



ANNUAL REPORT

2013

EPIGENETIC GENE REGULATION,
DNA REPAIR,
AND
DEVELOPMENTAL BIOLOGY.

IT ALL
COMES
TOGETHER
AT IMB.

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F O R E W O R D



In my third year as founding director, I was pleased that we again made great progress at IMB in 2013. Three new Group Leaders joined the institute, rounding out our scientific expertise in chromatin biology, proteomics and splicing regulation, and bringing the total number of labs at IMB to 13. We hosted a number of high-profile meetings, expanded the ranks of our International PhD Programme, and broke the 150-employee mark. This Annual Report brings you the highlights of another eventful year at the interface of epigenetics, DNA repair and developmental biology.

TRANSITIONS

IMB welcomed Falk Butter (from the Max Planck Institute of Biochemistry, Munich), Julian König (from the MRC Laboratory of Molecular Biology, Cambridge), and Petra Beli (from the Novo Nordisk Foundation Centre for Protein Research, Copenhagen) as new Group Leaders. Falk will also take the helm at our newly formed Proteomics Core Facility. His research group will investigate DNA and RNA-protein interactions using quantitative mass spectrometry. Julian, who as a postdoctoral fellow developed the iCLIP technology to identify protein-bound RNAs, will focus on the splicing of transposable elements. Petra is establishing her group at IMB with the help of a prestigious Emmy Noether Programme award from the German Research Foundation DFG. Her focus is DNA damage response.

We are also pleased to host Eva Wolf, who has recently been appointed professor at Johannes Gutenberg University (JGU) Mainz. Eva's research interests in chronobiology and circadian gene regulation nicely complement our work at IMB. As she is part of both our International PhD Programme and our International Summer School, we are delighted to offer her space while her laboratories at JGU are refurbished.

We closed 2012 with 99 staff, and continued to grow in 2013: IMB's total employees now number 154, and of our scientific staff 59% are international. On the administrative side, Roland Euler assumed the role of Business Director, while Ralf Eßmann became our Director of Administration. Changes were afoot in our Core Facilities as well, as Andreas Vonderheit, formerly Head of the Microscopy Core Facility at IMB, took over as director of all six core facilities.

With these new arrivals, we also bid farewell to a few colleagues who were instrumental to IMB's early years. We thank our departing Business Director, Götz Scholz, our former Director of Administration, Stephanie Oehl, and our former Director of Core Facilities, Bernhard Korn, who worked diligently to help build up IMB.

RESEARCH AND FACILITIES

Research at IMB continues to address key questions in epigenetics, developmental biology, DNA repair, and related fields. From revealing the epigenetic reprogramming of the genome to peering more deeply into cells with superresolution microscopes, IMB researchers are pushing the boundaries at molecular, cellular and systems fronts. Our groups investigate the epigenomic pathways that regulate cellular differentiation, the factors that influence splicing, as well as RNA interference and silencing mechanisms in different model organisms. Further, labs at IMB study DNA repair and genome stability, uncover why transcriptional cycling or aberrant gene expression may contribute to cancer, and reveal how the chromatin landscape changes during ageing. This research is changing our understanding of how we develop and adapt to our environments, and how we age or develop disease.

Scientists at IMB frequently use interdisciplinary approaches in tackling these questions. This is greatly aided by our strong Core Facilities, which employ dedicated experts and provide state-of-the-art equipment and services in bioinformatics, cytometry, genomics, proteomics, histology and microscopy. This ensures that ambitious projects can be driven forward rapidly and successfully even in highly competitive fields.

TRAINING AND EVENTS

Our International PhD Programme (IPP) forms a central part of our scientific mission, and continues to grow. Within the theme of *Dynamics of Gene Regulation, Epigenetics and DNA Damage Response*, 48 students are now conducting doctoral research in 27 labs at IMB and our IPP partner institutions JGU, the University Medical Centre, and the Max Planck Institute for Polymer Research. The IPP students, over 60% of whom now come from outside Germany, held their first student retreat in June, and a symposium in November at which they presented talks and posters.

To further encourage student research and open our labs to motivated junior researchers, we held our second International Summer School (ISS). Fourteen participants from eight countries attended training courses and lectures and undertook research projects within groups at IMB, JGU, and the University Medical Centre during July and August. Over the summer we also organised our first career day, which gave more than 100 students and junior researchers a chance to learn about non-academic jobs in the life sciences. Both the career day and the ISS will be staged again in 2014.

Outstanding international speakers joined us in October for our second annual IMB Conference, which this year focused on *Chromatin Dynamics & Stem Cells*. It was the culmination of a variety of scholarly events at IMB, including our regular research seminars with invited speakers, a workshop on the 4D nucleome, a joint EpiGeneSys/IMB workshop on epigenomics, and the kick-off meeting of the Federal Ministry of Research and Education's e:Bio Innovations in Systems Biology consortium.

