates. *Eur J Cell Biol* 2005; 84:137-49.

8. Costas E, Goyanes V. Architecture and evolution of dinoflagellate chromosomes: an enigmatic origin. *Cytogenet Genome Res* 2005; 109:268-75.

9. Roberson AE, Wolffe AP, Hauser LJ, Olins DE. The 5S RNA gene minichromosome of Euplotes. *Nucleic Acids Res* 1989; 17:4699-712.

10. Olins AL, Olins DE, Derenzini M, Hernandez-Verdun D, Gounon P, Robert-Nicoud M, Jovin TM. Replication bands and nucleoli in the macronucleus of Euplotes eurystomus: an ultrastructural and cytochemical study. *Biol Cell* 1988; 62:83-93.

11. Olins DE, Olins AL. The replication band of ciliated protozoa. *Int Rev Cytol* 1994; 153:137-70.

12. Bouchet-Marquis C, Dubochet J, Fakan S. Cryoelectron microscopy of vitrified sections: a new challenge for the analysis of functional nuclear architecture. *Histochem Cell Biol* 2006; 125:43-51.

13. Davies HG, Haynes ME. Light- and electron-microscope observations on certain leukocytes in a teleost fish and a comparison of the envelope-limited monolayers of chromatin structural units in different species. *J Cell Sci* 1975; 17:263-85.

14. Olins AL, Buendia B, Herrmann H, Lichter P, Olins DE. Retinoic acid induction of nuclear envelope-limited chromatin sheets in HL-60. *Exp Cell Res* 1998; 245:91-104.

15. Olins DE, Olins AL. Nuclear envelope-limited chromatin sheets (ELCS) and heterochromatin higher order structure. *Chromosoma* 2009; 118:537-48.

16. Baltzer F. Theodor Boveri, Life and Work of a Great Biologist 1862-1915. Berkeley: *University of California Press*, 1967.

Ada L. Olins and Donald E. Olins
Bowdoin College
Biology Department
Brunswick, Maine
aolins@bowdoin.edu
dolins@bowdoin.edu

34th Annual Meeting of the German Society for Cell Biology

29 March–2 April 2011
Bonn/Germany

Organiser

Prof. Dr. Dieter Fürst
Institute of Cell Biology
University of Bonn
Ulrich-Haberland-Straße 61a
53121 Bonn (Germany)
Phone +49 (0) 0228 73 53 01
Fax +49 (0) 0228 73 53 02
E-mail dfuerst@uni-bonn.de

Prof. Dr. Walter Witke
Institute of Genetics
University of Bonn
Römerstraße 164
53117 Bonn (Germany)
Phone +49 (0)228 73 42 11
Fax +49 (0)228 73 42 63
E-mail w.witke@uni-bonn.de

Topics

Plenary Sessions

- Cell cycle and cancer
- Cytoskeletal dynamics
- Stem cells
- Cellular mechanics
- Cell polarity

Symposia

- Molecular mechanisms of ageing
- Muscle cell organization
- Cellular immunity
- Inflammation
- Protein quality control
- Rho GTPases and signalling
- Host pathogen interactions
- Cellular neurobiology
- Biology of addiction
- Cell adhesion
- Nuclear envelope and NPCs
- Frontiers in microscopy
- Vesicle trafficking
- Intermediate filaments: novel functions
- Non-coding RNA

universitätbonn

Abstract deadline: 15 January 2011

www.dgz2011.de

Signal Transduction – Receptors, Mediators and Genes Meeting Report der 13. Jahrestagung der Gesellschaft für Signaltransduktion (STS) zusammen mit dem DGZ Arbeitskreis „Signaltransduktion“

Der Nobelpreis für Medizin im Jahr 2009 wurde an 3 Wissenschaftler (Elizabeth H. Blackburn (UCSF), Carol W. Greider (Johns-Hopkins-University, Baltimore), Jack W. Szostak (Harvard Medical School, Boston)) auf dem Gebiet der Telomerforschung im Rahmen der Erkenntnisse zu molekularen Mechanismen des zellulären Alterns verliehen und unter diesem Hauptthema "Aging and Senescence" stand auch die 13. Jahrestagung der Gesellschaft für Signaltransduktion (Signal Transduction Society STS) vom 28. bis 30. Oktober 2009 in Weimar in Zusammenarbeit mit den entsprechenden Signaltransduktion-Arbeitskreisen der Deutschen Gesellschaft für Zellbiologie (DGZ), der Deutschen Gesellschaft für Immunologie (DGfI) und der Gesellschaft für Biochemie und Molekularbiologie (GBM). Gerade diese zellbiologische Thematik („Aging“) auch im Hinblick auf ein sogenanntes „Reprogramming“ („Rejuvenation“) von Zellen, wobei zelluläre Prozesse wie Retrodifferenzierung und Transdifferenzierung das Spektrum zellulärer Entwicklungsmöglichkeiten und Plastizität deutlich erweitern durch natürliche oder induzierte Umprogrammierung zur Differenzierung entlang anderer Entwicklungswege, unterstreicht die Bedeutung dieses Forschungsgebietes und damit die Aktualität dieses Meetings.

