Institute of Molecular Biology

ANNUAL REPORT 2012


Molekulare
gefördert von der Boehringe

TABLE OF CONTENTS

Foreword 2

Research Groups

Christoph Cremer 4
René Ketting 6
Stefan Legewie 8
Christof Niehrs 10
George Reid 12
Holger Richly 14
Jean-Yves Roignant 16
Natalia Soshnikova 18
Vijay Tiwari 20
Helle Ulrich 22

Core Facilities

Introduction 24
Bioinformatics Core Facility 26
Cytometry Core Facility 27
Genomics Core Facility 28
Histology Core Facility 29
Microscopy Core Facility 30

Facts and Figures

Staff 32
External Funding 33
External Speakers 34
Training Activities 36
Publications 37
Research and Training Environment 40
Scientific Advisory Board 42
Contact 43
Imprint 44

FORE

"We shall not cease from exploration and the end of all our exploring will be to arrive where we started and know the place for the first time."

T. S. Elliot

The basic scientific questions addressed in IMB's second Annual Report are still the same as they were a century ago: How does an organism develop and how is its physiological homeostasis regulated? Science sets out with ever new expeditions to return with a bounty of new answers to the old questions. In this respect, IMB is a research vessel navigating between the fields of epigenetics, development, DNA repair and their unexplored interfaces.

The year 2012 was an eventful one for IMB and included the appointment of René Ketting (Hubrecht Laboratory, The Netherlands) and Helle Ulrich (Clare Hall Laboratories, United Kingdom) as Research Directors. René brings research into RNA biology during the gonadogenesis of zebrafish and *C. elegans* to IMB, while Helle is an internationally renowned expert on the regulation of DNA repair who has just been awarded a prestigious ERC senior investigator grant. They complete the list of IMB's senior scientists and bring exciting research themes to the institute. Both also hold a joint appointment with the Johannes Gutenberg University, Faculty of Biology. In January 2012 Jean-Yves Roignant, Natalia Soshnikova and Vijay Tiwari started their new research groups and quickly picked up speed, while in July Stefan Legewie acquired a € 1.14M grant from the BMBF to model biological networks.

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

WORD

A call for IMB proteomics Group Leaders was also successful and we hired Falk Butter, who will join us in 2013 after a successful postdoc with Matthias Mann. Falk uses mass spectrometry to identify and analyse DNA binding proteins.

A symposium celebrating the scientific inauguration of IMB took place in March and was graced by a cast of highly renowned speakers who discussed topics spanning from DNA repair to reprogramming. Among the speakers was Sir John Gurdon, who went on to win the Nobel Prize in Medicine later in the year. The symposium drew a large audience and saw the IMB auditorium packed. IMB also plans to run an annual scientific conference. The first of these took place in October and was the first ever meeting on the topic of DNA demethylation; a topic which constitutes one of the most recent frontiers of epigenetics. The lively conference featured pioneers of the field, was of a high calibre and set the standards for future IMB conferences. Besides the conference, IMB also ran regular research seminars with invited speakers, many of whom were curious to visit the institute.

IMB Group Leaders recruit their PhD students via our newly established International PhD Programme (IPP) that focuses on research regarding the *Dynamics of Gene Regulation, Epigenetics and DNA Damage Response*. This IPP, which is generously funded by the Boehringer Ingelheim Foundation, aims to attract top students to the Mainz campus. Group Leaders of the IPP are those of IMB as well as 14 members from the Johannes Gutenberg University, the University Medical Center, and the Max Planck Institute for Polymer Research. The IPP is therefore an important instrument for integration across the research campus. In fact, after only one and half years of existence, it is fair to say that IMB is already fully rooted on campus and through a variety of research initiatives and collaborations has become an important partner in the local biomedical research community.

In spring and autumn calls were made for applications to the IPP and more than half of the 18 students recruited were non-German, highlighting the international aspect of the programme. To support the IPP, we organized an International Summer School (ISS), during which university students spent six weeks at IMB. While here, they attended training courses and lectures, and undertook a small research project within the laboratory of one of the IPP Group Leaders. All of these scientific events where smoothly organized by Ralf Dahm and his team.

During a two day Group Leader retreat IMB faculty met to share research plans and discuss the institute's development.

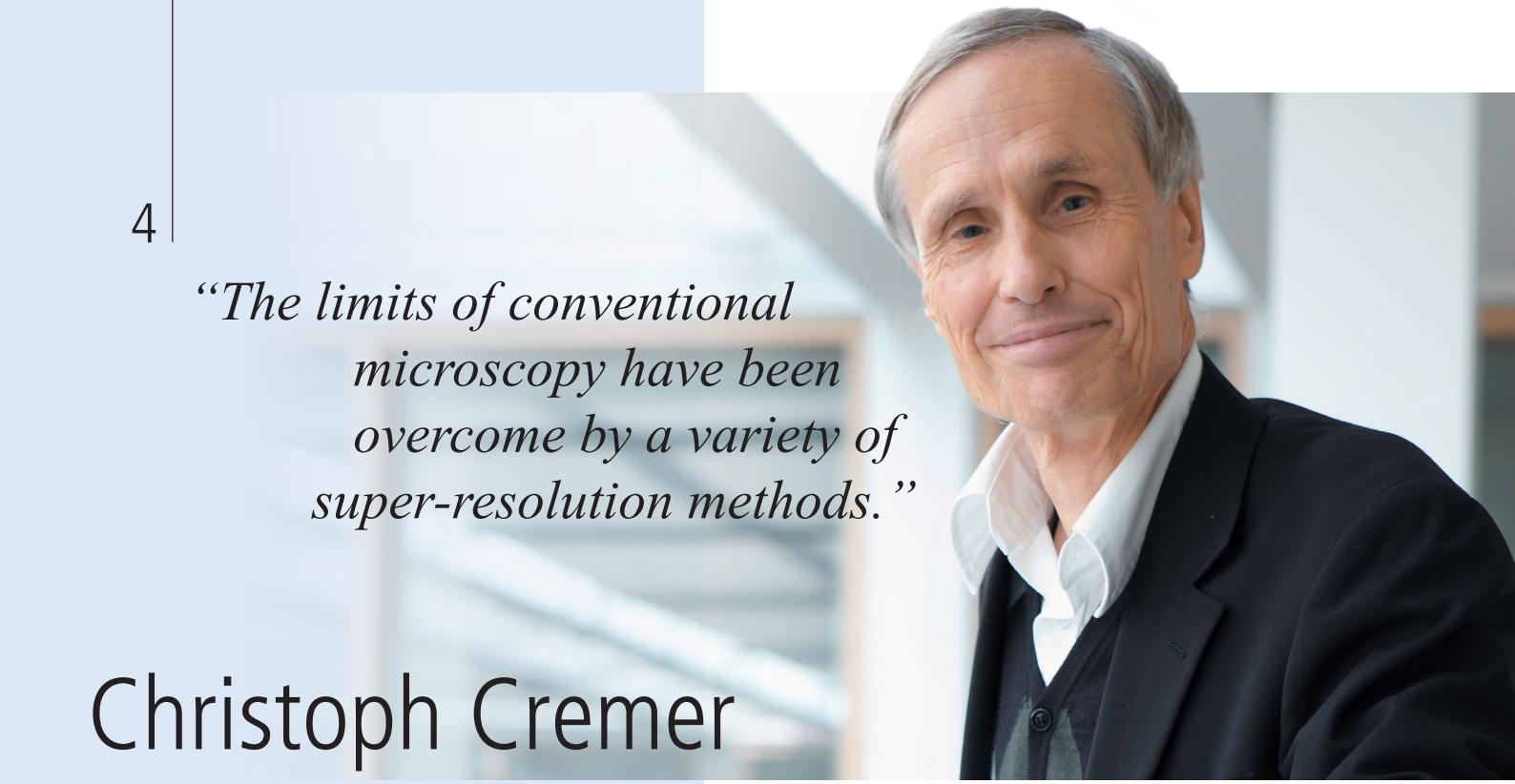
The IMB Core Facilities, supervised by Bernd Korn, were further consolidated with additional personnel and equipment acquisitions. The Core Facilities play an important role in enabling IMB scientists to get access to the latest equipment, supervised by technology experts. Notably, the large aquaria to house zebrafish and *Xenopus* frogs as model organisms were installed and the first inhabitants moved in. IMB Core Facilities are also used by colleagues from the University campus and as such they further contribute to the integration of the institute into the local research landscape.

Another notable event was the completion of the second phase of IMB's construction, and the resulting movement of researchers into the south wing. There were many hiccups which had to be dealt with in order to achieve this, but they were kept in check by the tireless efforts of Bernd Korn from the technical side and Stephanie Oehl from the legal side. The additional space will be required as the number of IMB employees hits the "100 mark" and continues to grow, mostly with scientific personnel and students.

We have great expectations for 2013. Helle Ulrich and Falk Butter will move in with their groups, a call for Group Leader recruitment has been advertised for further research expansion, and with the University Faculty of Biology we are planning the joint appointment of a Bioinformatics Professorship. In October, we will hold the second in our series of IMB conferences entitled *Chromatin Dynamics & Stem Cells*.

To continue with our quick start, we again relied on the effective team of administrators, managers, Core Facility experts, technicians, and not least a large number of colleagues from Johannes Gutenberg University Mainz. We are very grateful to all of them for their support. We are also indebted to Walter Kröll and Jörg Michaelis for their great engagement during the recruitment of IMB's Research Directors. We gratefully acknowledge our main sponsor, the Boehringer Ingelheim Foundation, and their commitment and genuine interest in IMB. It has again been a very good year for IMB and the institute has set sail for an exciting research voyage on the ocean of molecular life sciences.

CHRISTOF NIEHRS
Founding Director



"The limits of conventional microscopy have been overcome by a variety of super-resolution methods."

Christoph Cremer

EDUCATION

1970	Diploma in Physics, LMU, Munich
1976	PhD in Biophysics and Genetics, University of Freiburg
1983	Habilitation, University of Freiburg

POSITIONS HELD

1970 - 1983	Staff Scientist, Institute of Human Genetics, University of Freiburg
1983 - 1999	Managing/Deputy Director, Institute of Applied Physics I, University of Heidelberg
1983 - 2011	Professor of Applied Optics & Information Processing, University of Heidelberg
2005 - 2007	Deputy Director, Kirchhoff-Institute of Physics, University of Heidelberg
Since 2005	Director Biophysics of Genome Structure, Institute for Pharmacy and Molecular Biotechnology, University of Heidelberg
Since 2011	Group Leader, Institute of Molecular Biology (IMB), Mainz

GROUP MEMBERS

Udo Birk / Postdoc; since 02/2012

RESEARCH OVERVIEW

Until recently, the limits of conventional light microscopy (optical resolution about 200 nm in the object plane, 600 nm along the optical axis) have been a fundamental obstacle to the study of chromatin nanostructure and its importance to molecular epigenetics. This bottleneck has been overcome by a variety of super-resolution ("nanoscopy") methods. In 2012, we established various nanoscopy systems at IMB. Presently, these comprise a commercial laser scanning 4Pi microscope (Leica) and two devices (our own developments) for spectrally assigned localization microscopy (SALM). We have integrated the SALM technique of spectral precision distance microscopy (SPDM) into laterally structured interference illumination microscopy (SIIM). The combination of 'blinking' with other spectral signatures based on differences in the absorption/emission spectrum and the use of standard fluorophors and preparation conditions, makes the new SPDM techniques developed a significant improvement over the original PALM/STORM approaches.

RESEARCH HIGHLIGHTS

1) Spatial distribution of various histone types

Nuclear structure applications studied in 2012 in the Cremer-group include the spatial distribution of various histone types in normal/cancer cells before and after ionizing radiation exposure (collaborations: Prof. G. Dollinger, Munich, Prof. M. Hausmann and Prof. P. Huber, Heidelberg). Figure 1 shows an example for the spatial distribution of H2B – GFP and H4K20 obtained with the "Vertico-SPDM" at IMB. While in the conventional epifluorescence image, single molecule resolution was not possible, the two-colour SPDM image shows thousands of individually identified H2B and H4K20 molecules resolved with a mean localization precision of 18 nm.

For the first time, a single molecule resolution of individual γH2AX cluster tracks induced by individual accelerated heavy ions was obtained in nuclei of a glioblastoma cell line by three-colour SPDM-SIIM imaging.

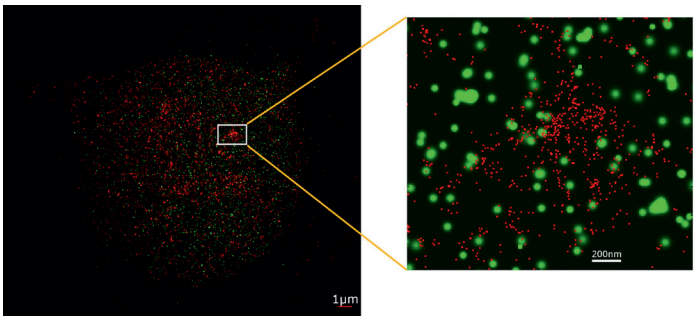


Figure 1. Two-colour SPDM of spatial "Histone Code" distribution in a human cell nucleus. Green: H2B – GFP; Red: H4K20 – Alexa-568 (immune-labelling); Collaboration M. Hausmann/KIP. Right magnified insert: The histone distribution is shown after blurring with the individual localization accuracy (frequently containing multiple GFP signals), while for H4K20, the Alexa-568 signal positions are shown without blurring.

2) Transcription and splicing

In collaboration with Prof. J. Gall (Baltimore), we have examined transcription loops on lampbrush chromosomes of the newt Notophthalmus using "Vertico-SPDM" (Kaufmann *et al.* 2012). Because of the favourable, essentially two-dimensional, morphology of these loops, an average optical resolution in the x–y plane of about 50 nm (~ 1/10th of the wavelength used) was achieved by two-colour SPDM. We analysed the distribution of the multifunctional RNA-binding protein CELF1 on specific loops. The CELF1 distribution observed is consistent with a model in which individual transcripts are tightly folded and hence closely packed against the loop axis (Figure 2).

3) Spatial distribution of SC35 splicing factors

Another research highlight (collaboration: Prof. T. Cremer, Munich) was the first two-colour SALM demonstration of the distribution of individual SC35 splicing proteins with respect to individual nucleosomes with a three-dimensional single molecule resolution in the few tens of nanometer range. Using a special 3D-SALM approach based on the astigmatic distortion of single molecule derived diffraction patterns, a lightoptical sectioning down to ~ 30 nm in intact cell nuclei was realized (for comparison: conventional confocal laser scanning microscopy allows optical sectioning only down to several hundred nm).

4) SPDM of gene expression

In addition to analysis of the spatial distribution of individual nuclear proteins, SPDM allows us to study the expression of cellular proteins at the molecular resolution level. In collaboration with Dr J. Piontek (Berlin), we performed SPDM of Tight Junction (TJ) proteins that regulate the paracellular permeability of tissue barriers, expressed by stable transfection of HEK293 cells with the barrier-forming Cld3 or Cld5. For an individual TJ network, up to about 1 million single expressed Claudin molecules were counted with a mean localization accuracy of ~ 20 nm, yielding a mean structural resolution of ~ 50 nm (Kaufmann *et al.* 2012).

In collaboration with Prof. G. Fricker (Heidelberg), we used SPDM to investigate the expression and spatial distribution of P-glycoproteins (Pgp; also known as MDR1, ABCB1) in the luminal plasma membrane of brain capillary endothelial cells. For this, immortalized human cerebral microvascular endothelial cells (hCMEC/D3) were transduced with a Pgp-green fluorescent protein (GFP) fusion protein incorporated in a lentivirus-derived vector (Huber *et al.* 2012).

A further application in collaboration with Dr C. Ehrhardt (Münster) examined the feasibility of using SPDM to monitor the expression and spatial distribution of individual fluorescence labelled HGFR receptor proteins in PR8 virus infected cells (Q. Wang *et al.* in preparation).

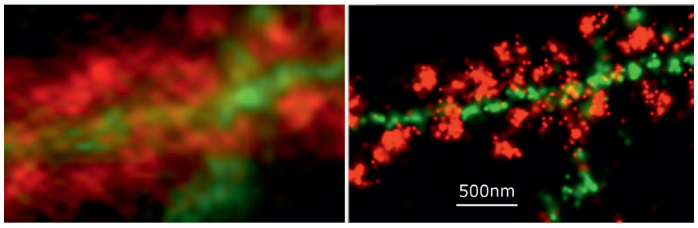


Figure 2: SPDM of a transcribed Lampbrush chromosome (LBC) loop and analysis of CELF1 distribution (detail). Green: Polymerase II; Red: CELF1. (Left) Conventional confocal microscopy. (Right) Two-colour SPDM. From Kaufmann *et al*. 2012.

FUTURE DIRECTIONS

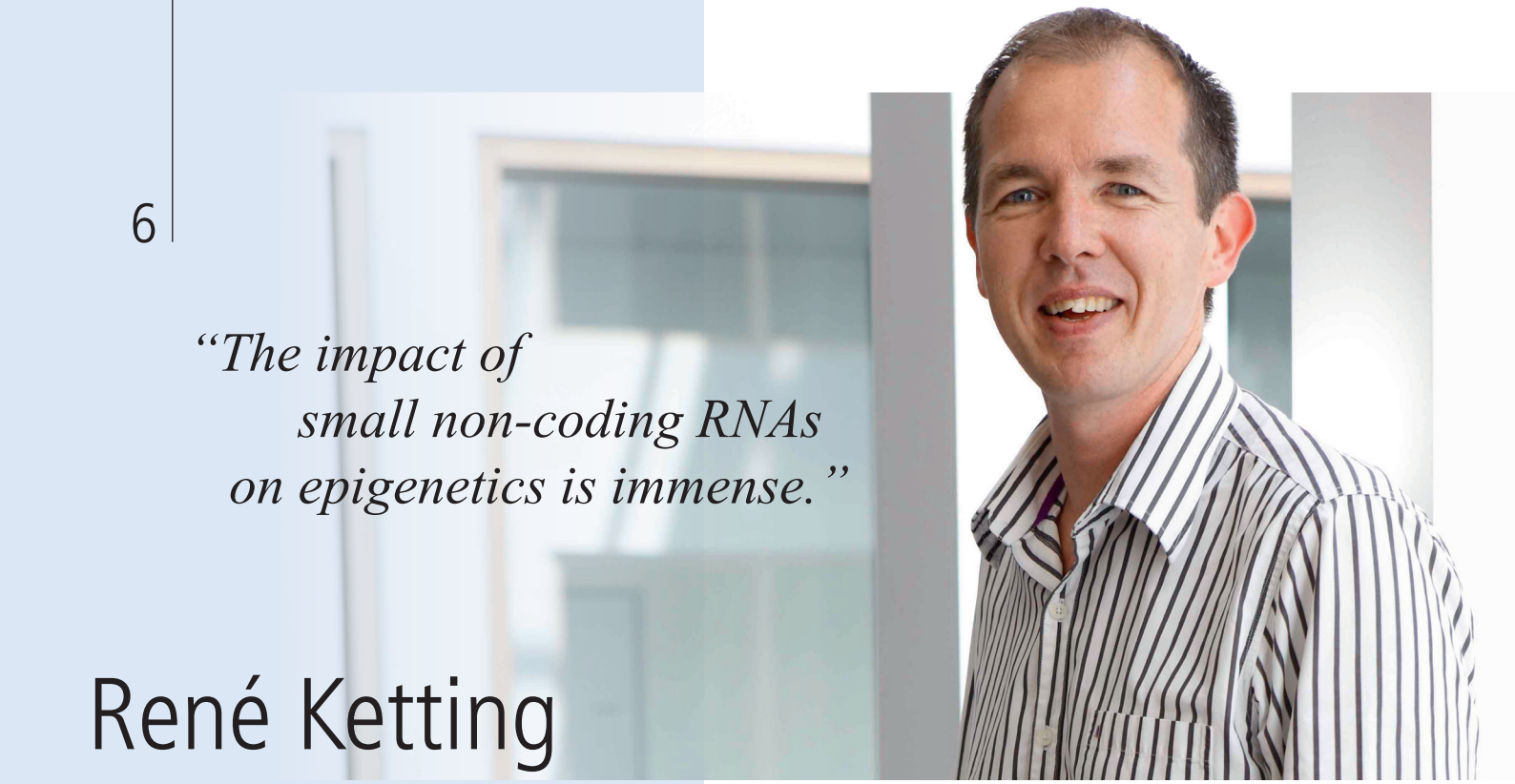
On the methodological side, it is planned to further consolidate the present super-resolution techniques and their application to chromatin nanostructure elucidation and other aspects of epigenetics and DNA repair. In particular, the 3D-SPDM system recently established at IMB will be further developed for three to four colour analysis and the "SPDM-Vertico" will be improved to allow high precision 3D-localization microsopy of single molecules (goal: a best 3D resolution limit of < 10 nm). On the application side, in addition to already ongoing collaborations, new projects are planned, especially with collegues from IMB (presently Prof. R. Ketting, Dr G. Reid, Dr H. Richly, Dr V. Tiwari) and Johannes Gutenberg University Mainz (Prof. T. Efferth, Prof. B. Lutz, Prof. G. Schoenhense, Prof. S. Strand, Prof. K. Wendt). Futhermore, in collaboration with Prof. H. Allgayer (Heidelberg), we shall apply SPDM to monitor the expression and spatial distribution of individual miRNAs in various types of cancer cell. A major longterm goal is to develop multi-colour super-resolution light microscopy methods to such a level of technical performance and resolution that in combination with new labelling tools, it will be possible to unravel the 3D folding of a chromatin fiber within chromosome territories/gene domains of intact nuclei. This information will then be correlated with epigenetic modifications, RNA and protein expression on the single cell/single molecule level.