COMING SOON

Upcoming highlights for 2014 include the ongoing recruitment for a full professorship in bioinformatics, a joint appointment between JGU's Faculty of Biology and IMB. GeneRED, short for Gene Regulation in Evolution and Development, is another exciting research initiative in collaboration with the Faculty of Biology that is set to launch next year. As a designated research focus of Rhineland-Palatinate's Ministry for Education and Research, GeneRED will build on the success of the IPP by awarding PhD positions in gene regulation research, and expanding the capacities for proteomics research in Mainz.

Our 2014 IMB Conference, *Nuclear RNA in Gene Regulation & Chromatin Structure*, will take place on 9-12 October. It will once again bring world-renowned speakers to Mainz, and promises to be an interactive and stimulating event.

All of this is of course enabled by the continued support of the Boehringer Ingelheim Foundation, for which we are very grateful, and the advice of the members of our outstanding Scientific Advisory Board. But it is the truly excellent team of researchers, students, technicians and administrators whose work makes IMB thrive.

I trust the reader will sense the vibrant spirit of IMB while leafing through the pages of this Annual Report.

Christof Niehrs
Founding Director



PETRA BELI

» *We use quantitative mass spectrometry to study the role of posttranslational modifications and protein-protein interactions in nuclear signalling.* «

EDUCATION

- 2007 Diploma in Molecular Biology, University of Zagreb
- 2011 PhD in Biology, Goethe University Frankfurt

POSITIONS HELD

- 2010-2013 Postdoctoral Researcher, The Novo Nordisk Foundation Centre for Protein Research, University of Copenhagen
- Since 2013 Group Leader, IMB

GROUP MEMBERS

Marina Borisova / PhD student; since 12/2013

RESEARCH OVERVIEW

The structure of eukaryotic chromatin has to be dynamically remodeled in response to DNA damage. Posttranslational modifications of chromatin-associated proteins play an essential role in this process by regulating protein activity and interactions. Protein ubiquitylation has emerged as an important mechanism that regulates the chromatin response to DNA damage. We have shown that DNA damage induces site-specific ubiquitylation and deubiquitylation of proteins, suggesting the widespread regulatory involvement of protein ubiquitylation in processes that maintain chromatin integrity. We plan to study the role of protein ubiquitylation in DNA repair and chromatin organisation using state-of-the-art mass spectrometry-based proteomics. In particular, we aim to investigate the function of site-specific protein ubiquitylation in DNA damage signalling and to identify the protein targets of nuclear ubiquitin ligases.

RESEARCH HIGHLIGHTS

Eukaryotic cells have evolved complex signalling pathways that coordinate the repair of DNA lesions. Posttranslational modifications (PTMs) of proteins play important regulatory roles in DNA damage signalling. Studies have demonstrated that modification of proteins with ubiquitin has essential regulatory functions in DNA repair processes. Prominent examples are monoubiquitylation of FANCI/FANCD2 and PCNA that play a central role in the repair of interstrand cross-links and in DNA damage bypass, respectively. The importance of protein ubiquitylation in DNA damage signalling was further emphasised by evidence that ubiquitin-modifying enzymes are recruited to lesion-flanking chromatin. Despite these advances, the regulatory scope of ubiquitylation in DNA damage signalling and the substrate spectrum of ubiquitin-modifying enzymes are currently incompletely understood. Recent advances in mass spectrometry (MS) as well as novel methods for the enrichment of ubiquitylated peptides now permit, for the first time, the systematic study of protein ubiquitylation after cellular perturbations. We have employed the method for analysis of protein ubiquitylation to identify 10,000 endogenous ubiquitylation sites in human cells. Our results demonstrate that a large part of the cellular proteome, including numerous cell surface receptors and nuclear proteins, is subjected to ubiquitylation. In a subsequent study, we have applied this method to investigate protein ubiquitylation in cells exposed to ultraviolet radiation (UV). We have identified numerous UV-induced ubiquitylation sites on known components of the DNA repair and chromatin organisation machinery, suggesting a widespread functional involvement of ubiquitin in processes that maintain chromatin integrity after DNA damage. The investigation of the precise functions of individual ubiquitylation events presents a future challenge and can provide a detailed mechanistic understanding of how ubiquitin signalling contributes to the maintenance of overall cell homeostasis.

FUTURE DIRECTIONS

FUNCTIONAL CHARACTERISATION OF UBIQUITYLATION EVENTS AFTER DNA DAMAGE

We have previously demonstrated that protein ubiquitylation and deubiquitylation occur in a site-specific manner after treatment of cells with UV, and characterised the function of PAF15 deubiquitylation in DNA damage bypass. The functional role for most of the identified ubiquitylation events remains to be investigated. We will conduct both gain and loss-of-function experiments to study the role of selected proteins and ubiquitylation sites in relevant DNA repair or gene regulatory processes in mammalian cells. In particular, we will employ mutant forms of proteins that cannot be ubiquitylated or that mimic ubiquitylation to investigate the impact of protein ubiquitylation on protein-protein interactions, and protein localization and activity.