Dieser internationale Kongress zieht jährlich über 200 Wissenschaftler aus einem breit gefächerten Spektrum von Forschungsgebieten aus dem In- und Ausland sowie über 20 Partner aus der Industrie an. Neben dem Hauptthema zu Alterungsmechanismen umfassten die weiteren Workshop-Themen die Immunologie und Tumorbiologie, sowie die Pharmakologie bis zu mathematischen und strukturellen Modellen neuer Signaltransduktionswege. Neben diversen Fachvorträgen wurden über 80 Abstractbeiträge auch als Poster präsentiert. Jeder Workshop begann mit Übersichtsvorträgen von eingeladenen, international renommierten Experten, alle weiteren Vorträge wurden aus den eingereichten Kongressbeiträgen ausgewählt. Von einigen repräsentativen “Highlights” des 13. STS Joint-Meeting “Signal Transduction – Receptors, Mediators and Genes” soll hier kurz berichtet werden.

Das wissenschaftliche Programm der Tagung startete mit dem Workshop 'Immune Signaling', welcher von der DGfI gemeinsam mit Wissenschaftlern des SFB/Transregio 52 "Transcriptional Programming of Individual T Cell Subsets" aus Würzburg, Mainz und Berlin co-organisiert wurde. Der einleitende Übersichtsvortrag wurde von Arthur Weiss (Howard Hughes Medical Institute, San Francisco), dem vergangenen Präsidenten der American Association of Immunology (AAI) und herausragenden Pionier in der Signaltransduktionsforschung von T-Lymphzyten gehalten. Professor Weiss diskutierte die Eigenschaften und notwendigen Einsatzgebiete von bestimmten Tyrosinkinase-Inhibitoren im Rahmen klinischer Anwendungen und fokussierte dabei auf immunmodulatorische Wirkungen bei der Tyrosinkinase ZAP-70. Spezifische Inhibitoren können hier einen signifikanten Konformationswechsel der Kinase bewirken, sodass damit der Aktivierungsstatus und gezielte intra- wie auch intermolekulare Protein-Protein-Interaktionen gesteuert werden können. Im Rahmen des 'Immune Signaling Workshops' wurde ein weiterer Hauptvortrag von Anjana Rao (Dana-Farber Cancer Institute, Harvard Medical School, Boston) präsentiert über Bindungseigenschaften und Funktionalität von Foxp3. Die DNA-Bindungsregion in der sogenannten 'Forkhead domain' bei diesem Transkriptionsfaktor unterscheidet sich in 3 Aminosäuren von denen in Foxp2 und nach Integration dieser Varianten in Foxp3 und entsprechender Überexpression konnte Professorin Rao eine Inhibition der supprimierenden Aktivität, aber überraschender Weise keine Änderung in den DNA Bindungseigenschaften zeigen, wobei dieses Motif für die Foxp3 Dimerisierung wichtig ist und damit 2 DNA Stränge strukturell assoziieren kann. Weitere Untersuchungen müssen zeigen, inwieweit Foxp3-vermittelte 'long-ranged' DNA Interaktionen zur Genregulation in regulatorischen T-Zellen und damit zur Funktionalität dieser Zellen beitragen.