SELECTED PUBLICATIONS

Kaufmann R, Müller P, Hildenbrand G, Hausmann M and Cremer C (2011). Analysis of Her2/neu membrane protein clusters in different types of breast cancer cells using localization microscopy. *J Microsc*, 242, 46-54.

Bohn M, Diesinger P, Kaufmann R, Weiland Y, Muller P, Gunkel M, von Ketteler A, Lemmer P, Hausmann M, Heermann DW and Cremer C (2010). Localization Microscopy Reveals Expression-Dependent Parameters of Chromatin Nanostructure. *Biophys J*, 99, 1358-1367.

Baddeley D, Batram C, Weiland Y, Cremer C and Birk UJ (2007). Nano-structure analysis using spatially modulated illumination microscopy. *Nat Protoc*, 2, 2640-2646.



"The impact of small non-coding RNAs on epigenetics is immense."

René Ketting

EDUCATION

1994	Masters in Chemistry, University of Leiden
2000	PhD in Molecular Biology, Netherlands Cancer Institute, Amsterdam

POSITIONS HELD

2000	Postdoctoral Researcher, Cold Spring Harbor Laboratories
2000 - 2004	Postdoctoral Researcher, Hubrecht Laboratory, Utrecht
2005 - 2012	Group Leader, Hubrecht Institute, Utrecht
2010 - 2013	Professor of Epigenetics in Development, Hubrecht Institute, Utrecht
Since 2012	Director, Institute of Molecular Biology (IMB), Mainz

GROUP MEMBERS

Bruno Albuquerque / PhD student; since 10/2012
Sabine Dominitzki / Technician; since 10/2012
Ricardo Rodrigues / PhD student; since 12/2012
Nadine Wittkopp / Postdoc; since 11/2011

RESEARCH OVERVIEW

The human genome encodes tens of thousands of genes and all these genes have to be properly regulated in order to sustain a healthy human being. In addition, our genome is home to a multitude of selfish elements that multiply themselves: In fact, these elements make up most of the DNA we carry in our cells. We study how the presence of these endogenous genomic parasites influences gene expression and how their mobility is controlled. We discovered that a process named RNA interference, or RNAi, plays an important role in this control. Our goals are to fully understand how RNAi affects the mobile elements in our genome and how this mechanism affects gene expression. We focus our efforts on the cells that propagate our genomes across generations, the germ cells, and on early embryogenesis. For this we make use of two model systems; the zebrafish and *C. elegans*.

RESEARCH HIGHLIGHTS

We have found that one of the small RNA pathways, the so-called Piwi pathway, can induce an epigenetic switch that can be stably remembered across generations. We identified this phenomenon in the nematode *C. elegans*. While analysing strains that are transgenic for a GFP transgene that is silenced by the Piwi pathway, we noticed that in a more or less stochastic manner, the transgene silencing becomes independent of the Piwi pathway, a state we named RNAe (RNA induced epigentically silenced). In other words, while in the normal state the transgene is activated upon disruption of Piwi, in the RNAe state the transgene remains silent upon loss of Piwi. Interestingly, the RNAe state is faithfully inherited across generations. We were able to establish that in response to the Piwi pathway, a secondary RNAi pathway is triggered that in turn modifies the chromatin status at the targeted locus. While the secondary RNAi pathway is required for the transgenerational inheritance, we showed it is not required for maintenance of the RNAe state within one generation. These results show that in animals extremely stable epigenetic states can be induced by small non-coding RNAs and that these states can even be inherited through the germline.

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

In addition, we have analysed how the small RNAs associated with the Piwi pathway, piRNAs, are inherited from one generation to the next. Using zebrafish as a model, we could show that genetically distant strains are characterized by quite distinct piRNA populations. Interestingly, progeny obtained from crosses between these strains show two types of piRNA inheritance. Firstly, piRNA abundance is largely set by the zygotic genome. Secondly, the ratio between sense and anti-sense piRNAs from a given element experiences significant maternal influence that lasts for at least two generations. These results imply that the maternally inherited piRNA populations are able to affect piRNA expression from the zygotic genome and thus that the experiences of the female germline can be epigenetically transmitted to the progeny.

Finally, we identified a protein that is required for the proper maternal inheritance of piRNAs. Interestingly, this same factor is also required for proper assembly of the germ plasm, a cytoplasmic region in the embryo that can induce germ cell fate in blastomeres. These results imply a mechanistic link between germ cell specification in the embryo and their protection from the harmful activities of mobile genetic elements.

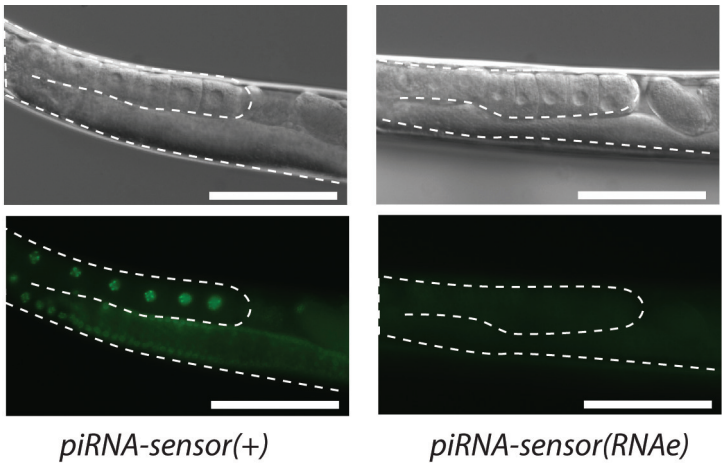


Figure 1. Nomarski (Top) and fluorescence (Bottom) images of *C. elegans* individuals carrying active (Left) and RNAe-silenced (Right) transgenes, coding for GFP fused to Histone 2B. The germlines are outlined in white. The RNAe state can be inherited virtually indefinitely and with extremely high penetrance.

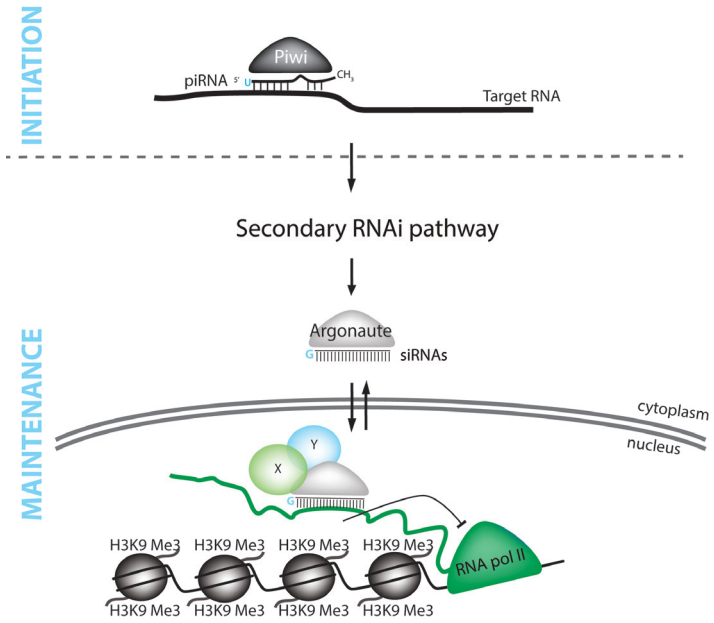


Figure 2. Model for initiation and maintenance of RNAe in *C. elegans*.

FUTURE DIRECTIONS

The finding that a small RNA pathway can initiate such a stable form of epigentic gene silencing is intriguing and prompts us to investigate this further. Through novel genetic screens in *C. elegans* we aim to identify the links between small RNA pathways and chromatin, and to decipher how these factors interact. Furthermore, we plan to establish a reporter system in zebrafish that will allow us to test the generality of our findings in a vertebrate model. In addition, we aim to develop tools that will allow us to follow small RNA pathway activity in real time in zebrafish embryos.

Finally, since epigenetic effects in germ cells clearly affect the epigenetic state of the early embryo, we will extend our efforts and start to study chromatin dynamics during early development. We will do this by investigating both DNA methylation and histone marks during the early embryonic stages of zebrafish development.

SELECTED PUBLICATIONS

Huang H, Houwing S, Kaaij LJT, Meppeling A, Redl S, Gauci S, Vos H, Draper BW, Moens C, Burgering BM, Ladurner P, Krijgsveld J, Berezikov E and Ketting RF (2011). Tdrd1 acts as a molecular scaffold for Piwi proteins and piRNA targets in zebrafish. *EMBO J*, 30, 3298-3308.

van Wolfswinkel JC, Claycomb JM, Batista PJ, Mello CC, Berezikov E and Ketting RF (2009). CDE-1 affects chromosome segregation through uridylation of CSR-1-bound siRNAs. *Cell*, 139, 135-148.

Houwing S, Kamminga LM, Berezikov E, Cronembold D, Girard A, van den Elst H, Filippov DV, Blaser H, Raz E, Moens CB, Plasterk RH, Hannon GJ, Draper BW and Ketting RF (2007). A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in zebrafish. *Cell*, 129, 69-82.

"New therapeutic approaches for cancer must take network structures into account."

Stefan Legewie

EDUCATION

2004	Diploma in Biochemistry, University of Witten/Herdecke
2008	PhD in Biophysics, Humboldt University Berlin

POSITIONS HELD

2008 - 2009	Postdoctoral Researcher, Institute for Theoretical Biology, Humboldt University Berlin
2009 - 2010	Group Leader 'Theoretical Systems Biology', Department of Theoretical Bioinformatics, German Cancer Research Center (DKFZ), Heidelberg
Since 2010	Group Leader, Institute of Molecular Biology (IMB), Mainz

GROUP MEMBERS

Stephan Baumgärtner / PhD student; since 11/2011
Bhashar Gosh / Visiting Postdoc; 12/2012 - 02/2013
Lu Huang / Postdoc; since 10/2012
Matthias Jeschke / Postdoc; since 07/2011
Monika Kuban / Research Assistant; since 10/2012
Tamara Milhaljev / Postdoc; since 11/2010
Uddipan Sarma / Postdoc; since 08/2012

RESEARCH OVERVIEW

Our group employs mathematical modelling to gain insights into the dynamics of biological networks. Data-based models are developed in close collaboration with experimental partners, and model predictions are verified using wet lab experiments. Our research focusses on cell-to-cell variability in cellular signal transduction and on quantitative modelling of gene expression responses.

RESEARCH HIGHLIGHTS

Modelling gene regulatory networks

One of the major focuses of our group is the quantitative modelling of gene regulation. We use various modelling approaches to describe different aspects of this process. Network models taking into account the wiring of many genes are derived based on large-scale datasets. We also study small genetic modules, and use models to describe mechanistic details at the single promoter level.

Gene expression responses to external stimulation are coordinated by complex networks of transcription factors. The wiring and the dynamics of such networks are incompletely understood. We characterized a medium-scale transcription factor response that mediates oncogenic transformation upon overexpression of oncogenic Ras (Stelniec-Klotz *et al.* 2012). The network dynamics were analysed by systematically perturbing transcription factor levels, and subsequently measuring gene expression responses. A mathematical model was applied to distinguish direct and indirect perturbation effects. As a result, regulatory interactions within the network could be reconstructed from the data and the wiring of the network controlling tumour growth clarified. The model-predicted network structure was validated experimentally using double perturbation experiments as well as phenotypic analyses. Contrary to current assumptions, the results show that no superordinate transcription factor exists that controls the activity of other factors as a master regulator. Instead, two hierarchical groups of interacting factors exist. Each of them activates gene sets needed for the growth and cancer-specific properties of the cells. The results indicate that new therapeutic approaches against tumours must target multiple rather than single factors and consider network structures.

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

Genes are often subject to complex combinatorial control by multiple transcription factors. Mathematical modelling approaches at the single-promoter level are required to quantitatively understand the dynamics of signal integration. Hepcidin, the central regulator of systemic iron homeostasis, is transcriptionally controlled by BMP/SMAD and IL6/STAT signalling cascades. Deregulation of either signalling pathway alters hepcidin expression, thereby causing iron storage diseases such as haemochromatosis and anaemia of inflammation. We quantitatively analysed how BMP and IL6 signal co-ordinately to control hepcidin expression in human hepatoma (HuH7) cells. We measured reporter gene expression and transcription factor phosphorylation under co-stimulation conditions, perturbed the promoter by systematic mutagenesis and applied mathematical modelling. Our results revealed that hepcidin cross-regulation primarily arises at the level of combinatorial SMAD/STAT transcription factor binding to the promoter, whereas signalling crosstalk plays only a minor role. We found that hepcidin expression responds with high sensitivity towards BMP stimulation owing to the simultaneous presence of two BMP-responsive elements in the promoter. Using mathematical modelling, we demonstrated that such a steep promoter response optimises the performance of the iron/BMP/hepcidin negative feedback loop controlling systemic iron homeostasis. These results reveal that the hepcidin promoter loses the ability to sense iron blood level changes in disease conditions like anaemia of inflammation, and that this leads to a disturbance of systemic iron homeostasis.

Gene expression is frequently controlled at the post-transcriptional level. We recently studied post-transcriptional regulation by small RNAs, and found that they are particularly efficient in robustly co-ordinating the expression of multiple target mRNAs (Schmiedel *et al.* 2012).

Control of cell-to-cell variability in signalling

Cellular signalling networks must function reliably despite noise from intracellular events and fluctuating environments. Conversely, signalling systems may also exploit noise to ensure that only a fraction of the cell population enters a new fate. It is thus important to understand how the heterogeneity of cellular signalling can be modulated. Molecular noise arises from stochastic dynamics of signal transduction processes ("intrinsic noise") or from cell-to-cell variability in the copy number of signalling proteins ("extrinsic noise"). Intrinsic fluctuations in signal transduction typically occur on a time scale of minutes, and are thus too fast to affect downstream cell fate decisions. Our research thus focusses on cell-to-cell variability due to noise in signalling protein concentrations.

In cooperation with the Niehrs group, we previously showed that negative feedback loops in the BMP signalling cascade expand the dynamic range of the system and at the same time suppress fluctuations (Paulsen *et al.* 2011; Blüthgen *et al.* 2012). We confirmed the robustness-promoting role of this feedback *in vivo* by showing that BMP-dependent morphological features of *Xenopus* embryos are significantly more variable when the feedback loops were perturbed. In our current research efforts we are analysing how variability can be modulated in more complex, multi-step signalling cascades like the mammalian and yeast MAPK pathways (Blüthgen *et al.* 2012; manuscript in preparation). We are applying this theory to interpret live-cell imaging data of yeast MAPK signalling networks.

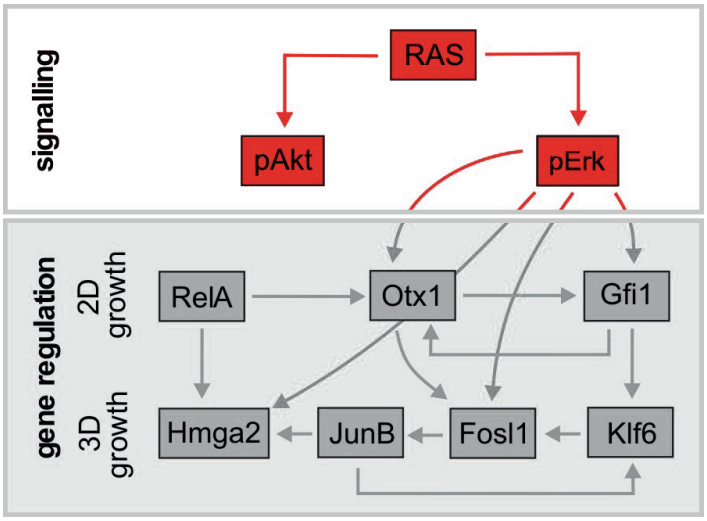


Figure 1. Transcription factor network that mediates oncogenic transformation by a constitutively active RAS mutant. RAS triggers the activation of AKT and ERK signalling pathways which in turn control the gene regulation network transcriptionally and post-transcriptionally (only a subset of regulatory interactions is shown). The transcription factor network decomposes into two hierarchical layers, each of which controls different aspects of oncogenic transformation.

FUTURE DIRECTIONS

We recently extended our gene expression analyses at the single promoter level to the temporal dynamics of transcription in collaboration with the Reid group. Specifically, we are interested in how transcriptional bursts at the single-cell level give rise to population-level cycling dynamics, and how these phenomena relate to the transcriptional readout.

SELECTED PUBLICATIONS

Schmiedel J, Axmann I and Legewie S (2012). Multi-target regulation by small RNAs synchronizes gene expression thresholds and may enhance ultrasensitive behavior. *PLoS ONE*, 7, e42296.

Stelniec I*, Legewie S*, Tchernitsa O, Bobbe S, Sers C, Herzel H, Blüthgen N and Schäfer R (2012). Reverse engineering a hierarchical regulatory network downstream of oncogenic KRAS. *Mol Syst Biol*, 8, 601 (* joint first authors).

Paulsen M*, Legewie S*, Eils R, Karaulanov E and Niehrs C (2011). Negative feedback in the bone morphogenetic protein 4 (BMP4) synexpression group governs its dynamic signalling range and canalizes development. *Proc Natl Acad Sci USA*, 25, 10202-10207 (* joint first authors).

10

"Knock-out mice are always full of surprises. Gadd45g mutants led us to sex determination."

Christof Niehrs

EDUCATION

1985	Diploma in Biochemistry, Free University of Berlin
1990	PhD, EMBL, Heidelberg
1997	Habilitation, Faculty of Biology (Zoology), University of Heidelberg

POSITIONS HELD

1990 - 1993	Postdoctoral Fellow, University of California Los Angeles
Since 1994	Head of Division "Molecular Embryology", German Cancer Research Center (DKFZ), Heidelberg
2000	Professor of Molecular Embryology, DKFZ, Heidelberg
Since 2010	Professor, Faculty of Biology, University of Mainz Founding Director, Institute of Molecular Biology (IMB), Mainz

GROUP MEMBERS

Khelifa Arab / Postdoc; since 11/2011
Mathias Gierl / Postdoc; since 07/2011
Wolfram Gruhn / PhD student; since 09/2010
Dandan Han / Research Assistant; since 12/2012
Sabine Karl / Postdoc; since 07/2011
Manuel Leichsenring / PhD student; since 04/2011
Medhavi Mallick / PhD student; since 08/2011
Konstantina Marinoglou / Postdoc; since 09/2011
Bernadette Mekker / PhD student; since 07/2010
Michael Musheev / Postdoc; since 07/2011
Sandra Rölle / Technician; since 04/2011
Andrea Schäfer / Postdoc; since 09/2010
Lars Schomacher / Postdoc; since 07/2011
Dominik Sebastian / PhD student; since 07/2011
Ulrike Stapf / Technician; since 05/2011
Annika von Seggern / Technician; since 06/2011

RESEARCH OVERVIEW

In the DNA of many multicellular organisms, DNA methylation is a common epigenetic mark associated with gene silencing. DNA methylation is dynamic and can be reversed by enzymatic demethylation, a process which is still incompletely understood. DNA demethylation is a widespread phenomenon in both plants and animals that occurs during development, in adults and during the somatic cell reprogramming of pluripotency genes. We showed that Growth Arrest and DNA Damage 45a (Gadd45a) is a key player in active DNA demethylation and acts via DNA repair. The goal of our research is to analyse the mechanism of DNA demethylation as well as the role played by Gadd45 in development. We study these questions by employing the mouse model as a genetic system and by using molecular biological, biochemical and cell biological approaches.

RESEARCH HIGHLIGHTS

Role of Gadd45g in mouse sex determination

Gadd45a,b,g comprise a small family of stress-response proteins that mediate diverse cellular processes, including DNA repair, apoptosis, cell cycle arrest, and senescence. They also function in gene activation by promoting DNA demethylation as well as MAPK signalling. In DNA demethylation, Gadd45 recruits DNA repair factors to gene specific loci to initiate demethylation and gene activation. In MAPK signalling, Gadd45g binds and activates Map3k4 to promote phosphorylation and activation of the p38 and c-Jun N-terminal (Jnk) MAP kinases. We aim to understand the mechanism of action and biological roles of the Gadd45 gene family. In this context we analysed Gadd45g mutant mice and discovered that the gene plays a critical role in male embryonic sex determination.

We discovered that Gadd45g mutant mice display complete male-to-female sex-reversal (Figure 1). Gadd45g and the sex determination gene Sry have a strikingly similar expression pattern in the genital ridge, and they are co-expressed in gonadal somatic cells. The Sry transcription factor is well known to direct the initially bipotential gonadal anlage towards a male fate and suppresses the female

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

programme. Without normal Sry expression gonads develop as ovaries or ovotestes. Thus, a key to induce the male programme is that the Sry expression level must exceed a specific threshold during this window of development, otherwise sex-reversal occurs. In Gadd45g mutants, Sry expression is delayed and reduced, and yet Sry seems to remain poised for expression, since its promoter is demethylated on schedule and is occupied by active histone marks. Furthermore, p38 MAPK signalling is impaired in Gadd45g mutants. Moreover, the transcription factor Gata4, which is required for Sry expression, binds to the Sry promoter *in vivo* in a MAPK dependent manner. The results reveal a signalling cascade, involving Gadd45g -> p38 MAPK -> Gata4 -> Sry, which regulates male sex determination (Figure 2).

Targeting of Gadd45 in DNA demethylation

Gadd45a mediated demethylation involves recruitment of the DNA repair machineries to excise 5mC. A common observation is that demethylation by Gadd45 is a highly selective process: Not only is it gene-specific, but within a given gene it typically affects distinct mCpGs, often in the promoter region. This specificity highlights a set of general, unresolved key questions in DNA demethylation: What determines the target site specificity of DNA demethylation? Is there a relationship between site specific DNA demethylation and the epigenetic landscape? What may be the cofactors involved in regulating targeting?

We identified a histone mark reader that interacts with Gadd45a to promote gene specific DNA demethylation. The reader binds Gadd45a and recruits it to sites marked by histone H3 lysine 4 trimethylation (H3K4me3) via its PHD domain. We found that reduced H3K4 methylation impairs recruitment of Gadd45a and the demethylation of target genes. Genome wide profiling revealed novel target genes of Gadd45a which are targeted via H3K4me3. The results indicate that histone methylation determines DNA demethylation.

Figure 1. Male-to-female sex-reversal in Gadd45g mutant mice. Anatomy of reproductive organs from adult Gadd45g mutant and control mice.

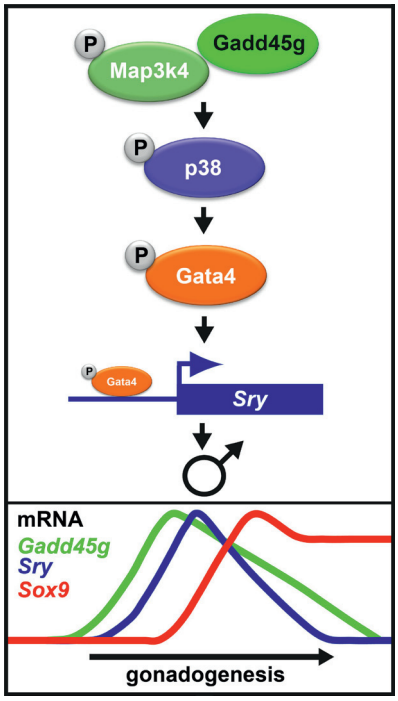


Figure 2. Model for Gadd45g action in sex determination. (Top) Gadd45g is specifically expressed in the gonadal somatic cells and activates Map3k4, leading to p38 and Gata4 phosphorylation. Gata4 directly binds and transactivates Sry. (Bottom) Schematic expression kinetics of key sex determination genes regulated by Gadd45g.

FUTURE DIRECTIONS

The targeting and specificity of Gadd45 action is still poorly understood. We will analyse the mechanism and biological role of Gadd45 targeting co-factors in mutant mice. Also, we described previously that Gadd45 is an RNA binding protein and so we will analyse which RNAs are physiologically bound to Gadd45 and determine what their roles are. This will determine if the role of Gadd45 bound RNAs is purely structural or if they play a specific role e.g. in the targeting of Gadd45 to demethylated DNA regions. Furthermore, we will analyse what significance ncRNAs may have in Gadd45 mediated DNA methylation, and using genome-wide approaches will identify the target genes of this process.

SELECTED PUBLICATIONS

Gierl MS, Gruhn WH, von Seggern A, Maltry N and Niehrs C (2012). GADD45G Functions in Male Sex Determination by Promoting p38 Signaling and Sry Expression. *Dev Cell*, 23, 1032-1042.

Niehrs C and Schaefer A (2012). Active DNA demethylation by Gadd45 and DNA repair. *Trends Cell Biol*, 22, 220-227.

Barreto G, Schaefer A, Marhold J, Stach D, Döderlein G, Maltry N, Swaminathan SK, Lyko F and Niehrs C (2007). Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. *Nature*, 445, 671-675.

"We have identified novel small molecules that provoke re-expression of silenced tumour suppressor loci."

George Reid

EDUCATION

1984	BSc in Biochemistry, University of Strathclyde
1988	PhD in Biochemistry, University of Strathclyde

POSITIONS HELD

1984 - 1985	Research Assistant, Turing Institute, Glasgow
1988 - 1993	Postdoctoral Researcher, MRC Retrovirus Research Laboratory, Glasgow
1994 - 1996	Senior Scientist, Pfizer Central Research, Sandwich
1996 - 1998	Postdoctoral Researcher, Beatson Institute for Cancer Research, Glasgow
1999 - 2004	Postdoctoral Researcher, EMBL, Heidelberg
2004 - 2010	Staff Scientist, EMBL, Heidelberg
Since 2006	Co-founder and Executive of Elara Pharmaceuticals
Since 2010	Group Leader, Institute of Molecular Biology (IMB), Mainz

GROUP MEMBERS

Iryna Charapitsa / Postdoc; since 07/2011
Christof Fritzsch / PhD student; since 09/2012
Wolf Gebhardt / Postdoc; since 08/2011
Ina Kirmes / PhD student; since 06/2012
Monika Kuban / Research Assistant; since 10/2012

RESEARCH OVERVIEW

The functional template of gene expression is chromatin. This provides multiple regulatory barriers that have to be overcome prior to the initiation of RNA synthesis. Dynamic methylation of DNA has been demonstrated by us to be an inherent process in the expression of tightly regulated genes. Using estrogen mediated gene expression as a model system, we are describing, on a genome-wide scale, changes on the chromatin template that determine the timing and output of transcriptional processes. Moreover, based on our detailed description of these dynamics we have devised a high-throughput screen to discover novel small molecules that perturb dynamic methylation. In addition to being useful biotools with which to further explore transcriptional regulation, they may define new therapeutic approaches for controlling tumour growth by provoking re-expression of silenced tumour suppressor loci.

RESEARCH HIGHLIGHTS

As a partner within a pan-european consortium of experimental and computational biologists (SYNERGY), we have generated global profiles of chromatin in estrogen dependent breast cancer cells following their synchronous release from estrogen starvation. This massively parallel sequence dataset includes time series of RNA expression, polymerase II, estrogen receptor alpha and variant histone H2AZ occupancy and DNA methylation profiles. Data have also been generated on covalent modifications to histone tails, in particular acetylation and methylation. By combining recent technical improvements in sequencing with state-of-the-art bioinformatics and computational biology with a tightly regulated pathway that signals to chromatin, these integrated and comprehensive datasets provide a wealth of information that promises to provide profound insight into eukaryotic gene regulation. Additionally, in collaboration with the Legewie group, we are using ChIP on synchronized cell populations, fluorescently labelled reporter arrays and transcription factors in single cells to further explore and model the dynamics of transcriptional cycling.

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

Our finding that the proximal promoter DNA of certain genes can undergo rapid DNA demethylation, mediated by base excision repair, suggested that it should be possible to discover small molecules that act to provoke DNA demethylation. We devised a phenotypic screen where fully methylated plasmid DNA was transfected into cells which were then incubated with test compounds to discover small molecules that could reactivate expression of a reporter gene. Two chemically distinct series of compounds were discovered that induce active demethylation. However, as a cell based screen was employed, the target of each series could not be inferred directly. Subsequent studies have made progress towards target identification. Genome-wide comparison of RNA profiles between treated and untreated cells predominantly show up-regulation of a significant number of genes, which are centred on the transcription factors *CREB1* and *EGR1*. Genome-wide analysis of methylated gDNA using the methyl binding domain (MBD) of MeCP2 discovered around 2700 regions that are significantly demethylated upon treatment with compound IMBMZ-703625 at 24 hours, with around 500 regions becoming methylated. Western blot analysis of selected active and repressive histone marks also shows significant changes in cells treated with either compound. Active marks (H3K4me3, H3K36me3, H3K14ac and panH3ac) are increased within the first hours after IMBMZ-744363 treatment. This is subsequently followed by an increase in the repressive mark H3K27me3. Whole genome ChIP-seq profiling of these histone marks demonstrates that changes in expression are concomitant with local changes at the promoter of responding genes (Figure 1).

CREB requires phosphorylation on serine 133 to achieve transcriptional activation and we found that IMBMZ-744363 induces prolonged phosphorylation of CREB at this residue. Furthermore, co-expression of dominant negative CREB, where ser133 is mutated to valine, abrogated induction of EGR1 promoter activity upon treatment with IMBMZ-744363. Moreover, pathway analysis demonstrates a clear induction and response in kinase signalling pathways, and methylation profiling of genomic DNA indicates that phosphorylation cascades can result in active demethylation of DNA containing CREB EGR1 modules. Collectively, these observations suggest that each compound series acts to stimulate a kinase cascade. Through screening a panel of kinase inhibitors that block the action of IMBMZ-744363, we have identified that the phosphinositol kinase pathway has a central role in the action of this compound. Our results are summarized in Figure 2.

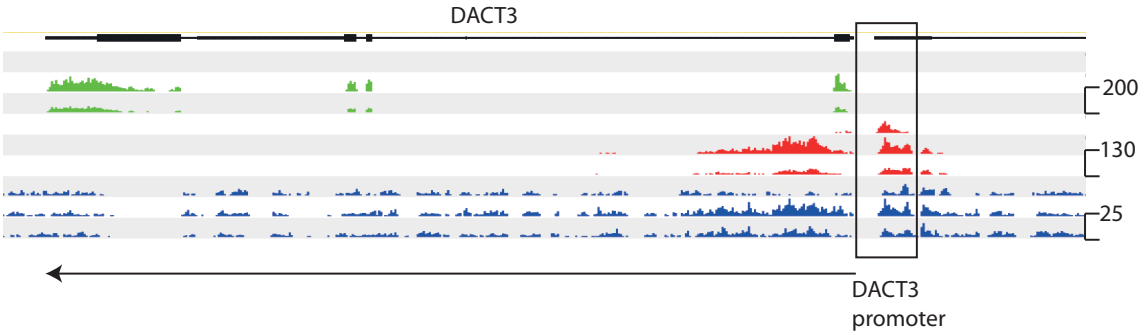


Figure 1. The induction of DACT3 expression (green) by IMBMZ-744363 correlates with a local increase in promoter H3K4 trimethylation (red) and of H3 acetylation (blue). Time points at 0, 6 and 24 hours post treatment are shown for each analysis.

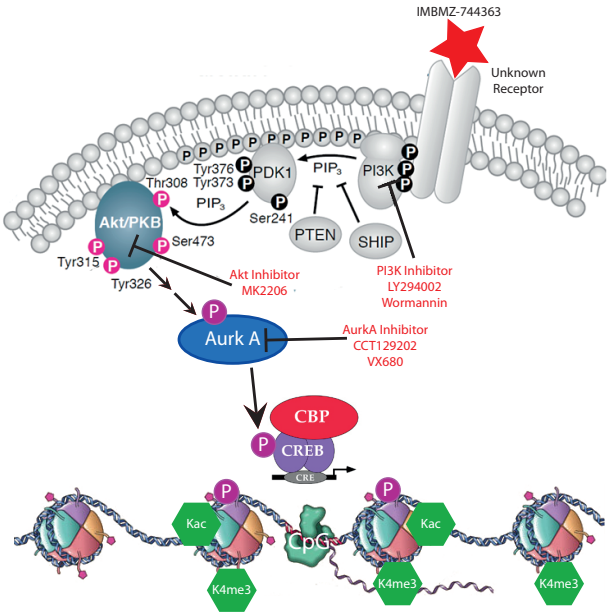


Figure 2. Working model of how IMBMZ-744363 signals to chromatin. The compound may bind to an unknown receptor that activates phosphoinositol (PI3K) signalling (inhibition of PI3K activity blocks the action of 744363), which in turn results in the essential activation of akt (akt inhibitors again block 744363). Activation of akt then results in the phosphorylation of aurora kinase A, which in turn phosphorylates the transcription factor CREB. Transcription factors targeted by this signalling cascade then effect local changes in chromatin, including post translation histone modifications and where present, local DNA demethylation.

FUTURE DIRECTIONS

Reflecting the potential therapeutic use of approaches that directly target the regulation of gene expression, we found that IMBMZ-403625 strongly inhibits the growth of the prostate carcinoma cell line CWR22 in nude mice. This encouraging result provides a starting point for the chemical optimization of this series in terms of activity and pharmacokinetic parameters, with the aim of providing a clinical candidate influencing the epigenetic landscape of tumours.

SELECTED PUBLICATIONS

Kangaspeska S, Stride B, Métivier R, Ibberson D, Carmouche RP, Benes V, Gannon F and Reid G (2008). Transient cyclical methylation of promoter DNA. *Nature*, 452, 112-115.

Métivier R, Gallais R, Tiffoche C, Le Péron C, Jurkowska R, Carmouche R, Barath P, Demay F, Reid G, Jeltsch A, Gannon F and Salbert G (2008). Cyclical DNA methylation of a transcriptionally active promoter. *Nature*, 452, 45-50.

"We aim to understand the elusive roles of ncRNA during DNA repair and ageing."

Holger Richly

EDUCATION

2000	Diploma in Biochemistry, Ruhr University, Bochum
2005	PhD in Biochemistry, Ludwig Maximilians University, Munich

POSITIONS HELD

2005 - 2011	Postdoctoral Researcher, Center for Genomic Regulation (CRG), Barcelona
Since 2011	Group Leader, Institute of Molecular Biology (IMB), Mainz

GROUP MEMBERS

Jonathan Byrne / PhD student; since 08/2011
Katerina Gracheva / PhD student; since 08/2011
Fabian Jung / Research Assistant; since 09/2012
Thaleia Papadopoulou / PhD student; since 10/2011
Stephanos Papaefstathiou / Research Technician; since 10/2011
Mieun Lee Theilen / Postdoc; 05/2012 – 12/2012
Thomas Wilhelm / PhD student; since 11/2011

RESEARCH OVERVIEW

Epigenetic modifications are a hallmark of gene regulation and thus govern a wide range of biological processes. We are interested in revealing the molecular interplay of chromatin-modifying proteins, chromatin-reading proteins and epigenetic marks to understand the molecular mechanisms underlying stem cell differentiation, ageing and DNA repair. It is intriguing how the environment impacts on epigenetic regulation and we therefore study how signalling pathways crosstalk with chromatin. Central to our research is the question of how epigenetic components are recruited to chromatin and how epigenetic marks are specifically read (Figure 1). Our research makes use of next-generation-sequencing, chromatin biochemistry, high-resolution-microscopy, *C. elegans* biology and genetics, and aims to understand the molecular orchestration of epigenetic events.

RESEARCH HIGHLIGHTS

Epigenetic mechanisms of stem cell differentiation

ncRNAs have been called the dark matter of the genome due to their abundance and very poorly understood function. We have focused on the role of long non-coding RNAs (lncRNAs) at the onset of embryonic stem cell differentiation. We have identified different ncRNA protein complexes that fulfil specific functions in reprogramming the epigenome during the differentiation of stem cells. We are currently characterizing these complexes and their physiological role in more detail to unravel genome-wide functions of lncRNAs. Our aim is to elucidate molecular mechanisms of gene activation that rely on the action of ncRNA protein complexes.

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

Impact of epigenetic components during UV-type DNA repair

Research on DNA repair has classically been devoted to investigating the protein complexes involved in repairing DNA lesions. Our research targets DNA repair in the context of chromatin and the epigenetic events that occur during UV-type DNA repair. Lately, we have elucidated a novel molecular mechanism demonstrating how DNA repair is carried out at chromatin. Our research illustrates a fine-tuned orchestration of epigenetic components that work in concert with specific DNA repair protein complexes. Our work also sheds light on how ubiquitylation signals govern the regulation of epigenetic components and repair factors. The main goal of this project is to understand the complex interplay of chromatin and DNA repair factors and to decode the meaning of differently linked ubiquitin-chains that decorate epigenetic components as well as DNA repair proteins.

Epigenetic determinants of ageing

Ageing is certainly one of the most interesting but less understood processes of multi-cellular organisms. Although in the last decade great advances have been made highlighting particular determinants of ageing, the contribution of epigenetic mechanisms is still elusive. Nematodes can be considered a model organism for ageing research and have been utilized in screenings to reveal genes that regulate the lifespan of the animal. We have set out to study the epigenetic landscape of *C. elegans* during ageing. The central questions in this project consist of which epigenetic components govern the ageing of nematodes and how these components are interpreted in the organism over their lifespan. We have therefore used several high-throughput next-generation techniques to get insights into the epigenetic landscape of organismal ageing.