Der Workshop 'Growth Factors, Cytokines and Chemokines' wurde mit einem Plenarvortrag von Ivo Touw (Erasmus University Medical School, Rotterdam) zur Regulation des 'granulocyte colony stimulating factor receptor' (G-CSF-R) eingeleitet. Professor Touw zeigte nach G-CSF Stimulation und nachfolgender Endozytose eine SOCS3-abhängige Ubiquitinierung des Rezeptors. an Lys632, die wichtig ist für eine lysosomale Adressierung des Komplexes mit nachfolgendem Abbau. Eine Mutation des Lys632 im G-CSF-R verhindert den Abbau und hält eine langanhaltende Phosphorylierungssignalkaskade aufrecht über STAT5 und Erk, aber nicht PKB. Neuere Daten zeigen eine weitere Wirkmöglichkeit von G-CSF, was ebenso zur verstärkten Expression von DUB2A – einem deubiquitinierenden Enzym – führen kann und somit zur Stabilisierung von G-CSF-R in Endosomen beiträgt durch teilweise Neutralisierung der SOCS3-abhängigen Rezeptorubiquitinierung, wobei die genaue integrative Regulationen dieser entgegengesetzten Signalwege noch unklar sind.

Ein weiterer Workshop 'Migration, Adhesion and Intercellular Signaling' wurde von Stephen Ward (University of Bath, UK) eingeleitet. Professor Ward beschäftigte sich mit der Rolle der Phosphatidylinositol-3-Kinase (PI3-K) während der Polarisierung und Migration von Leukozyten in entzündlichen Prozessen und bei Autoimmunerkrankungen. Klinische Relevanz für Interventionen in diese Signalwege könnten laut Professor Ward neben spezifischen PI3-K Inhibitoren ggf. auch Aktivatoren der antagonistisch wirkenden Phosphatase SHIP (SH2-containing inositol-5-phosphatase) darstellen als pharmakologisches Target für hämatopoetische oder immunologische Erkrankungen mit einem fehlgesteuerten PI3-K Signalweg.

Michael Naumann (Universität Magdeburg) leitete mit einem Übersichtsvortrag zu immunmodulatorischen Eigenschaften von Bakterien und Viren über den NF-kB Signalweg den Workshop über 'Pathogens and Disease' ein. Professor Naumann stellte die Bedeutung von NF-kB exemplarisch für das

angeborene, unspezifische ('innate immune system') und das im Rahmen einer spezifischen Anpassung an Antigene 'adaptive' Immunsystem vor, wobei viele Komponenten des NF-kB Signalweges durch post-transkriptionale Modifikationen reguliert werden. Ubiquitinierung mit nachfolgendem Abbau oder ubiquitinähnliche Modifizierer (bspw. Nedd8 oder SUMO) des NF-kB-assoziierten Inhibitors IkB sind häufige Aktivierungssequenzen für den Transkriptionsfaktor, wobei bestimmte pathogene Bakterien die Expression deubiquitinierender Enzyme vermitteln können. So exprimiert bspw. Chlamydia trachomatis, ein humanpathogener Erreger, der u.a. zur Erblindung führen kann, das deubiquitinierende Protein ChlaDub1, welches die IkB Ubiquitinierung verhindert und damit eine NF-kB vermittelt Entzündungs- und Immunantwort inhibiert. Diese generelle Strategie zur Modulation des Ubiquitinsystems wird auch von einigen Viren übernommen, so bspw. das Kaposisarkoma-assoziierte Herpesvirus (KSHV), sodass diese Deubiquitinierungsfaktoren ggf. ein molekulares Target für therapeutische Ansätze dieser Art von Bakterien- oder Vireninfektionen darstellen.

Der Hauptfokus des Meetings in 2009 lag – wie eingangs bereits erwähnt – auf der Thematik 'Aging and Senescence'. Dieses Symposium wurde organisiert vom Düsseldorfer SFB 728 "Environmentally-induced Aging Processes" gemeinsam mit dem DGZ-Arbeitskreis 'Signaltransduktion', zu dem 3 international renommierte Keynote Sprecher eingeladen wurden. Einleitend zu diesem Symposium stellte dabei Alexander Bürkle (Universität Konstanz) das durch die EU-Kommission geförderte Projekt MARK-AGE vor. Professor Bürkle als Koordinator dieses multinationalen Projekts präsentierte diese Populationsstudie, die dazu dienen soll, Biomarker des humanen Alterungsprozesses zu identifizieren, um mit einem umfangreichen Panel verschiedener zellbiologischer, biochemischer, immunologischer und genetischer Marker ein biologisches Alter besser abschätzen zu können bspw. im Hinblick auf alterungsassoziierte Krankheiten.