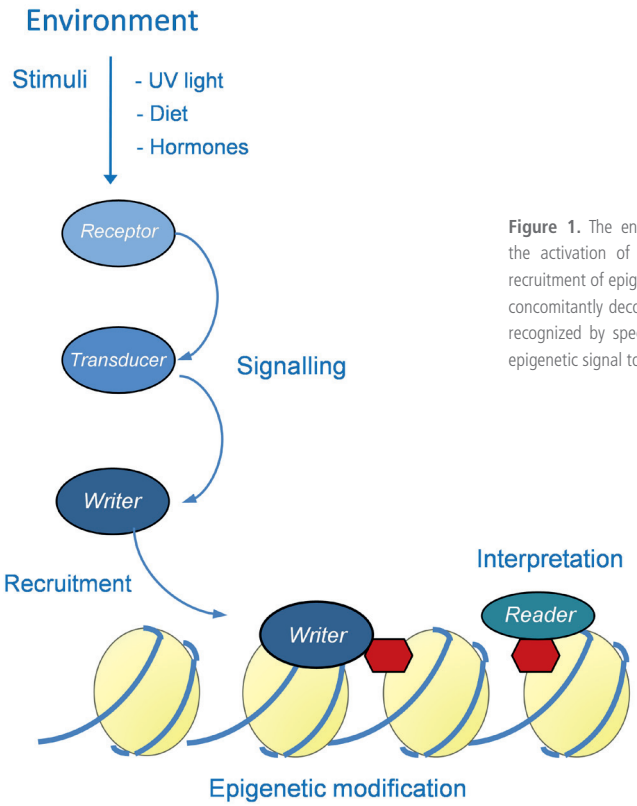


Figure 1. The environment provides stimuli that lead to the activation of signalling cascades and ultimately the recruitment of epigenetic components to chromatin, which is concomitantly decorated by epigenetic marks. The latter are recognized by specific proteins (readers) that transmit the epigenetic signal to downstream factors.

FUTURE DIRECTIONS

Further to our findings concerning the impact of ncRNAs in stem cell differentiation, we and others have discovered a role for RNA at chromatin during DNA repair. Also the process of ageing seems to be accompanied by specific functions of various classes of RNA. We will set out to understand the so far elusive roles of ncRNA species in these physiological processes.

SELECTED PUBLICATIONS

Richly H, Aloia L and Di Croce L (2011). Roles of the Polycomb group proteins in stem cells and cancer. *Cell Death & Disease*, 2, e204.

Richly H and Di Croce L (2011). The flip side of the coin. Role of ZRF1 and histone H2A ubiquitination in transcriptional activation. *Cell Cycle*, 10, 745-750.

Richly H, Rocha-Viegas L, Ribeiro JD, Demajo S, Gundem G, Lopez-Bigas N, Nakagawa T, Rospert S, Ito T and Di Croce L (2010). Transcriptional activation of polycomb-repressed genes by ZRF1. *Nature*, 468, 1124-1128.

"Drosophila provides the ideal model to study how epigenetic processes affect alternative splicing."

Jean-Yves Roignant

EDUCATION

1997	BSc in Molecular and Cellular Biology, Rennes
2003	PhD in Developmental Biology, Jacques Monod Institute, Paris

POSITIONS HELD

2003 - 2008	Postdoctoral Fellow, Skirball Institute of Biomolecular Medicine, New York University
2008 - 2011	Research Associate, Skirball Institute of Biomolecular Medicine, New York University
From 2012	Group Leader, Institute of Molecular Biology (IMB), Mainz

GROUP MEMBERS

Junaid Akhtar / Postdoc; since 02/2012
Christine Bauer / Technician; 01/2012 – 12/2012
Pia Deinhard / Intern; 05/2012 - 09/2012
Cristiana Lungu / PhD student; since 07/2012
Claire Mestdagh / PhD student; since 01/2012

RESEARCH OVERVIEW

In the human genome, the majority of genes are subjected to alternative splicing (AS), a process that increases the diversity of the transcriptome and ensures the complexity of the organism. Its mis-regulation is the leading cause of genetic diseases and is also associated with various tumours and cancer conditions. AS is controlled by the binding of splicing factors to *cis*-acting sequences within the pre-mRNA. The transcriptional machinery can also modulate splicing since both processes are tightly linked in the nucleus. Interestingly, recent lines of evidence support an important role for chromatin and associated factors in the coupling between transcription and AS. However, despite its potential significance, a comprehensive picture of this interplay is still lacking. The goal of our research is to understand the role and mechanisms of alternative splicing during cell fate decision and the relative contribution of epigenetic modifications to this process. To study these questions we are employing a wide range of genomic, molecular and cellular approaches using the *Drosophila* model as a genetic system.

RESEARCH HIGHLIGHTS

Role of chromatin in alternative splicing regulation during cell fate decision

AS is controlled by the binding of splicing factors to *cis*-acting sequences within the pre-mRNA. Since splice sites do not contain enough information to explain the complex regulation of AS it is clear that other factors must play important roles (Figure 1). Emerging evidence suggests that epigenetic mechanisms influence the splicing machinery in the recognition of small exons within much larger introns. Two main chromatin features, nucleosome positioning and histone marks, were shown to be linked to exon-intron architecture in many species. Exons containing weak splice sites or surrounded by larger introns have stronger nucleosome occupancy. This is indicative of a strong correlation between nucleosome occupancy and splicing,

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

but how nucleosomes influence splicing remains unclear. Another intriguing connection is the role of specific chromatin modifications. Several histone marks were found preferentially enriched on exons. However, their causal role in alternative splicing remains to be clearly demonstrated. Also, a clear genome-wide picture investigating this relationship *in vivo* is definitively lacking. Using a combination of genomic approaches such as RNA-seq and ChIP-seq experiments we are investigating in *Drosophila melanogaster* the contribution of histone marks to splicing events in several cell types. Computational analysis is used to establish initial correlations, which are subsequently validated *in vivo* using the power of *Drosophila* genetics.

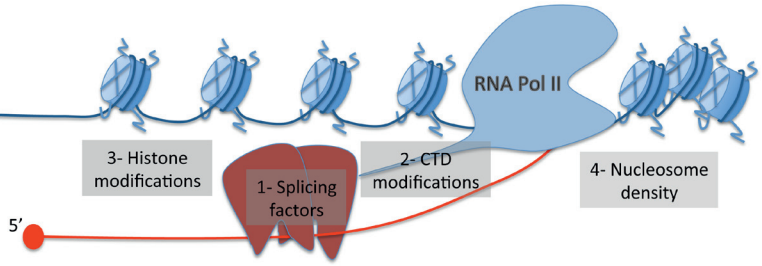


Figure 1. Co-transcriptional regulation of splicing integrates signals from chromatin, DNA and the nascent RNA. Such signals include splicing factors (1), CTD modification (2), histone tail modification (3) and chromatin compaction (4).

Heterochromatin and pre-mRNA splicing

Another major focus in our lab is to understand how heterochromatin influences pre-mRNA splicing. To tackle this question we are investigating the function of the exon junction complex (EJC) in *Drosophila*. The EJC is a highly conserved ribonucleoprotein complex which plays a major role in post-transcriptional regulation. It is loaded onto mRNA upstream of exon-exon junctions during splicing, and is transported to the cytoplasm together with its bound mRNAs. Here it influences the localization, translation and decay of the bound mRNAs. In addition to these cytoplasmic functions, we have discovered a novel important function for the nuclear EJC (pre-EJC) in regulating the splicing of a subset of primary transcripts, including *Mitogen-activated protein kinase* (MAPK). MAPK is expressed from constitutive heterochromatin and contains very large introns. We found in a genome-wide survey of pre-EJC-regulated genes that many genes sharing these features were overrepresented (Figure 2). These results support a model by which heterochromatic transcripts containing large introns require a distinct mechanism for their splicing. However, the mechanism underlying this specificity remains to be understood. We are currently testing which features of heterochromatin are providing this specificity. Introns of heterochromatic genes are rich in repetitive DNA composed of transposons, retrotransposons and satellite sequences. Insertion of these sequences within introns may interfere with splicing by mutating *cis*-regulatory splice sites or conversely by generating novel splice sites. Furthermore, heterochromatin genes are embedded in a very compact chromatin structure, which can also directly or indirectly influence pre-mRNA splicing. We are currently investigating both the contribution of *cis*-acting sequences and chromatin structure to the requirement of the pre-EJC for splicing heterochromatic transcripts. Additionally, a high-throughput screen using RNA interference in *Drosophila* cells to systematically knockdown the expression of nuclear proteins is being performed to specifically identify novel factors required for splicing large heterochromatic transcripts. The role of newly identified components will be confirmed *in vivo* using loss of function studies in *Drosophila*. We expect that these studies will reveal important insights into the interplay of chromatin and pre-mRNA splicing, and may also reveal novel splicing mechanisms.

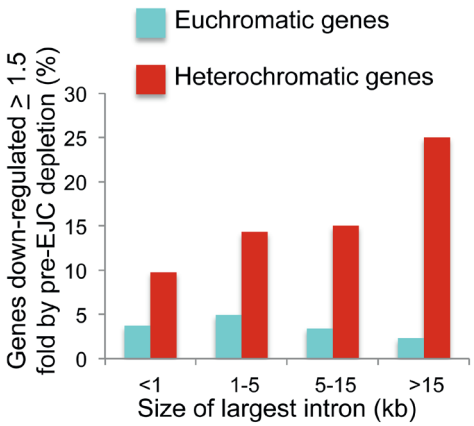


Figure 2. Genes downregulated >1.5-fold in absence of the pre-EJC, broken down by location in euchromatin or heterochromatin and by the size of their largest introns, are shown as a percentage of the total number of genes in each category.

FUTURE DIRECTIONS

The recent discovery that nucleosome density and specific patterns of histone modification may help delineate where the intron-exon junction lies and facilitate exon definition and alternative splicing raises many outstanding questions. What are the histone marks that play a direct and preponderant role in splicing? How are these marks deposited in the first place? Does splicing also influence chromatin structure? How do repressive marks affect splicing of heterochromatic transcripts? The answer to these questions will increase our understanding of the connection between chromatin and splicing and should lead to alternative therapeutic strategies which will involve the modulation of chromatin to treat human diseases that are a consequence of aberrant splicing.

SELECTED PUBLICATIONS

Roignant JY, Legent K, Janody F and Treisman JE (2010). The transcriptional cofactor Chip acts with LIM-homeodomain proteins to set the boundary of the eye field in Drosophila. *Development*, 137, 273-281.

Roignant JY and Treisman JE (2010). Exon junction complex subunits are required to splice Drosophila MAP kinase, a large heterochromatic gene. *Cell*, 143, 238-250.

Miura GI, Roignant JY, Wassef M and Treisman JE (2008). Myopic acts in the endocytic pathway to enhance signaling by the Drosophila EGF receptor. *Development*, 135, 1913-1922.

"We study the transcriptional differences between embryonic endoderm and adult intestinal stem cells."

Natalia Soshnikova

EDUCATION

1998	Diploma with honour in Molecular Biology, Novosibirsk State University
2004	PhD in Molecular Biology, Humboldt University, Berlin

POSITIONS HELD

2004 - 2011	Postdoctoral Researcher, Department of Zoology, University of Geneva
From 2012	Group Leader, Institute of Molecular Biology (IMB), Mainz

GROUP MEMBERS

Valerio del Vescovo / Postdoc (Marie Curie Fellowship); since 12/2012
Stefanie Grimm / Research Technician; since 01/2012
Elif Güney / Summer student (Erasmus); 06/2012-09/2012
Sabina Jakob / Student assistant; 06/2012-10/2012
Juri Kazakevych / PhD student; since 09/2012
Julia Koehn / PhD student; 01/2012-05/2012
Christina Krienke / Diploma student; since 04/2012
Eduarda Mazzagao Guerreiro / MSc student (Erasmus); since 09/2012

RESEARCH OVERVIEW

We are interested in the regulatory mechanisms underlying vertebrate pattern formation during embryogenesis and tissue homeostasis in adults. Our current focus is on the endodermal stem cells of the small intestine, and their mechanisms of maintenance, differentiation and alteration during the life of an animal. We initiated an ambitious programme aimed at dissecting these various aspects of endodermal stem cell biology using both mouse genetics and functional genomics, including ChIP-, MBD- and RNA-sequencing technologies. This combination of technological approaches provides us with a unique opportunity to solve questions regarding the relationship between chromatin structure and gene regulation; questions that have become increasingly important over the last few years.

RESEARCH HIGHLIGHTS

Molecular characterization of mouse endodermal stem cells

Stem cells are defined by two characteristics; the ability to self-renew and the capacity to give rise to one or more differentiated cell types. Small intestine epithelium homeostasis requires rapid and continuous regeneration and differentiation of specific cell types from precursor cells referred to as intestinal stem cells (ISCs). The generation of specific cell types from the ISCs is dictated by the combinatorial actions of diverse signals that regulate the expression of transcription factors and impose developmental restrictions on progenitor cells. In the adult small intestinal epithelium, differentiated progenitor cells of enterocytic, goblet and enteroendocrine lineages reside within villi, whereas proliferating stem cells are found within the intervilli/ crypt compartment. In contrast, the embryonic intestine appears as a primitive tube of polarized epithelium with cytologically identical endodermal cells until at least E14.5. At this time point a drastic remodelling process leads to villi formation. Little is known about the molecular changes that occur in the intestinal epithelium during embryogenesis before villus emergence begins. During the past year we have been particularly active in establishing specific technologies, including isolation of endodermal stem cells by Fluorescence Activated Cell Sorting, RNA-sequencing, MBD-sequencing and ChIP-sequencing, using small

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

amounts of primary cells from either mouse embryos or adults (Figure 1). We have defined the whole transcriptome of both embryonic endoderm from small intestine cells at E12.5 and of adult intestinal stem cells (Figure 2). Furthermore, we have learned about differences at the level of chromatin between embryonic and adult intestinal stem cells by applying MBD-sequencing and ChIP-sequencing technologies (Figure 2). Our studies have provided evidence that the embryonic endoderm and adult stem cells have different transcriptional programmes. Moreover, our histological studies using molecular markers have shown that there are different endodermal cell populations within the primitive small intestine as early as E12.5.

Functions of chromatin marks in the regulation of gene expression

Recent genome-wide studies mapping the distribution of modified histones revealed a complex epigenetic landscape on eukaryotic chromosomes. Distinct chromatin signatures were associated with potential enhancers, which represent the largest class of distal regulatory elements reported to date. According to the prevailing model, multiple enhancer elements, often located at a great distance from their target genes, activate genes by making direct physical contact with their promoters. Evidence for a requirement of chromatin modification at enhancers during this process is presently lacking. To define the biological significance of histone modifications covering enhancer sequences *in vivo* we have generated transgenic mice that allow us to target histone-modifying activities at enhancers using a recently introduced technology based on transcription activator-like effectors (TALEs). Phenotypic and molecular characterization of these mouse lines is in progress.

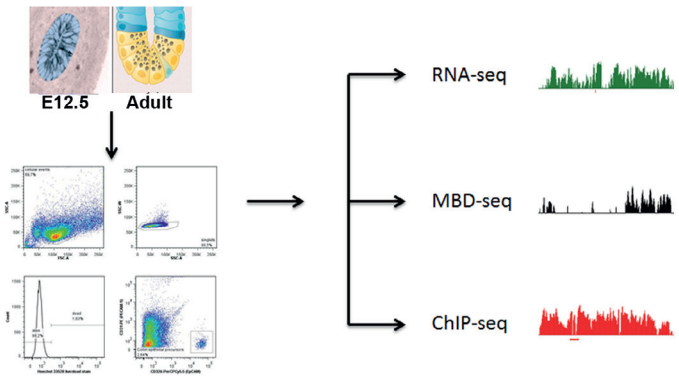


Figure 1. Schematic of the workflow aiming to address the dynamics of chromatin marks, including DNA methylation (MBD-seq) and histone modifications (ChIP-seq), accompanying changes in transcription (RNA-seq) during maintenance and differentiation of mouse embryonic (E12.5) or adult intestinal stem cells isolated by FACS.

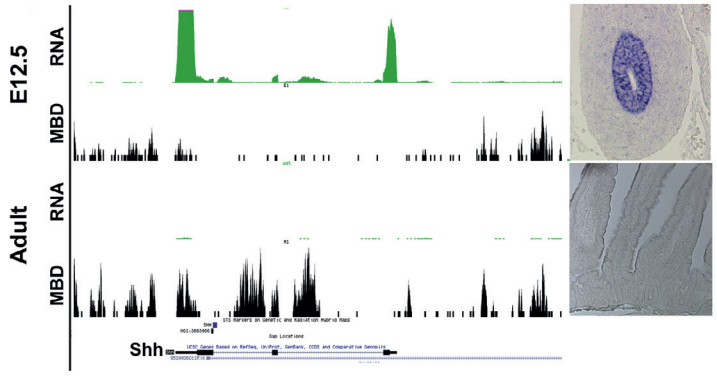


Figure 2. Repression of Sonic hedgehog (Shh) gene transcription in adult ISCs (RNA, green) correlates with an increase in DNA methylation over the gene body (MBD, black). The spatial distribution of Shh transcripts is assessed by *in situ* hybridization on tissue sections of mouse embryonic (E12.5) and adult small intestine (right panels).

FUTURE DIRECTIONS

We will further address the mechanistic basis of transcriptional differences between embryonic endoderm and adult intestinal stem cells. Moreover, we will characterize the novel cell populations identified within the embryonic endoderm at E12.5 using lineage tracing analysis. Our analysis of the transgenic mice will tell us how changes in histone marks over enhancer sequences affect the transcriptional status of the endogenous target genes. The results of our studies will be an important contribution to the fields of gene regulation, and chromatin, stem cell and developmental biology.

SELECTED PUBLICATIONS

Montavon T, Soshnikova N, Mascrez B, Joye E, Thevenet L, Splinter E, de Laat W, Spitz F, Duboule D (2011). A Regulatory Archipelago Controls Hox Genes Transcription in Digits. *Cell*, 147, 1132–1145.

Soshnikova N, Montavon T, Leleu M, Galjart N, Duboule D (2010). Functional analysis of CTCF during mammalian limb development. *Dev Cell*, 19, 819-830.

Soshnikova N and Duboule D (2009). Epigenetic temporal control of mouse Hox genes in vivo. *Science*, 324, 1320-1323.

"We study how cell-fates are specified during development and miss-specified in disease."

Vijay Tiwari

EDUCATION

2002	MSc in Molecular and Human Genetics, Banaras University, Varanasi
2006	PhD in Developmental Biology, Uppsala University

POSITIONS HELD

2006 - 2008	Postdoctoral Researcher, Johns Hopkins University School of Medicine, Baltimore
2008 - 2011	Postdoctoral Researcher, Friedrich Miescher Institute for Biomedical Research (FMI), Basel
Since 2011	Group Leader, Institute of Molecular Biology (IMB), Mainz

GROUP MEMBERS

Angela Garding / Postdoc; since 04/2012
Johannes Jung / Diploma student; since 07/2012
Federico Marini / PhD student; since 01/2012
Anke Salzer / Lab Manager; since 07/2012
Sandra Schick / PhD student; since 06/2012
Sudhir Thakurela / PhD student; since 01/2012

RESEARCH OVERVIEW

The research in my lab is aimed at achieving an integrated molecular and systems-level understanding of the mechanisms by which epigenetic machinery and regulatory factors contribute to the transcriptional reprogramming that defines cell-type identity during development, and how this communication is altered in diseases such as cancer. To investigate these questions, we employ a multidisciplinary approach combining cutting-edge epigenetics, genomics and proteomics together with computational biology tools in sophisticated and defined models of cellular differentiation and carcinogenesis. Our primary research interests include:

- Signalling to chromatin crosstalk during cellular differentiation
- Epigenetic regulation of neurogenesis
- Transcription factors and lineage-specification
- Epigenomics of cell-type specification
- Systems biology of gene regulatory networks

RESEARCH HIGHLIGHTS

A MAP kinase binds promoters and regulates transcription by directly modifying histones

Signalling mediates cellular responses to extracellular stimuli. A generally accepted concept in the field is that MAP kinases act via other downstream effector proteins in a cascade to bring about transcriptional changes, rather than by directly influencing transcription events. Using genome-wide binding assays, we have shown *in vivo* that a MAP kinase, JNK, directly binds to a large set of active promoters during the differentiation of stem cells into neurons. We identified the NF-Y complex as the mediator of JNK recruitment to target promoters. We further discovered that histone H3 Ser10 (H3S10) is a substrate for JNK kinase activity and that JNK-bound promoters are enriched for H3S10 phosphorylation. Inhibition of JNK signalling in post-mitotic neurons reduces phosphorylation at H3S10 and the expression of target genes. These results established MAP kinases as a novel set of epigenetic regulators that function by directly binding and modifying chromatin for transcriptional regulation during cellular differentiation (Figure 1).

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

Targeting of a Topoisomerase isoform to regulatory regions in the genome to govern the transcriptional programme associated with neurogenesis

Topoisomerases are essential for DNA replication in dividing cells, but their function in postmitotic cells remained poorly understood. We discovered, both *in vitro* and *in vivo*, that the induction of neuronal differentiation accompanies a switch in expression from the classical Topoisomerase IIα (Top2α) to another isoform, Topoisomerase IIβ (Top2β). A global analysis revealed that Top2β binds to genomic sites that are defined by a combination of distinct histone modifications. Top2β preferentially targets sites that are promoters and occupies these during the transition from neuronal progenitors to neurons, at a time when cells become postmitotic. Furthermore, the target neuronal genes require Top2β for chromatin accessibility of their promoters as well as the transcription state during neurogenesis. Top2β deficiency does not impair stem cell properties and the early steps of neuronal differentiation but does cause premature death of postmitotic neurons. This neuronal degeneration is caused by up-regulation of Ngfr p75, a gene bound and repressed by Top2β. These findings revealed chromatin-based targeting of a topoisomerase isoform to regulatory regions in the genome to govern the transcriptional programme associated with neuronal differentiation and longevity (Figure 2).

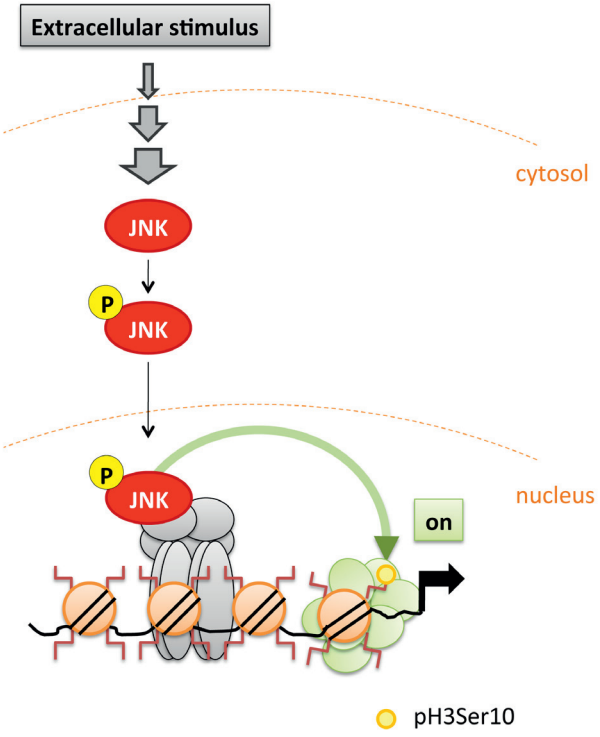


Figure 1. A MAP kinase, JNK, is recruited to gene promoters during stem cell differentiation for the transcriptional regulation of developmental genes via histone phosphorylation.

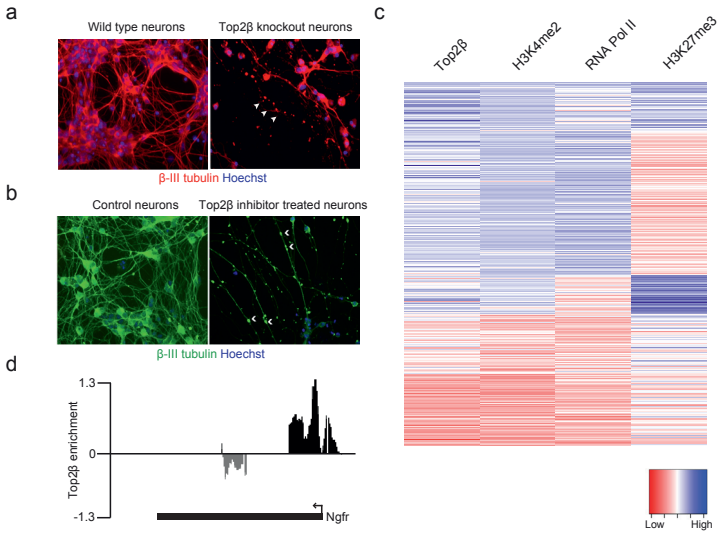


Figure 2. Targeting of Top2β to regulatory regions in the genome to govern the transcriptional programme associated with neuronal differentiation and longevity. (A) Immunofluorescence analysis using a specific antibody (red) shows significant neuronal degeneration in cells lacking Top2β. The white arrowheads mark axonal degeneration. (B) A similar phenotype was obtained upon chemical inhibition of Top2β activity. (C) The heat map depicts Top2β enrichments as well as H3K4me2, H3K27me3, and RNA Pol II levels, showing that Top2β targets are embedded in H3K4-marked chromatin and that Top2β's occupancy correlates positively with an active transcription state. (D) One Top2β target is Ngfr, neurotrophin receptor p75, which has previously been implicated in neuronal death. We further found that up-regulation in p75 levels underlies neuronal death in the absence of Top2β.

FUTURE DIRECTIONS

Breaking dogmas, our findings have revealed novel proteins and pathways that directly modulate chromatin at regulatory regions for transcriptional regulation. We will investigate if comparable principles of chromatin binding and activity are utilized by other proteins in the genome, especially in the context of cellular differentiation and cancer.

SELECTED PUBLICATIONS

Tiwari VK*, Burger L, Nikoletopoulou V, Deogracious, Thakurela S, Wirbelauer C, Hoerner L, Mielke C, Boege F, Murr R, Peters A, Barde YA and Schübeler D* (2012). Target genes of Topoisomerase IIbeta regulate neuronal survival and are defined by their chromatin state. *Proc Natl Acad Sci USA*, 109, E934-43 (*co-corresponding authors).

Tiwari VK, Stadler M, Wirbelauer C, Paro R, Beisel C and Schübeler D (2012). A Chromatin-modifying Function of JNK during Stem Cell Differentiation. *Nat Genet*, 44, 94-100.

Stadler MB, Murr R, Burger L, Ivanek R, Lienert F, Schöler A, Wirbelauer C, Oakeley EJ, Gaidatzis D, Tiwari VK and Schübeler D (2011). Transcription factor binding dynamically shapes the mouse methylome at distal regulatory regions. *Nature*, 480, 490-495.

"PCNA modifications exemplify the diversity of ubiquitin and SUMO signalling."

Helle Ulrich

EDUCATION

1992	Diploma in Biology, Georg-August-University Göttingen
1996	PhD in Chemistry, University of California, Berkeley
2004	Habilitation, Faculty of Biology (Genetics), Philipps University Marburg

POSITIONS HELD

1997 - 1998	Postdoctoral Fellow, University of Heidelberg
1998 - 2000	Postdoctoral Fellow, Max Planck Institute for Biochemistry, Martinsried
2000 - 2004	Group Leader, Max Planck Institute for Terrestrial Microbiology, Marburg
2004 - 2012	Group Leader, Cancer Research UK London Research Institute, Clare Hall Laboratories
From 2013	Director, Institute of Molecular Biology (IMB), Mainz

RESEARCH OVERVIEW

DNA is susceptible to many insults that threaten genome stability. Dealing with damage during replication is particularly important because DNA polymerases cannot cope with defective templates. In order to avoid a permanent arrest, cells employ mechanisms of damage bypass, which ensure complete replication of damaged DNA and are therefore essential for survival of a cell in the presence of genotoxic agents. However, as lesion bypass is often associated with damage-induced mutations, the pathway is also a potential source of genome instability in itself and therefore needs to be tightly controlled. Our research aims at understanding the mechanisms by which the post-translational modifiers ubiquitin and SUMO promote damage bypass and limit the accumulation of unwanted mutations. Via modification of an essential replication factor, PCNA, they activate two different modes of damage bypass that differ significantly in their accuracy, and prevent homologous recombination as an alternative means of resolving replication problems.

RESEARCH HIGHLIGHTS

Function of Mgs1 in DNA damage bypass

Whereas monoubiquitylation of PCNA activates a set of damage-tolerant but error-prone DNA polymerases to replicate across damaged DNA, modification with a K63-linked polyubiquitin chain is required for an error-free pathway of damage avoidance that is mechanistically poorly understood. We have now identified a DNA-dependent ATPase, Mgs1, as a downstream factor that recognises ubiquitylated PCNA. The protein is a PCNA interactor that contributes to genome maintenance in a poorly defined manner and harbours a ubiquitin-binding zinc finger (UBZ) motif. We were able to show that that this domain facilitates the recruitment of Mgs1 to chromatin at sites where PCNA is ubiquitylated in response to DNA damage. By means of genetic analysis, we found that those activities of Mgs1 that become apparent in response to DNA damage indeed depend on the ubiquitylation of PCNA, thus confirming that Mgs1 acts as a downstream effector of the modified clamp. Using linear fusions of ubiquitin and PCNA as mimics of ubiquitylated PCNA, we demonstrated that the interaction

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

between Mgs1 and PCNA is enhanced by monoubiquitin and even more so by polyubiquitin chains on PCNA (Figure 1). Thus, our findings indicate that Mgs1 might contribute to DNA damage bypass in a regulatory manner. However, as we also know that linear polyubiquitin chains cannot substitute for the genuine K63-linked chains that are attached to PCNA *in vivo*, the rather non-specific manner in which Mgs1 recognises ubiquitin makes it unlikely that the action of Mgs1 alone initiates the polyubiquitin-dependent template switching pathway.

Crosstalk between SUMO and ubiquitin on PCNA

Sumoylation of PCNA in budding yeast occurs constitutively during S phase and - by means of recruiting an antirecombinogenic helicase, Srs2 - prevents unscheduled recombination at replication forks. The notion that this modification is targeted predominantly towards the same site as ubiquitylation raises the question of how the transition between the replication-associated sumoylated form of PCNA to the damage-induced ubiquitylated form is managed. We have previously reconstituted PCNA modifications with purified proteins *in vitro* and confirmed that the requirements for each of these modifications closely match the physiological situation in yeast cells. To our surprise, we now found that in this purified system ubiquitylation and sumoylation of PCNA did not compete with each other, but that the presence of the sumoylation enzymes instead enhanced the efficiency of PCNA monoubiquitylation. Experiments using a linear fusion of SUMO to the N-terminus of PCNA revealed that this stimulatory effect was attributable to sumoylation of PCNA itself (Figure 2). Consistent with this notion, we identified a SUMO-interacting motif (SIM) in the ubiquitin ligase Rad18, responsible for PCNA monoubiquitylation. We found that this motif indeed mediated the enhanced activity of Rad18 towards sumoylated PCNA. The positive effect of the SIM-SUMO interaction was reproduced *in vivo*, where mutation of the SIM or abolishment of PCNA sumoylation led to significant defects in PCNA ubiquitylation and damage bypass, implying that the PCNA-SUMO conjugate is Rad18's physiological target. Hence, our findings have identified Rad18 as an additional effector of sumoylated PCNA and at the same time a member of a recently identified class of enzymes, the so-called SUMO-targeted ubiquitin ligases. Our results suggest a new mechanism by which damage-induced ubiquitylation is specifically targeted to those molecules of PCNA that are actively engaged in replication.

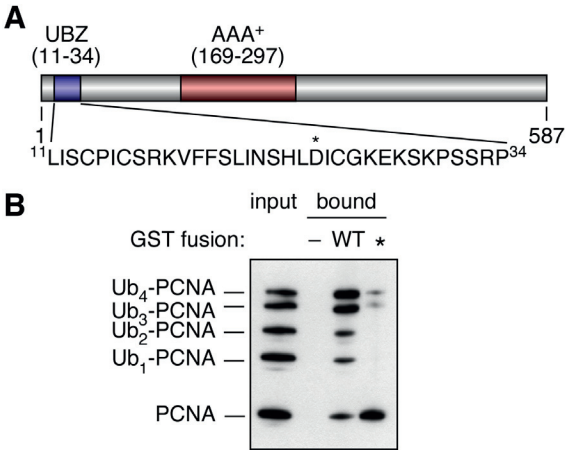


Figure 1. Mgs1 preferentially binds to polyubiquitylated PCNA. (A) Domain structure of Mgs1. The asterisk indicates the conserved residue mutated to alanine in the UBZ mutant Mgs1*. (B) Interactions of GST-tagged Mgs1 or Mgs1* with linear fusions of ubiquitin to PCNA, analysed by pull-down assays *in vitro*.

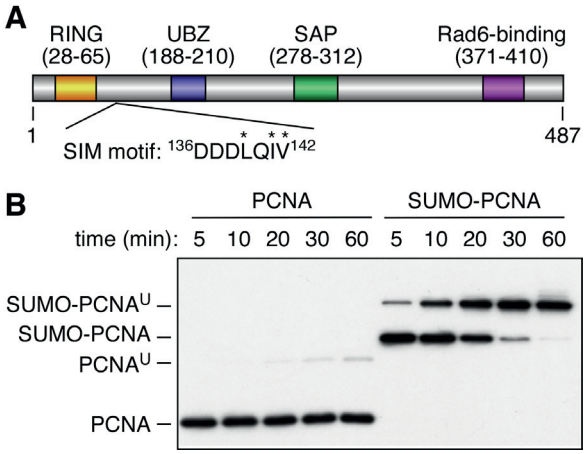


Figure 2. Rad18 preferentially ubiquitylates sumoylated PCNA. (A) Domain structure of Rad18. Asterisks indicate conserved residues mutated to alanine in the SIM* mutant. (B) Activity of Rad6-Rad18 towards PCNA versus a linear SUMO-PCNA fusion construct, assayed *in vitro* with purified proteins.

FUTURE DIRECTIONS

Our identification of Mgs1 as a downstream effector of ubiquitylated PCNA raises the question of how this ATPase modulates damage bypass. The protein exhibits DNA strand annealing activity *in vitro* and appears to exert a negative effect on the interaction of PCNA with the replicative polymerase δ, but it is unclear which of these activities is relevant for damage bypass. By establishing *in vitro* translesion synthesis assays with ubiquitylated PCNA, we hope to address the role of Mgs1 in this reaction biochemically. In parallel, we hope to gain insight into the mechanism of error-free bypass by identifying additional effectors of modified PCNA that would selectively recognise its polyubiquitylated form. Finally, we are interested in elucidating the functions of sumoylated PCNA in higher eukaryotes, where this modification is likely to serve an alternative purpose. We are therefore screening for proteins that preferentially interact with sumoylated PCNA in *Xenopus laevis* egg extracts.

SELECTED PUBLICATIONS

Daigaku Y, Davies AA and Ulrich HD (2010). Ubiquitin-dependent DNA damage bypass is separable from genome replication. *Nature*, 465, 951-955.

Parker JL and Ulrich HD (2009). Mechanistic analysis of PCNA poly-ubiquitylation by the ubiquitin protein ligases Rad18 and Rad5. *EMBO J*, 28, 3657-3666.

Davies AA, Huttner D, Daigaku Y, Chen S and Ulrich HD (2008). Activation of ubiquitin-dependent DNA damage bypass is mediated by Replication Protein A. *Mol Cell*, 29, 625-636.

CORE FACILITIES

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

BERNHARD KORN
Director of Core Facilities and Technology

OVERVIEW

The Core Facilities at IMB are set up to allow IMB Research Groups smooth, fast and economical access to up-to-date technologies that are supported by in-depth expertise from dedicated service personnel. The remit of the Core Facilities is completely driven by demand for in-house research facilities, and they ensure a short turn-around time from initial project discussions to sample supply and the return of data. Service groups are only set up if the highest quality of service and a high demand for the facility can be foreseen. Therefore, we make sure that investments are made in the most efficient way. All Core Facility personnel are dedicated to both service and training. The Core Facilities provide equal access to all IMB members on a first-come-first-serve basis and access is provided as a service or through collaborative research projects.

The set-up of strong in-house Core Facilities allows all IMB groups to undertake ambitious projects with high-throughput designs. Project support is provided from the initial phase of grant applications (e.g. feasibility studies, consulting, application support), via experimental design and experimentation, to data management and analysis. Core Facility support is provided in various ways. Full service is given for next-generation-sequencing (NGS), microarray analysis and cell sorting. In these cases IMB members provide samples, which are quality controlled by the Core Facilities, and a complete service is then provided up to the point of data production. Basic data analysis is also conducted to ensure good quality and accuracy of the information being returned to the in-house user. To be able to offer this type of cutting-edge service, we have entered into cooperations with other academic institutes such as EMBL and Johannes Gutenberg University Mainz. Public-private partnerships have also been initiated such as the establishment of IMB as a reference centre for high-resolution microscopy with Leica microsystems. A very strong part of the Core Facilities covers informatics of various flavours, from basic front-end IT support, server administration and intranet provision, to bioinformatics, statistics and on-demand programming.

Whenever possible, IMB outsources services as long as sufficient quality and a fast turn-around time can be guaranteed by external providers. However, for strategic reasons we keep certain technologies and their development in-house so that we can provide sufficient capacity, training and education to IMB students and personnel in these areas. We also provide assisted access to equipment and facilities within IMB. In this respect the Cytometry, Genomics, Histology and Microscopy Core Facilities provide specific training and assistance in the use of their equipment. Furthermore, Core Facility personnel provide training courses for the use of any other Core Equipment that is available at IMB, e.g. plate readers, ultra centrifuges etc. Finally, the Core Facilities provide a wide range of additional support, encompassing a media kitchen, animal care, management of in-house enzymes and clone/vector/cell line storage.

BIOINFORMATICS
CORE FACILITY

PEOPLE: EUGEN EIRICH, EMIL KARAULANOV, HOLGER KLEIN, NASTASJA KREIM, JOERN TOEDLING

CORE FACILITY OVERVIEW

The Bioinformatics Core Facility provides different levels of advanced data analysis tools and support to IMB scientists; this support ranges from experimental design to data evaluation. The facility is committed to finding and providing optimal solutions for bioinformatics issues that arise in the course of research projects. The rapidly changing landscape of biomedical research and the ever increasing flow of high-throughput datasets necessitate dedicated and flexible bioinformatic support for the many projects at IMB. The facility enables researchers to analyse experimental data themselves by providing access to state-of-the-art software tools with workflows for standard analyses that can be utilized and adapted to specific problems. Additionally, the facility offers long-term support and analytical expertise for individual research projects on a collaborative basis.

In 2012 the facility extended its hardware infrastructure with a file server of 140TB raw capacity. This is mainly used for next-generation-sequencing datasets.

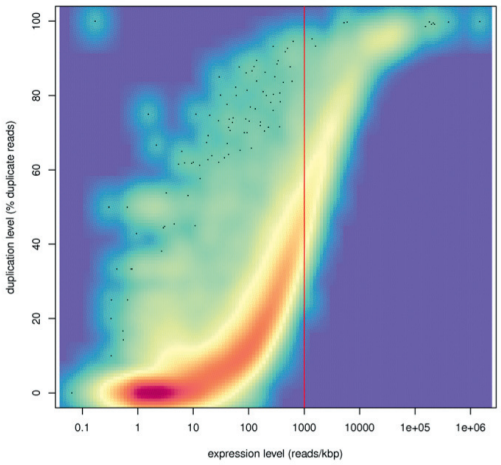


Figure 1. Duplication rate analysis of RNA-seq data. Highly expressed genes have high duplication rates, which dominate the overall duplication rate of a dataset. New quality control tools allow this effect to be distinguished from PCR artefacts which might have been caused by a low amount of input material.

SELECTED PUBLICATIONS

Chen CJ, Servant N, Toedling J, Sarazin A, Marchais A, Berthet ED, Cognat V, Colot V, Voinnet O, Heard E, Ciaudo C, Barillot E (2012). ncPRO-seq: a tool for annotation and profiling of ncRNAs in sRNA-seq data. *Bioinformatics*, 28, 3147-3149.

Stumm M, Entezami M, Trunk N, Beck M, Löcherbach J, Wegner RD, Hagen A, Becker R, Hofmann W (2012). Noninvasive prenatal detection of chromosomal aneuploidies using different next generation sequencing strategies and algorithms. *Prenatal Diagnosis*, 32, 569-577.