Jose Vina (University of Valencia, Spain) präsentierte Eigenschaften von „Langlebigkeitsfaktoren" (longevity-associated factors), wobei p53/p16 und GRF-1 (guanosine releasing factor-1) eine wichtige Rolle spielen könnten. Direkte Longevity-Gene, deren Expressionsniveau unmittelbar die organismische Lebensdauer beeinflussen, wurden bei C.elegans gefunden. Eine wichtige Rolle bei Alterungsprozessen spielen auch DNA Schäden und assoziierte Reparaturprozesse. Ian Hickson (University of Oxford, UK) stellte dabei den wichtigen Status der ReqQ Helicasen für die Erhaltung der genomischen Stabilität vor. Diese DNA Modifikationsenzymfamilie spielt daher funktionell nicht nur bei Alterungsprozessen eine wichtige Rolle, sondern hat auch eine besondere Bedeutung bei der Verhinderung von Krebserkrankungen und bestimmte ReqQ Helicasen sind involviert beim Bloom's und Werner's Syndrom.

In dem sich anschliessenden Workshop 'Tumor Biology' fokussierte Stefan Knapp (University of Oxford, U.K.) auf die Regulation bestimmter Kinaseinhibitoren als Tumortherapeutika. So zeigten einige klinische Studien, dass Interventionspunkte in einer Signalkaskade mit Hilfe von Kinaseinhibitoren nicht die erwünschte Spezifität bzgl. der entsprechend zu bekämpfenden Krankenheit zeigen, oder sich zu viele unerwünschte Nebeneffekte einstellen. Professor Knapp stellte eine Reihe neuartiger potenzieller Strukturen für Kinaseinhibitordomänen vor und ihre Komplexe mit den katalytischen Kinasezentren, die für ein spezifisches Signaltargeting an gezielteren Interventionspunkten wirksamer als bisherige Inhibitoren sein könnten.

Wie bereits in den Jahren zuvor wurde insgesamt auch bei dieser STS Jahrestagung 2009 ein breites Spektrum von Forschungstätigkeiten auf dem Gebiet der Signaltransduktion abgedeckt, obgleich nicht jedes Jahr alle Themengebiete voll-umfänglich abgebildet werden können. Daher variieren einige Workshops thematisch von Jahr zu Jahr und insbesondere der Schwerpunkt wechselt. Entsprechend wird der Schwerpunkt des diesjährigen kommenden Joint Meeting der Arbeitskreise „Signaltransduktion" der DGZ, DGfI und GBM zusammen mit der STS auf das Thema 'Drug Design / Drug Discovery' fokussieren. Das Meeting dieses Jahr wird vom 18. bis. 20. Oktober wiederum in Weimar stattfinden mit Details zu Programm und Anmeldung unter www.sigtrans.de.

Prof. Dr. Ralf Hass
AG Biochemie und Tumorbiologie
Zentrum Frauenheilkunde
Medizinische Hochschule Hannover
Carl-Neuberg-Str. 1
D–30625 Hannover
Germany
E-mail: hass.ralf@mh-hannover.de

The EMBO Meeting 2010
4. – 7. September 2010, Barcelona
www.the-embo-meeting.org

International Meeting of the DGZ on „Actin Dynamics"
15. – 18. September 2010, Jena
Information/Registration: www.zellbiologie.de
Sekretariat der DGZ: dgz@dkfz.de

40. Jahrestagung der Deutschen Gesellschaft für Immunologie
22. – 25. September 2010, Leipzig
www.immunologie2010.de

Symposium des Sonderforschungsbereiches 590: "Membrane Dynamics of the Cell"
4th International Symposium SFB 590
23. – 25 September 2010, Düsseldorf
www.membrane.uni-duesseldorf.de

5. Kongress der Mitteldeutschen Chirurgen
23. – 25. September 2010, Magdeburg
www.conventus.de/mdcv2010/

7. Jahrestagung der Deutschen Vereinten Gesellschaft für Klinische Chemie und Laboratoriumsmedizin
29. September – 2. Oktober 2010, Mannheim
www.dgkl-kongress.de

5. Jahrestagung der Deutschen Gesellschaft für Stammzellforschung (GSZ)
30. September – 2. Oktober 2010, Lübeck
www.conventus.de/gsz2010

6th International F.C. Behr Symposium on Stem Cells and Cancer
3. – 5. Oktober 2010, Heidelberg
www.dkfz.de/SCC2010

Wnt Symposium 2010 DFG Forschergruppe 1036 „Mechanisms, functions and evolution of Wnt-signaling pathways"
25. – 26. Oktober 2010, Heidelberg
www.wnt.uni-hd.de

Jahrestagung der Deutschen Gesellschaft für Altersforschung (DGfA)
(organisiert durch FLI und DGfA)
29. – 30. Oktober 2010, Jena
www.fli-leibniz.de/news/meetings.php; abstract submission to:
Lenhard.Rudolph@uni-ulm.de