Toedling J, Servant N, Ciaudo C, Farinelli L, Voinnet O, Heard E, and Barillot E (2012). Deep-Sequencing Protocols Influence the Results Obtained in Small-RNA Sequencing. *PLoS ONE*, 7, e32724.

In 2012 the Bioinformatics Core Facility analysed datasets in several in-house collaborations. Data analysed included that from ChIP-seq (histone modifications and transcription factors, time series), strand-specific and agnostic RNA-seq (differential expression and isoform usage, time-series), MBD-seq, Bisulfite-seq, microarray and proteomics experiments. In the course of the analyses the facility extended its standard pipelines and engaged in the development of new quality control tools for the assessment of read duplication rates in RNA-seq. Members of the facility engaged in consultations regarding experimental design and data interpretation as well as user training and education through the provision of the following lectures and practical courses:

- International PhD Programme Lectures: Genomics, ChIP-seq, RNA-seq, methlylomics
- International PhD Programme Practical Courses: Galaxy, Chipster, Genomatix
- International Summer School: Bioinformatics for next-generation-sequencing, data analysis
- Other Seminars: Four lectures on various sequencing technologies

SERVICES OFFERED

The Bioinformatics Core Facility provides assistance on two levels:

1. General support to the IMB user base:

- Assistance with experimental design of high-throughput assays such as ChIP-seq, RNA-seq or Bisulfite-seq
- In-house training and tutorials on bioinformatic tools and databases to facilitate data access and analysis
- Implementation and adaptation of open-source tools and commercial software solutions for "omics" data interpretation
- Setup and development of analysis pipelines for sequencing, microarray, cytometry and microscopy data
- Quality assessment and pre-processing of raw data coming from the sequencing instruments

2. In-depth assistance for individual projects:

- Comprehensive high-throughput data analyses, interpretation and presentation of results
- Development of custom tools to address specific bioinformatic needs
- Exploration and implementation of novel analysis methods and pipelines
- Data mining of "omics" databases and published high-throughput datasets

The following bioinformatic resources were installed or updated to newer versions at IMB in 2012:

- R-Studio: new installation. A server frontend for the statistical programming language R and BioConductor packages
- GALAXY-LIMS: new installation. A customized version of the Galaxy laboratory information management system
- GALAXY: customization, upgrade and tighter infrastructure integration. An open-source environment for genomics data analysis
- GENOMATIX: upgrade and tighter infrastructure integration. Commercial software for performing standard next-generation-sequencing data mapping and analysis tasks
- CHIPSTER: upgrade. Open-source data analysis platform for various types of microarray

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

CYTOMETRY
CORE FACILITY

MALTE PAULSEN

EDUCATION

2006 Diploma in Molecular Biology, University of Constance
2011 PhD in Molecular Biology, German Cancer Research Center (DKFZ), Heidelberg

POSITIONS HELD

Since 2011 Head of Cytometry, Core Facilities and Technology, Institute of Molecular Biology (IMB), Mainz

CORE FACILITY OVERVIEW

The mission of the Cytometry Core Facility is to provide state-of-the-art services and training for IMB personnel and collaborating institutions. The facility provides assisted access to the newest generation of high-end FACS analysers and cell sorters operated by dedicated staff.

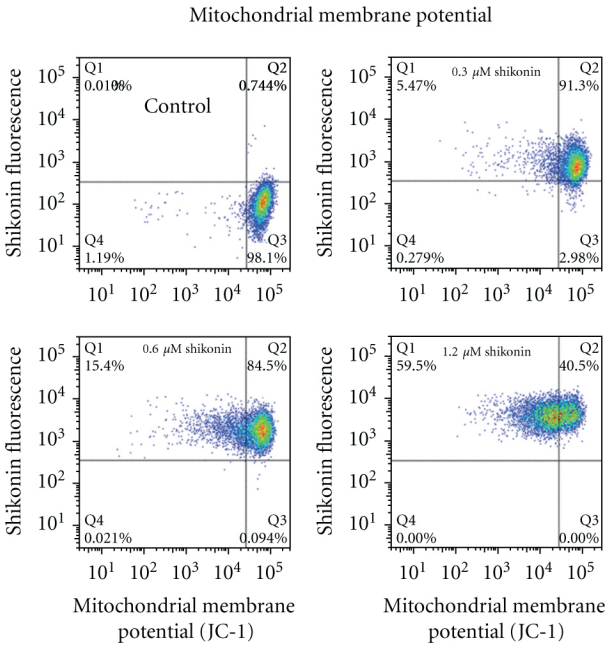


Figure 1. FACS based analysis of shikonin's effect on the mitochondrial membrane potential (MMP) in U937 cells. Shikonin resembles an intrinsic e- carrier and a potent ROS inducer and leads to a dose dependent decrease of the MMP. This renders the mitochondria inactive. From Wiench B *et al*, 2012.

SERVICES OFFERED

The Core Facility cytometer and sorter are both optically identical and harbour a five laser excitation suite and 18 parameter detector bench (Excitation Lasers 355/405/488/561/640 nm; Emission Detection (PMTs):2/5/3/5/3) providing users with the broadest range of usable dye and application choices. Routine checking and servicing of the cytometers and laboratory equipment ensures that scientists perform experiments with reliable and up-to-date instruments.

Levels of staffing within the facility allow for the proper training of users, help with experimental design and hands-on support during measurements. The facility offers IMB scientists cell sorting possibilities with high flexibility in experimental planning and very short waiting periods as well as dedicated help planning and analysing experiments based on flow cytometry. Machines in the Cytometry Core Facility were purchased with support from DFG.

SELECTED PUBLICATIONS

Wiench B, Eichhorn T, Korn B, Paulsen M* and Efferth T* (2012). Utilizing inherent fluoresecence of therapeutics to analyze real-time and multi-parametric effector kinetics. *Methods*, 57, 376-382 (*co-corresponding authors).

Wiench B, Eichhorn T, Paulsen M, and Efferth T (2012). Shikonin directly targets mitochondria and causes mitochondrial dysfunction in cancer cells. *Evid Based Complement Alternat Med*, doi:10.1155/2012/726025.

GENOMICS
CORE FACILITY

BERNHARD KORN

EDUCATION

1989	Diploma in Biology and Biochemistry, University of Kaiserslautern
1994	PhD in Genomics, German Cancer Research Center (DKFZ), Heidelberg

POSITIONS HELD

1994	Postdoctoral Researcher, Imperial Cancer Research Fund, Genome Analysis Laboratory, London
1995	Postdoctoral Researcher, DKFZ, Heidelberg
1996-2000	Head of Contract Research, German Resource Center for Genome Research, DKFZ, Heidelberg
2000-2005	CSO and Head of Heidelberg node of the German Resource Center for Genome Research, DKFZ, Heidelberg
2006-2010	Head of Division, Genomics and Proteomics Core Facilities, DKFZ, Heidelberg
Since 2010	Director of Scientific Core Facilities and Technology, Institute of Molecular Biology (IMB), Mainz

CORE FACILITY OVERVIEW

The Genomics Core Facility provides a full service for next-generation-sequencing (NGS) and microarray technologies. We primarily provide NGS services using the 454/Roche and Illumina platforms. In terms of microarrays we offer Agilent and some Illumina services. Moreover, the facility provides hands-on training and access to central quality control (QC) systems such as qPCR, Bioanalyzer and Nanodrop.

SERVICES OFFERED

The facility has been established according to the needs of the current Research Groups at IMB. As not all groups have started yet, spare capacity is provided to the wider Mainz scientific community.

All genomics services start with a mandatory project discussion between the user, facility staff and associated bioinformatic support. This ensures optimal experimental design and tailored support to any given project. All services have a defined start and end point for facility support. This typically starts with nucleic acids of a given quality and quantity and finishes with the provision of raw data (that has passed all predefined QC measures) to the user or a member of the Bioinformatics Core Facility.

In terms of NGS, we perform all applications available on the 454/Roche platform, but with a clear focus on amplicon sequencing of human and mouse, including methylation analyses of promoters. Services established and in routine use include microarray expression profiling of mRNA and miRNA from mouse and human. With respect to NGS, we currently offer mRNA-seq, ribo-depletion sequencing, ChIP-seq, MBP-seq and MeDIP-seq. With this technology, all library preparation and QC steps are performed in-house, and the final sequencing is carried out in collaboration with Johannes Gutenberg University, using their Illumina HiSeq2000 system. This ensures Illumina services can be provided to IMB Research Groups with very short turn-around times.

Whole genome bisulfite sequencing and the sequencing of small RNAs has been established and developed for the provision of routine service applications. Moreover, in December 2012 we purchased an Illumina MiSeq system. This instrument will be used for small service projects and Core Facility QC measures. It is also available to all IMB members for assisted access.

SELECTED PUBLICATIONS

Bartholomae CC, Arens A, Balaggan KS, Yáñez-Muñoz RJ, Montini E, Howe SJ, Paruzynski A, Korn B, Appelt JU, Macneil A, Cesana D, Abel U, Glimm H, Naldini L, Ali RR, Thrasher AJ, von Kalle C and Schmidt M (2011). Lentiviral vector integration profiles differ in rodent postmitotic tissues. *Mol Ther*, 19, 703-710.

Breitling LP, Yang R, Korn B, Burwinkel B and Brenner H (2011). Tobacco-smoking-related differential DNA methylation: 27K discovery and replication. *Am J Hum Genet*, 88, 450-457.

Meder B, Haas J, Keller A, Heid C, Just S, Borries A, Boisguerin V, Scharfenberger-Schmeer M, Stähler P, Beier M, Weichenhan D, Strom TM, Pfeufer A, Korn B, Katus HA and Rottbauer W (2011). Targeted next-generation sequencing for the molecular genetic diagnostics of cardiomyopathies. *Circ Cardiovasc Genet*, 4, 110-122.

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

HISTOLOGY
CORE FACILITY

MATHIAS GIERL

EDUCATION

2002	Diploma in Biology, Free University of Berlin
2007	PhD in Developmental Biology, Max Delbrück Center for Molecular Medicine & Free University of Berlin

POSITIONS HELD

2007	Postdoctoral Researcher, Max Delbrück Center for Molecular Medicine, Berlin
2007-2011	Postdoctoral Researcher, German Cancer Research Center (DKFZ), Heidelberg
Since 2011	Postdoctoral Researcher, Institute of Molecular Biology (IMB), Mainz
Since 2011	Head of Histology, Core Facilities and Technology, Institute of Molecular Biology (IMB), Mainz

CORE FACILITY OVERVIEW

The Histology Core Facility primarily provides equipment, reagents, consumables and support for tissue processing, embedding and sectioning. In addition, there is lab space, equipment and reagents to perform routine stainings (e.g. H&E staining, *in situ* hybridization, immunofluorescence). General advice on staining techniques is given on request.

SERVICES OFFERED

The Histology Core Facility can be used by the whole scientific community in Mainz. The machines can be booked electronically, are accessible 24/7 for trained users and are operated on a self-service basis. Currently the facility is equipped with the following machines for routine tissue processing, embedding and sectioning:

- Leica TP1020: Automated tissue processor for tissue fixation, dehydration and infiltration
- Leica EG1150 (including stereo-microscope): Tissue embedding center for paraffin embedding
- Leica RM2255 (including waterbaths and heating plates): Rotary microtome for motorized paraffin sectioning
- Leica CS3050 S: Cryostat for routine cryo-sectioning
- Leica VT1000 S: Vibrating-blade microtome (vibratome) to generate slices/sections of fresh (living) and fixed/stained samples
- Leica DM750: Routine microscope for checking the sections

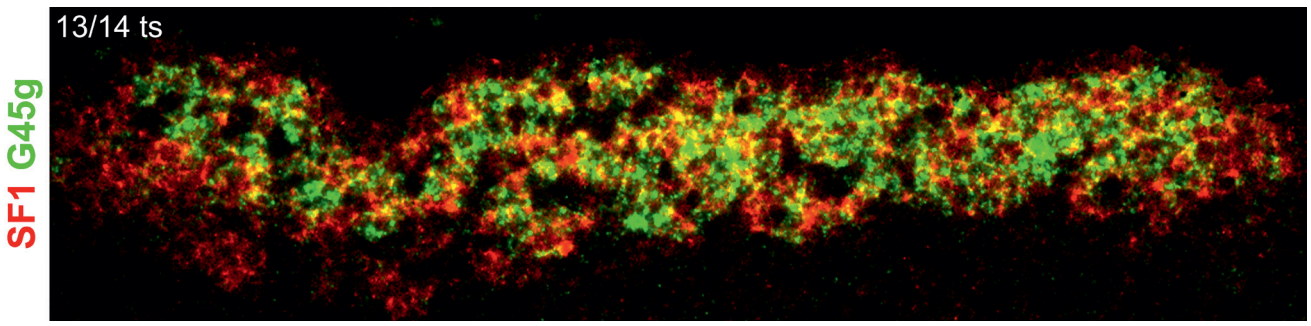


Figure 1. Double fluorescence *in situ* hybridization with probes for *SF1* (red) and *Gadd45g* (green) on sagittal cryo-sections of wild-type embryos at 13/14 ts (E11.25). Both markers are expressed in the gonads and co-localized in many cells. Sections were generated on the Leica CS3050 S cryostat by A. von Seggern, staining was performed by M. Gierl in the Niehrs lab and images were taken by M. Gierl in the Microscopy Core Facility using the Leica SP5 confocal laser scanning microscope.

SELECTED PUBLICATIONS

Gierl MS, Gruhn WH, von Seggern A, Maltry N and Niehrs C (2012). GADD45G Functions in Male Sex Determination by Promoting p38 Signaling and Sry Expression. *Dev Cell* 23,1032-1042.

MICROSCOPY
CORE FACILITY

ANDREAS VONDERHEIT

EDUCATION

2000	Diploma in Molecular Biology, ZMBH, University of Heidelberg
2004	PhD in Biochemistry, ETH, Zürich

POSITIONS HELD

2005-2008	Postdoctoral Researcher, Institute of Nanotechnology, ETH, Zürich
2008-2011	Screening Scientist at the RNAi Image-based Screening Center (RISC) at the Light Microscopy Centre (LMC), ETH, Zürich
Since 2011	Head of Microscopy, Core Facilities and Technology, Institute of Molecular Biology (IMB), Mainz

CORE FACILITY OVERVIEW

The Microscopy Core Facility not only provides hands-on training and access to various state-of-the-art microscopes, but also offers further education through the provision of a variety of practical courses and lectures. Training lectures provided range from a general "*Introduction to Microscopy*" to "*Pitfalls in Image Acquisition*" and "*Ethics in Image Acquisition and Processing*". Lectures, courses and the scientific services available are all open to Mainz' scientific community. To date the lectures have attracted a large number of non-IMB researchers, with about 80% of the audience coming from Johannes Gutenberg University and the University Medical Center. The microscopes provided are utilized equally by IMB staff and external users.

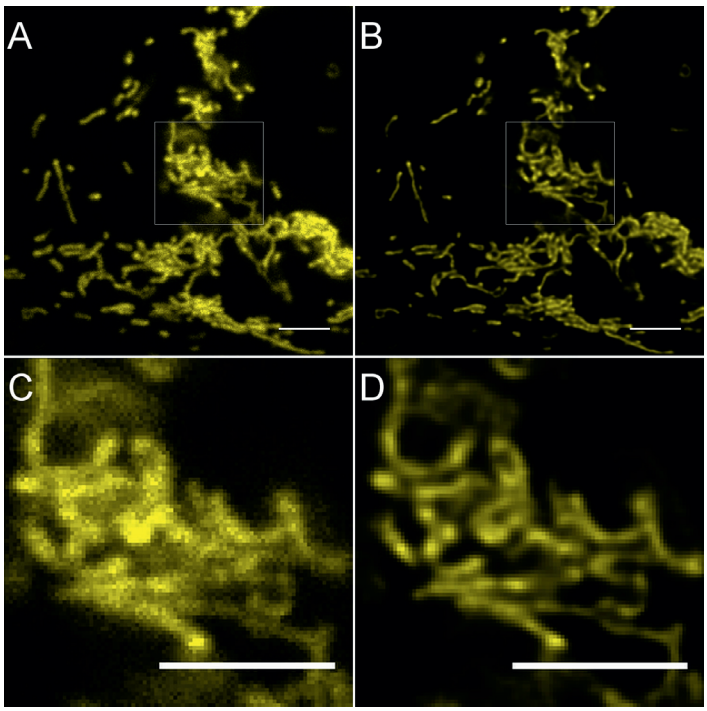


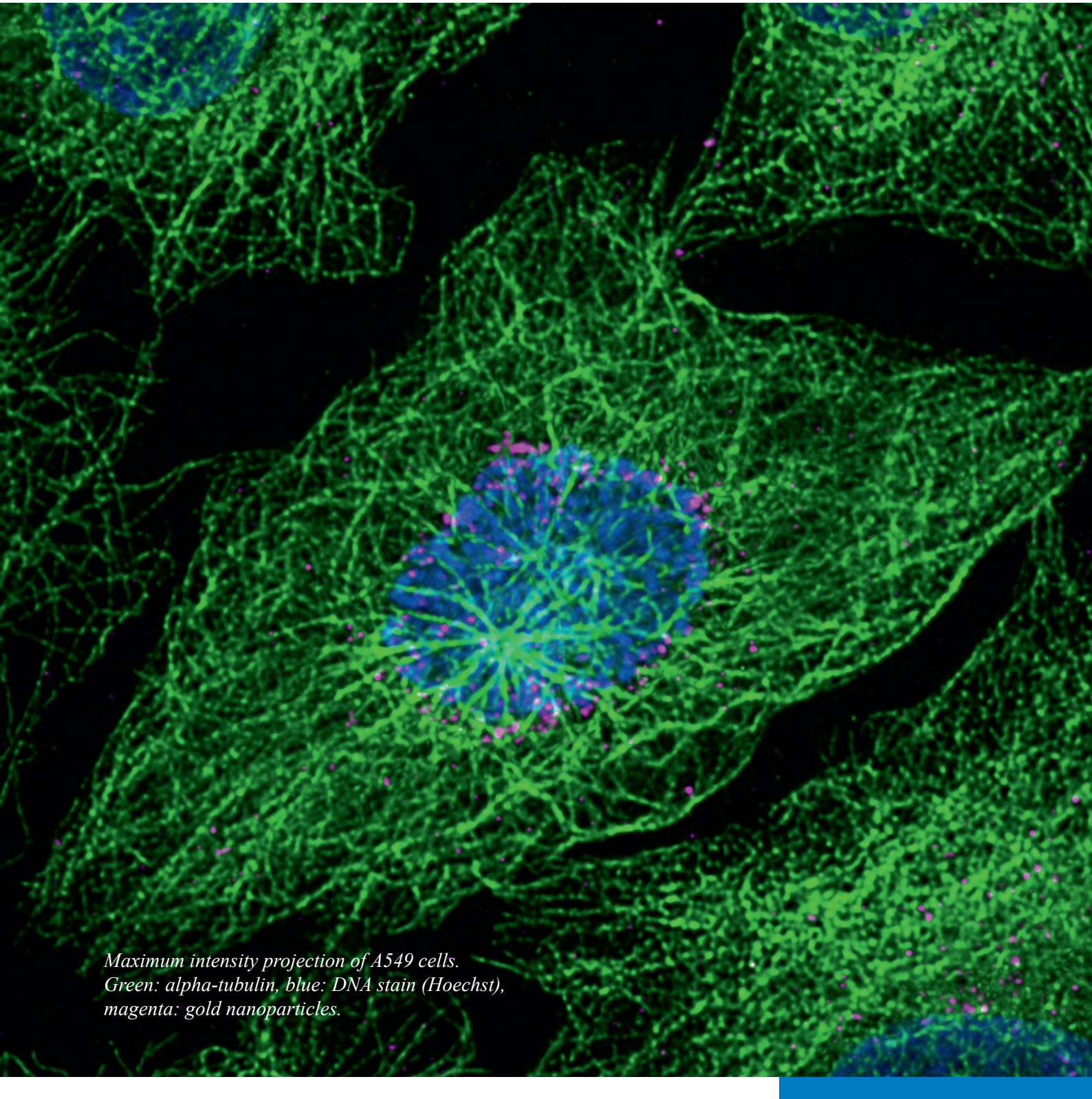
Figure 1. Mitochondria network in CHO cells labeled with Mitotracker® and imaged with a confocal microscope. (A) One plane of a z-stack. (B) Same plane after deconvolution. (C) Cutout of A. (D) Cutout of B. Scalebar: 4 µm. Imaged by A. Vonderheit.

SERVICES OFFERED

The facility provides hands-on training and access to various ultra-modern confocal laser scanning microscopes, super-resolution microscopes and motorized fast-flourescence microscopes for live-cell microscopy. There are also PCs for image processing equipped with software for deconvolution and 3D-rendering. The following microscopic equipment, a large part of which was financed by DFG, is available:

- M80 Demonstration Stereo Microscope: Equipped with a camera and a 24" monitor for teaching
- M205 FA Fluorescence Stereo Microscope: Equipped with a camera, fluorescence light source and three filters for UV, green, and red fluorophores
- DM2500 Fluorescence Upright Microscope: Equipped with a colour camera, fluorescence light source and three filters for UV, green, and red fluorophores and with five air objectives (this microscope is perfectly suited for histology)
- AF7000 Widefield Fluorescence Microscope: This widefield microscope is equipped with an incubator box for live-microscopy, fast filter wheels and a fast camera
- TCS LSI Macro Zoom Confocal: This microscope combines a stereo microscope with a confocal. Organisms like *C. elegans* or *Drosophila* larvae can be scanned as a whole or zoomed down to single cell level in confocal mode
- TCS SPE Confocal Microscope: An upright confocal microscope with one detector
- TCS SP5 Confocal Microscope: An inverse microscope with four PMTs, four lasers, and a fast resonance scanner
- TCS STED CW Super-Resolution Microscope: A super-resolution microscope which allows live microscopy and FCS. Equipped with an incubation box, two normal PMTs and two HyD detectors
- SR GSD Super-Resolution Microscope (localization method): A super-resolution microscope on which TIRF is possible
- Image Processing Station

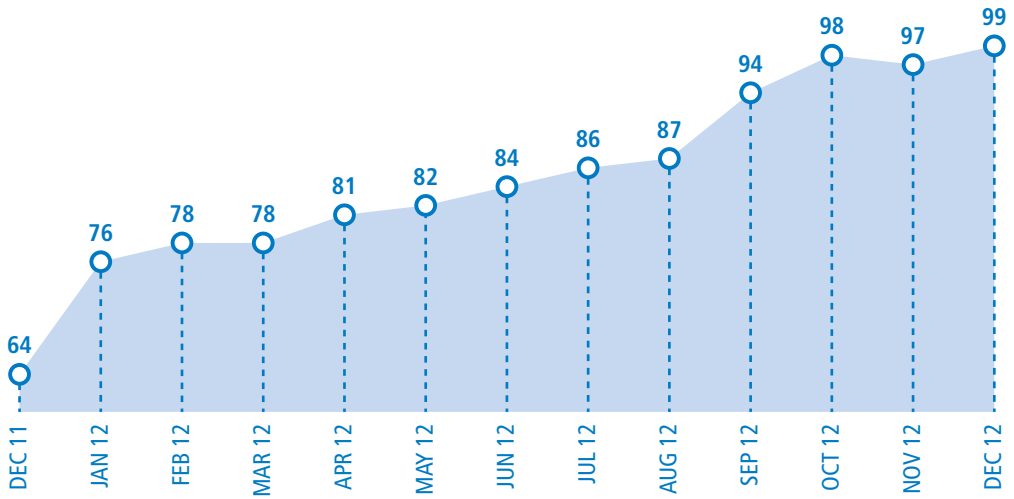
Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures



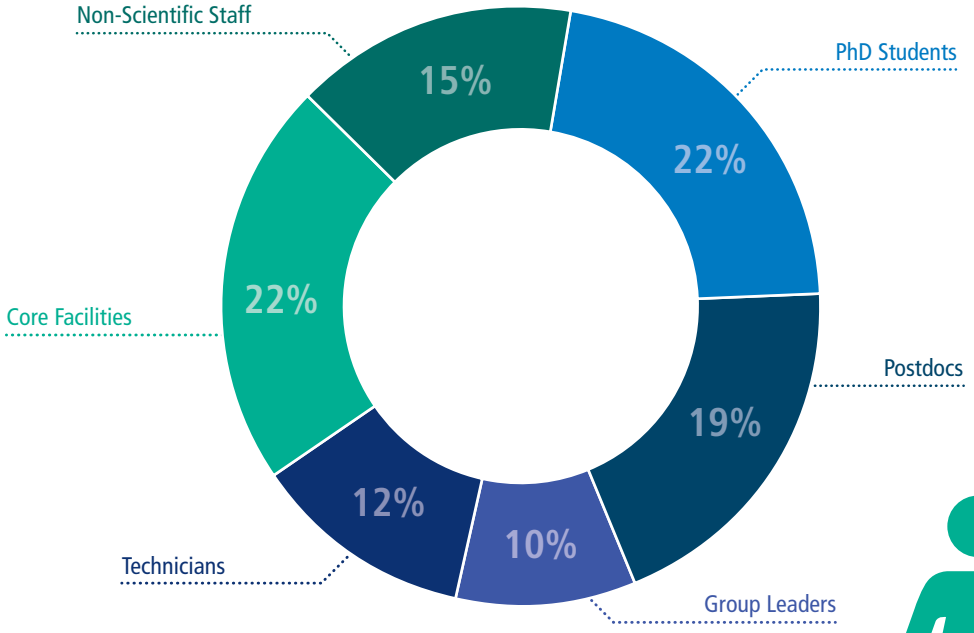
Maximum intensity projection of A549 cells. Green: alpha-tubulin, blue: DNA stain (Hoechst), magenta: gold nanoparticles.

32 Staff

PERSONNEL DEVELOPMENT: DECEMBER 2011 TO DECEMBER 2012



EMPLOYEES BY STAFF CATEGORY



GENDER DISTRIBUTION

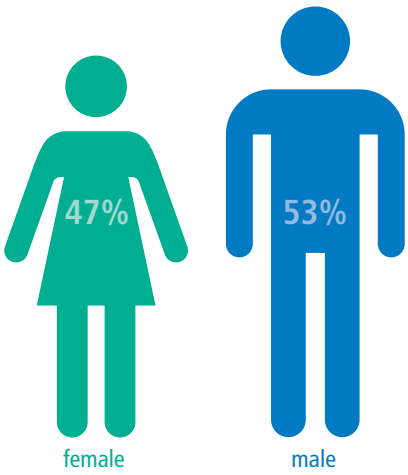
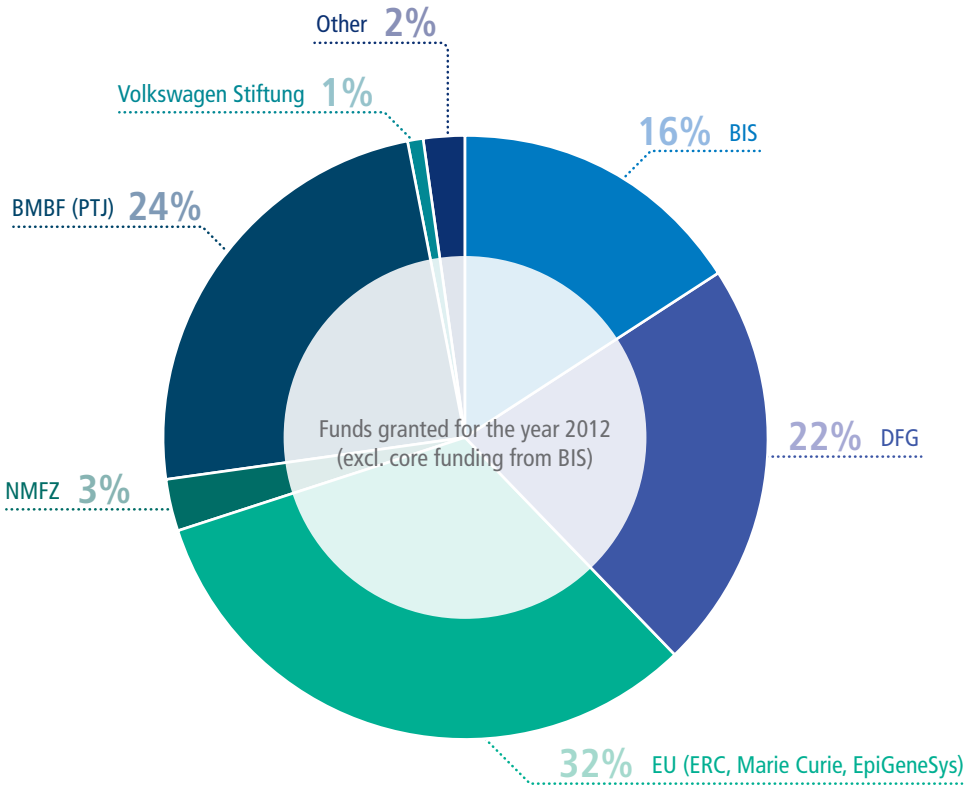


Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

External Funding

RESEARCH GRANTS



SOURCES OF FUNDING

Boehringer Ingelheim Stiftung (BIS)

Deutsche Forschungsgemeinschaft (DFG)

European Research Council (ERC)

EpiGeneSys

Federal Ministry of Education and Research (BMBF)

German National Academy of Sciences Leopoldina

Marie Curie Actions

Naturwissenschaftlich-Medizinisches Forschungszentrum (NMFZ)

Projektträger Jülich (PTJ)

Stiftung Rheinland-Pfalz für Innovation

Volkswagen Stiftung

Wilhelm Sander-Stiftung

34 External Speakers 2012

Date	Event	Speaker	Institution	Title
				JANUARY
31st	TechTalk	Andreas Hildebrandt	Johannes Gutenberg University, Mainz	Bioinformatics methods in computer aided drug design
				FEBRUARY
17th	Seminar	Andy Greenfield	MRC Harwell	Sex determination in mice and humans
23rd	Seminar	Valerio Orlando	Dulbecco Telethon Institute, Rome	Epigenetic control of noncoding elements contributes to skeletal muscle cell differentiation and disease
28th	TechTalk	Lars Knoch	Cellectis bioresearch	TALENs®: genome modifications in any organism, in any gene ... at any position
29th	Seminar	Antonio Giraldez	Yale University, New Haven	Micro RNAs, mechanism and function during vertebrate development
				MARCH
1st	Seminar	Stefano Piccolo	University of Padua	Role of the Hippo pathway effectors YAP and TAZ in mechanotransduction, cell polarity and cancer stem cells
13th	TechTalk	Wolfgang Wurst	Institute for Developmental Genetics, Helmholtz Center Munich	Extending the genetic toolkit for functional genome annotation
20th	TechTalk	Wolfgang Huber	EMBL Heidelberg	Statistics of expression analysis with RNA-Seq
				APRIL
12th	Seminar	Alexander Meissner	Harvard Stem Cell Institute, Cambridge	DNA methylation dynamics in development
13th	Tech Talk	Moran Jerabek-Willemsen	NanoTemper Technologies GmbH	Immobilization-free analytics of biomolecules using Microscale Thermophoresis
24th	TechTalk	Frank Lyko	DKFZ, Heidelberg	Cancer epigenetics: paradigms and drugs
				MAY
8th	TechTalk	Stefan Wiemann	DKFZ, Heidelberg	miRNA regulation of cell signaling in breast cancer
15th	TechTalk	Michael Stadler	Friedrich Miescher Institute, Basel	Whole-genome DNA methylation profiling by bisulfite-sequencing
29th	TechTalk	Caroline Friedel	Ludwig Maximilians University Munich	Ultra short and progressive 4sU-tagging reveals key characteristics of RNA processing at nucleotide resolution
30th	IPP Lecture	Barry R. Masters	Massachusetts Institute of Technology, Cambridge	Responsible conduct of research: How to avoid the pitfalls of plagiarism, flawed data or conflicts of interest
31st	Seminar	Peter Mombaerts	Max Planck Institute of Biophysics, Frankfurt	Coding olfaction

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

Date	Event	Speaker	Institution	Title
JUNE				
5th	TechTalk	Sven Nahnsen	Eberhard Karls-University, Tübingen	Large-scale proteomics: From cultivating cells to high-performance-computing
15th	Seminar	Olivier Pourquié	IGBMC, Strasbourg	Epigenetic control of embryonic symmetry
19th	TechTalk	Bertil Schmidt	Institute of Informatics, Johannes Gutenberg University, Mainz	Parallel algorithms and tools for NGS and sequence analysis
21st	Seminar	Dirk Schübeler	Friedrich Miescher Institute, Basel	Genetic determinants of the epigenome
22nd	Seminar	Helle Ulrich	Cancer Research UK London Research Institute	Control of DNA damage bypass by ubiquitin and SUMO
JULY				
5th	Seminar	Orlando D. Schärer	Stony Brook University, New York	Regulating nuclease activity in human DNA repair pathways
AUGUST				
28th	TechTalk	Antje Krause	University of Applied Sciences Bingen	Bioinformatics education in Germany
30th	Lecture	Huck Hui Ng	Genome Institute of Singapore	Systems biology of stem cells
SEPTEMBER				
7th	TechTalk	Andreas Keller	Saarland University, Saarbrücken	Precision diagnostics: high-throughput sequencing and bioinformatics on the way to clinical routine
18th	Seminar	Rudi Balling	Luxembourg Centre for Systems Biomedicine	Systems approaches to Parkinson's disease
OCTOBER				
4th	Seminar	Attila Becskei	Biozentrum, University of Basel	The role of chromatin and transcriptional networks in cellular differentiation and memory
9th	TechTalk	Lars Ohl and Michael Liebler	Merck Millipore	Amnis imaging flow cytometry: Applications of high speed high content image analysis of cells in suspension
11th	Seminar	Mischa Bonn	Max Planck Institute for Polymer Research, Mainz	Label-free, quantitative microscopy of cells
25th	Seminar	Thomas Höfer	DKFZ, Heidelberg	Stochastic switches in mammalian gene induction
NOVEMBER				
8th	Lecture	Susan Gasser	Friedrich Miescher Institute, Basel	Organizing the genome for development: mechanisms that segregate hetero- and eu-chromatin
13th	Seminar	Paola Arlotta	Harvard Stem Cell Institute, Cambridge	Programming and reprogramming projection neuron subtypes in the cerebral cortex
DECEMBER				
6th	Lecture	Denis Duboule	University of Geneva	Large scale gene regulation at the HoxD cluster
18th	TechTalk	Harry Böltz	NanoString Technologies Europe	Gene expression analysis down to the single cell level by digital quantification of nucleic acids utilizing a color-coded barcode technology

36 Core Facility Training

LECTURES

PROVIDER	TITLE	TIMES HELD
BIOINFORMATICS CORE FACILITY	Bioinformatics for ChIP Sequencing	1
	Bioinformatics for NGS	1
	Bioinformatics for RNA Sequencing	1
	Bisulfite-Sequencing Tools in Galaxy	1
	DNA Methylation Profiling - Methods, Applications & Data Analysis	3
	Duplication Analysis in RNA-Seq	1
	Influence of Sequencing Protocols on Small RNA Analyses	1
	Overview of Some GUI Tools for NGS Data Analysis & Visualisation	1
	Sequencing	1
CYTOMETRY CORE FACILITY	Advanced Cytometry	1
	Basics of FACS	2
	Utilizing Inherent Fluorescence for FACS Based Drug Screening (RegioFlow)	1
MICROSCOPY CORE FACILITY	Image Processing	1
	Imaging Ethics	2
	Introduction to Microscopy I	2
	Introduction to Microscopy II	2
	Pitfalls in Image Acquisition	2
	Super-Resolution Microscopy	1

PRACTICAL COURSES

PROVIDER	TITLE	DURATION
BIOINFORMATICS CORE FACILITY	Chipster	1 day
	Galaxy	2 x 0.5 day
	Genomatix	2 days
CYTOMETRY CORE FACILITY	Advanced Cytometry	2 days
MICROSCOPY CORE FACILITY	Confocal and Live Microscopy	2 days
	Hands on ImageJ	1 day

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

Publications by IMB Members in 2012

CREMER

Ach T, Best G, Rossberger S, Heintzmann R, Cremer C and Dithmar S (2012). Autofluorescence imaging of human RPE cell granules using structured illumination microscopy. *Br J Ophthalmology*, doi: 10.1136/bjophthalmol-2012-301547.

Cremer C (2012). Optics far Beyond the Diffraction Limit: From Focused Nanoscopy to Spectrally Assigned Localization Microscopy. In: *Springer Handbook of Lasers and Optics*, 2nd edition (F Trager, Edit.), Springer Press, 1359-1397.

Cremer T, Markaki Y, Hubner B, Zunhammer A, Strickfaden H, Beichmanis S, He. M, Schermelleh L, Cremer M and Cremer C (2012). Chromosome Territory Organization within the Nucleus. In: *Encyclopedia of Molecular Cell Biology and Molecular Medicine: Epigenetic Regulation and Epigenomics*, 2nd edition (Meyers RA, Edit.), Wiley-VCH, 451-484.

Huber O, Brunner A, Maier P, Kaufmann R, Couraud P-O, Cremer C and Fricker G (2012). Localization microscopy (SPDM) reveals clustered formations of P-Glycoprotein in a human blood-brain barrier model. *PLoS ONE*, 7, e44776.

Kaufmann R, Cremer C and Gall JG (2012). Superresolution imaging of transcription units on newt lampbrush chromosomes. *Chromosome Research*, doi:10.1007/s10577-012-9306-z.

Kaufmann R, Piontek J, Grüll F, Kirchgessner M, Rossa J, Wolburg H, Blasig IE and Cremer C (2012). Visualization and quantitative analysis of reconstituted tight junctions using localization microscopy. *PLoS ONE*, 7, e31128.

Maruyama EO, Hori T, Tanabe H, Kitamura H, Matsuda R, Tone S, Hozak P, Habermann FA, von Hase J, Cremer C, Fukagawa T and Harata M (2012). The actin family member Arp6 and the histone variant H2A.Z are required for spatial positioning of chromatin in chicken cell nuclei. *J Cell Sci,* 125, 3739-3743.

KETTING

Kamminga LM, van Wolfswinkel JC, Luteijn MJ, Kaaij LJT, Bagijn MP, Sapetschnig A, Miska EA, Berezikov E and Ketting RF (2012). Differential impact of the HEN1 homolog HENN-1 on 21U and 26G RNAs in the germline of Caenorhabditis elegans. *PLoS Genetics*, 8, e1002702.

Luteijn MJ, van Bergeijk P, Kaaij LJ, Almeida MV, Roovers EF, Berezikov E and Ketting RF (2012). Extremely stable Piwi-induced gene silencing in Caenorhabditis elegans, *EMBO J*. 31, 3422-3430.

LEGEWIE

Blüthgen N and Legewie S (2012). Robustness of signal transduction pathways. *Cell Mol Life Sci*, doi:10.1007/s00018-012-1162-7.

Glenewinkel HS, Reinz E, Bulashevska S, Beaudouin J, Legewie S, Alonso A and Eils R (2012). Multiparametric image analysis reveals role of Caveolin1 in endosomal progression rather than internalization of EGFR. *FEBS Lett*, 586, 1179-1189.

Kallenberger S and Legewie S (2012). Modeling formalisms in systems biology of apoptosis. In: *Systems Biology of Apoptosis* (Lavrik I, Edit.), Springer Press, 1-32.

Schmiedel J, Axmann I and Legewie S (2012). Multi-target regulation by small RNAs synchronizes gene expression thresholds and may enhance ultrasensitive behavior. *PLoS ONE*, 7, e42296.