26. Ernst Klenk Symposium in Molecular Medicine
NOX Family NADPH Oxidases as Therapeutic Targets
4. – 6. November 2010, Köln
www.zmmk.uni-koeln.de

34. Jahrestagung der DGZ
30. März – 2. April 2011, Bonn
www.dgz2011.de

Proteomic Forum 2011
3. – 7. April 2011, Berlin
www.ctw-congress.de/ProteomicForum2011

Impressum

Zellbiologie *aktuell*

Herausgeber:
Deutsche Gesellschaft für Zellbiologie e.V. (DGZ)

Verantwortlicher Schriftleiter:
Prof. Dr. Harald Herrmann-Lerdon, Präsident
Prof. Dr. Ralph Gräf, Geschäftsführer

Redaktion/Anzeigen:
Sabine Reichel-Klingmann
Sekretariat der Deutschen
Gesellschaft für Zellbiologie e.V.
Deutsches Krebsforschungszentrum
Im Neuenheimer Feld 280
69120 Heidelberg
Tel.: 06221/42-3451
Fax: 06221/42-3452
E-mail: DGZ@DKFZ.de
Internet: www.Zellbiologie.de

Manuskripte:
Erbeten an das Sekretariat der DGZ

Anzeigen:
Vorlagen an das Sekretariat der DGZ

Produktion:
abcdruck GmbH
Waldhofer Str. 19
69123 Heidelberg
Tel.: 06221/8446-19
Fax: 06221/8406-00
Gestaltung: Heike Fischer

Auflage:
1800 Exemplare

Erscheinungsweise:
4 Ausgaben jährlich

Bezug:
Für Mitglieder der DGZ kostenlos

Haben Sie Interesse, in „Zellbiologie *aktuell*" eine Werbeanzeige zu schalten? Dann wenden Sie sich bitte an das Sekretariat der DGZ.

Personalien

Die DGZ begrüßt folgende neue Mitglieder:

Dr. Frank BRADKE, Dennis BREITSPRECHER, Dr. Diego J. WALTHER, M.Sc. Biochem. Sven EISELEIN, Johannes FÖRTSCH, Dr. rer. nat. Natja HAAG, Saskia Anne HUTTEN, Dr. Hellen ISHIKAWA-ANKERHOLD, Dr. Nicole KOCH, Dr. Guido KRUPP, Dr. Robert LINDNER, Joern LINKNER, Dr. Uta MELLERT, Benjamin NORDHOLZ, Prof. Dr. med. K. Lenhard RUDOLPH, Dr. rer. nat. Mario SCHELHAAS, Katharina SCHNEIDER, Dr. Andre SCHÖNICHEN, Dr. Katharina SCHÖNRATH, Dr. rer. nat. Susann SCHÜLER, M. Sc. Lukas SCHWINTZER, Dipl. Biochem. Adrian SPRENGER, Dr. Zuzana STORCHOVÁ, Sabine UNGER, Petra ZUGSCHWERDT

Wer kann uns helfen? Von den unten aufgeführten Mitgliedern fehlen uns die aktuellen Adressen. Wenn jemand uns diesbezüglich weiterhelfen kann, bitten wir um Mitteilung an unser Sekretariat. Wir sind für jeden Hinweis dankbar. Vielen Dank für Ihre Mithilfe!