Stelniec I*, Legewie S*, Tchernitsa O, Bobbe S, Sers C, Herzel H, Blüthgen N and Schäfer R (2012). Reverse engineering a hierarchical regulatory network downstream of oncogenic KRAS. *Mol Syst Biol*, 8, 601 (* joint first authors).

NIEHRS

Cruciat CM and Niehrs C (2012). Secreted and transmembrane Wnt inhibitors and activators. *Cold Spring Harbor Perspectives in Biology*, doi:pii: cshperspect.a015081v1.

El Wakila A, Guya N, Bandulikc S, Bendahhoub S, Zennaroe MC, Niehrs C, Maria B, Warth R, Barhanin J, Lallia E (2012). Dkk3 is a component of the genetic circuitry regulating expression of the Cyp11b2 rate-limiting enzyme of aldosterone synthesis in the adrenal cortex. *Hum Mol Genet*, 21, 4922-4929.

Gierl MS, Gruhn WH, von Seggern A, Maltry N and Niehrs C (2012). GADD45G Functions in Male Sex Determination by Promoting p38 Signaling and Sry Expression. *Dev Cell*, 23, 1032-1042.

Papatriantafyllou M, Moldenhauer G, Ludwig J, Tafuri A, Garbi N, Hollmann G, Küblbeck G, Klevenz A, Schmitt S, Pougialis G, Niehrs C, Gröne HJ, Hämmerling GJ, Arnold B and Oelert T (2012). Dickkopf-3, an immune modulator in peripheral CD8 T-cell tolerance. *Proc Natl Acad Sci USA*, 109, 1631-1636.

Teufel A, Becker D, Weber SN, Dooley S, Breitkopf-Heinlein K, Maass T, Hochrath K, Krupp M, Marquardt JU, Kolb M, Korn B, Niehrs C, Zimmermann T, Galle PR and Lammert F (2012). Identification of RARRES1 as a core regulator in liver fibrosis. *J Mol Med*, 90, 1439-1447.

Niehrs C (2012). The complex world of WNT receptor signalling. *Nat Rev Mol Cell Biol*, 13, 767-779.

Niehrs C and Acebron SP (2012). Mitotic and mitogenic Wnt signalling. *EMBO J*, 31, 2705-2713.

Niehrs C and Schaefer A (2012). Active DNA demethylation by Gadd45 and DNA repair. *Trends Cell Biol*, 22, 220-227.

RICHLY

Ribeiro J, Morey L, Mas A, Gutierrez A, Luis N, Mejetta D, Richly H, Benitah S, Keyes WM and Di Croce L (2012). ZRF1 controls oncogene-induced senescence through the INK4-ARF locus. *Oncogene*, doi: 10.1038/onc.2012.241.

TIWARI

Tiwari VK*, Burger L, Nikoletopoulou V, Deogracious R, Thakurela S, Wirbelauer C, Hoerner L, Mielke C, Boege F, Murr R, Peters A, Barde YA and Schübeler D* (2012). Target genes of Topoisomerase IIbeta regulate neuronal survival and are defined by their chromatin state. *Proc Natl Acad Sci USA*, 109, E934-43 (*co-corresponding authors).

Tiwari VK, Stadler M, Wirbelauer C, Paro R, Beisel C and Schübeler D (2012). A Chromatin-modifying Function of JNK during Stem Cell Differentiation. *Nat Genet*, 44, 94-100.

ULRICH

Davies AA and Ulrich HD (2012). Detection of PCNA modifications in Saccharomyces cerevisiae. *Methods Mol Biol*, 920, 543-567.

Finley D, Ulrich HD, Sommer T and Kaiser P (2012). The ubiquitinproteasome system of Saccharomyces cerevisiae. *Genetics*, 192, 319-360.

Parker JL and Ulrich HD (2012). In vitro PCNA modification assays. *Methods Mol Biol*, 920, 569-589.

Parker JL and Ulrich HD (2012). A SUMO-interacting motif activates budding yeast ubiquitin ligase towards SUMO-modified PCNA. *Nucleic Acids Res*, 40, 11380-11388.

Saugar I, Parker JL, Zhao S and Ulrich HD (2012). The genome maintenance factor Mgs1 is targeted to sites of replication stress by ubiquitylated PCNA. *Nucleic Acids Res*, 40, 245-257.

Ulrich HD (2012). Ubiquitin, SUMO and phosphate: how a trio of posttranslational modifiers governs protein fate. *Mol Cell*, 47, 335-337.

Ulrich HD (2012). Ubiquitin and SUMO in DNA repair at a glance. *J Cell Sci*, 125, 249-254.

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

CORE FACILITIES

Abba M, Laufs S, Aghajany M, Korn B, Benner A and Allgayer H (2012). Look who's talking: deregulated signaling in colorectal cancer. *Cancer Genomics Proteomics*, 9, 15-25.

Ammerpohl O, Haake A, Pellissery S, Giefing M, Richter J, Balint B, Kulis M, Le J, Bibikova M, Drexler HG, Seifert M, Shaknovic R, Korn B, Küppers R, Martín-Subero JI and Siebert R (2012). Array-based DNA methylation analysis in classical Hodgkin lymphoma reveals new insights into the mechanisms underlying silencing of B cell-specific genes. *Leukemia*, 26, 185-188.

Chen CJ, Servant N, Toedling J, Sarazin A, Marchais A, Berthet ED, Cognat V, Colot V, Voinnet O, Heard E, Ciaudo C and Barillot E (2012). ncPRO-seq: a tool for annotation and profiling of ncRNAs in sRNA-seq data. *Bioinformatics*, 28, 3147-3149.

Gierl MS, Gruhn WH, von Seggern A, Maltry N and Niehrs C (2012). GADD45G Functions in Male Sex Determination by Promoting p38 Signaling and Sry Expression. *Dev Cell*, 23, 1032-1042.

Spaich S, Will RD, Just S, Spaich S, Kuhn C, Frank D, Berger I, Wiemann S, Korn B, Koegl M, Backs J, Katus HA, Rottbauer W and Frey N (2012). Fbxl22, A Cardiac-Enriched F-Box Protein, Regulates Sarcomeric Protein Turnover and is Essential for Maintenance of Contractile Function In Vivo. *Circ Res*, in press.

Stumm M, Entezami M, Trunk N, Beck M, Löcherbach J, Wegner RD, Hagen A, Becker R, Hofmann W (2012). Noninvasive prenatal detection of chromosomal aneuploidies using different next generation sequencing strategies and algorithms. *Prenatal Diagnosis*, 32, 569-577.

Teufel A, Becker D, Weber SN, Dooley S, Breitkopf-Heinlein K, Maass T, Hochrath K, Krupp M, Marquardt JU, Kolb M, Korn B, Niehrs C, Zimmermann T, Godoy P, Galle PR and Lammert F (2012). Identification of RARRES1 as a core regulator in liver fibrosis. *J Mol Med (Berl)*, 90, 1439-1447.

Toedling J, Servant N, Ciaudo C, Farinelli L, Voinnet O, Heard E and Barillot E (2012). Deep-Sequencing Protocols Influence the Results Obtained in Small-RNA Sequencing. *PLoS ONE*, 7, e32724.

Wiench B, Eichhorn T, Korn B, Paulsen M* and Efferth T* (2012). Utilizing inherent fluoresecence of therapeutics to analyze real-time and multi-parametric effector kinetics. *Methods*, 57, 376-382 (*co-corresponding authors).

Wiench B, Eichhorn T, Paulsen M, Efferth T (2012). Shikonin directly targets mitochondria and causes mitochondrial dysfunction in cancer cells. *Evid Based Complement Alternat Med*, doi:10.1155/2012/726025.

RESEARCH AND TRAINING AT IMB

IMB is the place to be for scientists who want to carry out cutting-edge research in the fields of epigenetics, developmental biology, DNA repair and the interfaces between these fields. The research conducted by our multinational team of Group Leaders, postdocs and PhD students is vital to IMB's work in addressing key questions within these areas.

PhD students and postdocs at IMB are a key part of our research team and work on projects of fundamental biological importance or of relevance to human disease. Their research is conducted in a vibrant and highly interdisciplinary environment with leaders in the fields of epigenetics, DNA repair, developmental biology and systems biology.

At IMB we make sure our scientists can work productively. A key part of the support we offer comes from our state-of-the-art Core Facilities. They provide services in bioinformatics, cytometry, histology, genomics and microscopy, and are staffed by experts who are there to advise and assist our scientists during each step of their experiments; from the initial conception to the analysis of data. As part of the dynamic and collaborative spirit at IMB, all key equipment is shared between Research Groups and looked after by trained staff in the Core Facilities. This means our scientists will always have access to the most up-to-date and well maintained equipment that is required for their experiments.

In addition to the training available through our Core Facilities, IMB has training programmes for both specialist scientific techniques and key transferable skills. The scientific training we provide includes courses on key topics such as statistics, the analysis of large datasets (including those generated by microarrays and next-generation-sequencing methods) and advanced live and super-resolution microscopy. We also make sure our junior scientists learn the soft skills required for a successful career in an increasingly competitive scientific world both within and outside of academia. Courses we offer cover topics such as presentation skills, scientific writing and project management, as well as conflict management and leadership skills.

INTERNATIONAL PHD PROGRAMME

Our International PhD Programme on the "*Dynamics of Gene Regulation, Epigenetics and DNA Damage Response*" gives talented and enthusiastic students the opportunity to undertake PhD research at the cutting edge of modern biology.

Our groups cover a broad range of expertise and include leading biochemists; geneticists; cell and developmental biologists who study the molecular mechanisms of embryonic development, ageing and disease; bioinformaticians and systems biologists who analyse high-throughput datasets and model regulatory gene networks; and applied physicists who develop ground breaking super-resolution microscopes. This range of expertise and the open and vibrant atmosphere within the Programme encourages multidisciplinary collaborations and innovative research.

The International PhD Programme therefore has a clearly defined and unique profile that provides students with interdisciplinary education in the following fields:

- Epigenetics
- Gene Regulation
- DNA Repair
- Functional Morphology of the Nucleus
- Systems Biology and Bioinformatics

The Programme is coordinated by IMB and participating groups are located at the:

- Institute of Molecular Biology
- Johannes Gutenberg University
- University Medical Center
- Max Planck Institute for Polymer Research

www.imb-mainz.de/PhD

INTERNATIONAL SUMMER SCHOOL

The IMB International Summer School (ISS) is a six-week programme for outstanding and enthusiastic undergraduate, Masters and PhD students who want to acquire excellent practical skills and hands-on training from leading scientists in molecular biology. Research Groups participating in the ISS include Group Leaders at IMB, Johannes Gutenberg University (JGU) and Mainz' University Medical Centre.

The International Summer School offers an attractive framework to improve the training of prospective scientists in an informal and international atmosphere. This includes theory modules (lectures and discussion groups) and practical research projects. Lectures by Group Leaders and external speakers give students comprehensive insight into the latest research findings and identify key open questions in Gene Regulation, Epigenetics and DNA Damage. Furthermore, the ISS teaches students the complementary skills, such as presentation and communication techniques, that are required for a successful career as a scientist.

Beyond these specific events, ISS participants are also fully integrated into scientific life at IMB by participating in Lab Meetings and Journal Clubs. Furthermore, students work on a cutting-edge research project within the lab of one of the participating Research Groups.

www.imb-mainz.de/ISS

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

IMB'S RESEARCH ENVIRONMENT

IMB is embedded in a strong and dynamic research environment. It is located within the leafy campus of the Johannes Gutenberg University, just west of Mainz city centre. With 10 departments, 150 institutes and 36,000 students, the JGU is one of the largest German universities. In biomedical research, the University has built strong, interdisciplinary centres dedicated to cardiovascular medicine, neuroscience, immunology and oncology.

The University Medical Centre, which is located near the main university campus, has a strong focus on clinical and translational research and has researchers who also work in close contact with IMB. In addition to the University, IMB has two Max Planck Institutes (Max Planck Institute for Chemistry, Max Planck Institute for Polymer Research) and Mainz' University of Applied Sciences as immediate neighbours.

Mainz is also surrounded by a number of towns and cities with extensive research activities. For instance, Frankfurt is only 35 km away and is home to Goethe University, which has a total of 38,000 students and 10 research institutes within the Biochemistry, Chemistry and Pharmacy Department alone. Furthermore, there are several Max Planck Institutes in Frankfurt (including the Max Planck Institute for Biophysics, the Max Planck Institute for Brain Research and the Ernst Strüngmann Institute for Cognitive Brain Research). In addition to Frankfurt, nearby Darmstadt is home to both a Technical University, whose Department of Biology has a focus on synthetic biology and the biology of stress responses, and a University of Applied Sciences that includes a focus on biotechnology.

Furthermore, there is an extensive industry R&D presence, with the headquarters of Boehringer Ingelheim and the Merck Group both in close vicinity.

WHERE WE ARE

IMB is located in the city of Mainz, a charming, open-minded city that dates back 2,000 years to Roman times and still has a historic centre with a magnificent medieval cathedral. It was also here, in 1450, that Johannes Gutenberg invented modern book printing. The city is located at the confluence of two of the most important rivers in Germany, the Rhine and the Main, and has spectacular esplanades.

Mainz is within easy reach of both cosmopolitan Frankfurt, with its famous opera house, avant-garde museums and glass-and-steel banking district, and the Rhine valley region with its castles, vineyards and nature reserves that offer great outdoor activities. With Frankfurt airport – one of the largest airports in Europe – only 20 minutes away, countless European and overseas destinations are within easy reach.

IMB IS EXTREMELY THANKFUL

to the following outstanding scientists for the insight, guidance and advice that they have provided regarding IMB's foundation and our work to become a world leading research centre.

PROF. DR BRADLEY CAIRNS

Huntsman Cancer Institute
University of Utah
Salt Lake City, USA

PROF. DR INGRID GRUMMT

Deutsches Krebsforschungszentrum (DKFZ)
Heidelberg, Germany

PROF. DR RUDOLF JAENISCH

The Whitehead Institute for Biomedical Research
Cambridge, USA

PROF. DR JOSEF JIRICNY

Institute of Molecular Cancer Research
University of Zurich
Zurich, Switzerland

PROF. DR JÖRG MICHAELIS

Scientific Spokesperson for the Board of Directors
of the Boehringer Ingelheim Foundation
Mainz, Germany

PROF. DR RENATO PARO

Department of Biosystems Science and Engineering
Swiss Federal Institute of Technology
Basel, Switzerland

PROF. DR ERNST-LUDWIG WINNACKER

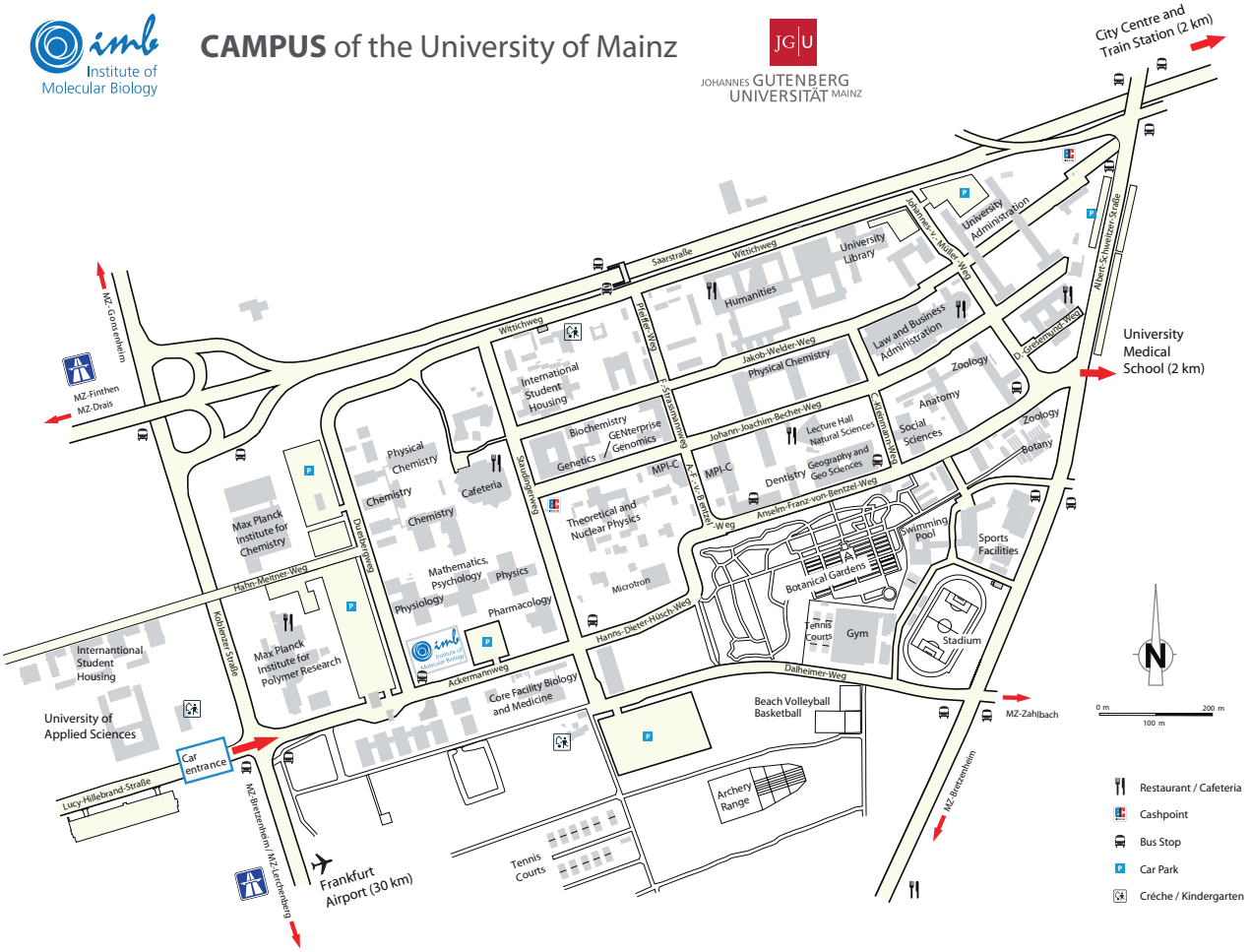
Secretary General International Human Frontier
Science Program Organization
Strasbourg, France

Table of Contents
Foreword
Research Groups
Core Facilities
Facts and Figures

imb Institute of Molecular Biology

CAMPUS of the University of Mainz

JG|U JOHANNES GUTENBERG UNIVERSITÄT MAINZ



Contact

Institute of Molecular Biology gGmbH
supported by the Boehringer Ingelheim Stiftung
Ackermannweg 4
55128 Mainz
Phone: +49-6131-39-21501
Fax: +49-6131-39-21521
www.imb-mainz.de
info@imb-mainz.de

Imprint

PUBLISHED BY

Institute of Molecular Biology gGmbH

RESPONSIBLE FOR CONTENT

Christof Niehrs and Ralf Dahm

COORDINATION

James Donald, Andrea Pollack and Ralf Dahm

EDITOR

James Donald

LAYOUT & DESIGN

cala media

SCIENTIFIC ILLUSTRATIONS

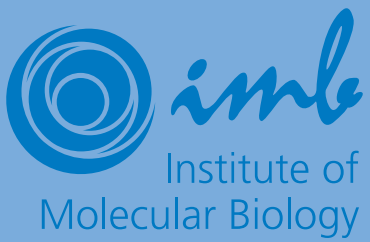
Provided by the respective Research Groups and Core Facilities

PORTRAITS OF GROUP LEADERS & HEADS OF CORE FACILITIES

Thomas Hartmann

OTHER PHOTOS

Photo of IMB on cover by Ule Ruhland, Adler & Olesch. Photo of IMB sign on page 0/1 by Thomas Hartmann. Photo of Cytometry Core Facility FACS machine on page 24 by Holger Klein. Image of A549 cells on page 31 captured by Katharina Böse (Microscopy Core Facility). Photos on pages 37 and 39 by Birthe Stiglegger. Portraits on page 42 were supplied by the respective SAB members. All other photos were from the IMB archive.



ANNUAL REPORT 2012

www.imb-mainz.de