Adelt Stephan, **Adjogble** Koku Zikpi, **Alev** Cantas, **Bajbouj** Khuloud, **Barendziak** Tanja, **Behrendt** Marc, **Bichelmeier** Ulrike, **Blase** Christopher, **Blume** Jessica, **Boshart** Michael, **Brandt** Peter, **Brigge** Theo, **Buchner** Klaus, **Bumann** Johann, **Carl-McGrath** Stacy, **Cerff** Rüdiger, **Conradi** Anne, **Dannhauser** Philip, **Dressler** Cathrin, **Eckert** Hans-Georg, **Eichstaedt** Stefanie, **Friedrich** Eckhard, **Geßner** Reinhard, **Grabenbauer** Markus, **Gruner** Karl Reinhold, **Gunzer** Matthias, **Hameister** Horst, **Hartmann** Kristina, **Hartwig** Benedikt, **Herbst** Detlev, **Hesse** Michael, **Hilker** Michael, **Hillje** Anna-Lena, **Hinz** Giselbert, **Hochhuth** Christa, **Hofmann** Wilma, **Huff** Thomas, **Jarchau** Thomas, **Kahl** Günter, **Kalbas** Matthias, **Kessels** Michael M., **Kettelhake** Antje, **Knoll** Gerhard, **Korfsmeier** Karl-Hermann, **Kralewski** Martina, **Kröger** Stephan, **Krüger** Bernd, **Kuchenbecker** Ralf, **Küchler** Michael, **Lahm** Harald, **Lange** Philipp, **Lehmann** Friederike, **Lehnert** Lasse, **Lucka** Lothar, **Mattsson** Mats-Olof, **Michel** Caroline, **Moodt** Susanne, **Motrescu** R. Elena, **Müller** Jens, **Murk** Kai, **Oberbäumer-Wesch** Ilse, **Pauli** Andrea, **Paulus** Gerd, **Peschke** Matthias, **Peters** Kirsten, **Peters** Winfried, **Petrovitch** Alexander, **Pohlner** Johannes, **Riehl** Astrid, **Röser** Kerstin, **Rudel** Thomas, **Scharf** Klaus-Dieter, **Schenk** Hainfried, **Schmidt** Annette, **Schreiber** Daniela, **Schwarz** Gerd, **Seedorf** Udo, **Seidl** Klaus, **Siemon** Marén, **Smolenová** Eva, **Sprick** Martin, **Spring** Karsten, **Thiel** Lena, **Traub** Otto, **Volknandt** Walter, **Von der Ahe** Dietmar, **Wacker-Schröder** Irene, **Walch-Solimena** Christiane, **Waldvogel** Horst, **Wolters** Hanno, **Zimmer** Klaus-Peter.

Beitrittserklärung/Adressenänderung

Beitrittserklärung

Hiermit erkläre ich mit sofortiger Wirkung meinen Beitritt zur Deutschen Gesellschaft für Zellbiologie. Der Jahresbeitrag in Höhe von EUR 52,00 für Vollmitglieder, EUR 35,00 für Doppelmitglieder in DGZ & GBM und EUR 18,00 für Studenten wird zu Beginn des Kalenderjahres erhoben, möglichst durch Lastschriftverfahren.

Name, Titel: .....

Vorname: .....

Univ., Firma: .....

Inst., Bereich: .....

.....

Straße, Postf.: .....

PLZ, Stadt: .....

E-mail: .....

☐ Vollmitglied ☐ Doppelmitglied ☐ Student

Ermächtigung

zum Einzug von Forderungen mit Lastschrift

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

☐ EUR 52,00 (Jahresbeitrag Vollmitglied)
☐ EUR 35,00 (Jahresbeitrag Doppelmitglied)
☐ EUR 18,00 (Jahresbeitrag Student)

Bankinstitut: .....

Kto.-Nr.: .....

BLZ: .....

Kontoinhaber: .....
(Name bitte in Druckbuchstaben oder Stempel)

.....
Datum, Unterschrift

Ich wurde geworben von (DGZ-Mitglied): .....

Gewünschtes Präsent des Werbers:

☐ *T-SHIRT (nur in Größe L)*
☐ *KAPPE Farbe:* ☐ *hellgrau oder* ☐ *schwarz*
☐ *ANSTECKNADEL*

Änderung der Mitgliedsdaten

Falls Ihre Adresse oder Teile Ihrer Adresse sich geändert haben, wenn Sie Mitglied in der GBM (Doppelmitglied) oder vom Studenten zum Vollmitglied geworden sind, bitten wir um Information an das DGZ-Sekretariat.

Name, Titel: .....

Vorname: .....

Univ., Firma: .....

Inst., Bereich: .....

.....

Straße, Postf.: .....

PLZ, Ort: .....

E-mail: .....

☐ Vollmitglied ☐ Doppelmitglied ☐ Student

Änderung der Bankverbindung

Falls Sie eine Einzugsermächtigung für Ihren Mitgliedsbeitrag erteilt haben und sich Ihre Bankverbindung geändert hat, teilen Sie uns diese bitte <u>umgehend</u> mit. Es erspart uns Mehrarbeit und Kosten, wenn Sie uns rechtzeitig informieren.

Bankinstitut: .....

Konto-Nr.: .....

BLZ: .....

Kontoinhaber: .....
(Name bitte in Druckbuchstaben oder Stempel)

<u>Kontakt / Sekretariat der DGZ:</u>

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

.....
Datum, Unterschrift