SELECTED PUBLICATIONS

Beli P*, Lukashchuk N*, Wagner SA, Weinert BT, Olsen JV, Baskcomb L, Mann M, Jackson SP, Choudhary C (2012). Proteomic investigations reveal a role of THRAP3 in DNA damage response. *Mol Cell*, 4, 212-25.

Povlsen LK*, Beli P*, Wagner SA, Poulsen SL, Sylvestersen KB, Poulsen JW, Nielsen ML, Bekker-Jensen S, Mailand N, Choudhary C (2012). Systems-wide analysis of ubiquitylation dynamics reveals a key role for PAF15 ubiquitylation in DNA-damage bypass. *Nat Cell Biol*, 14, 1089-98.

Wagner SA*, Beli P*, Weinert BT, Nielsen ML, Cox J, Mann M, Choudhary C (2011). A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles. *Mol Cell Proteomics*, 10, M111.013284.

(* joint first authors)

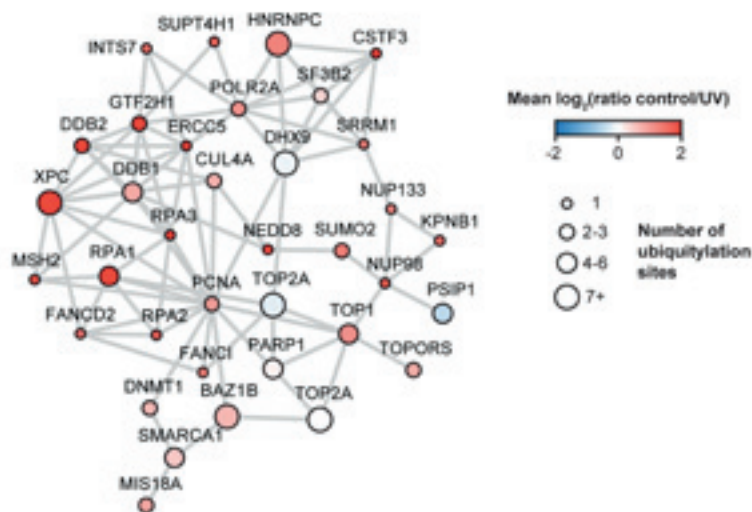


Figure 1. Systems-wide analysis of ubiquitylation events after DNA damage. A functional interaction network of proteins with UV-induced ubiquitylation sites, where the node size depicts the number of ubiquitylation sites on the indicated proteins. The colour code illustrates the mean SILAC ratio of all ubiquitylation sites quantified on the indicated proteins.

IDENTIFICATION OF TARGETS OF NUCLEAR UBIQUITIN LIGASES

Ubiquitin ligases play an integral role in DNA repair and chromatin organisation. Ubiquitin ligases including RNF8/168, BRCA1, and RAD18 are involved in the cellular response to DNA double-strand breaks and replication stress. Recent studies have implicated histone modifying ubiquitin ligases, which play essential roles in chromatin organisation during transcription, in the cellular response to DNA damage. For most of these enzymes, the protein substrates that are modified in response to DNA damage and the functional roles in DNA damage signalling remain obscure. We plan to combine siRNA-mediated knockdown with quantitative MS to identify the physiological substrates of nuclear ubiquitin ligases. If the ubiquitin ligase-substrate relation can be established *in vivo* and/or *in vitro*, we will aim to further characterise the physiological relevance of the modification for the target proteins.

FALK BUTTER

» *Quantitative mass spectrometry is a powerful tool for discoveries in biology and medicine.* «



EDUCATION

2006 Diploma in Biochemistry, University of Leipzig
 2010 PhD in Biochemistry, Ludwig Maximilian University Munich

POSITIONS HELD

2010-2013 Postdoctoral Researcher, Max Planck Institute for Biochemistry, Martinsried
 Since 2013 Group Leader, IMB

GROUP MEMBERS

Núria Casas Vila / PhD student; since 11/2013
 Anja Freiwald / Engineer; since 04/2013
 Marion Scheibe / Postdoc; since 06/2013

RESEARCH OVERVIEW

Mass spectrometry has evolved into a powerful tool to study proteins in an unbiased and global manner. The current improvements in identification accuracy, sample throughput, and data analysis allow us to observe changes in the proteome with unprecedented speed and detail. Our group applies quantitative approaches such as label free quantitation or SILAC (Stable Isotope Labelling with Amino acids in Cell culture) that enable us to directly compare thousands of proteins in complex mixtures. This lets us study changes in protein expression, and we also use this approach for interactomics to identify specific interactions of proteins with targets of interest within a vast number of background binders. We apply mass spectrometry in several biological areas to advance our knowledge of cellular processes such as telomere biology and RNA-mediated gene regulation.

RESEARCH HIGHLIGHTS

HOT1 IS A DIRECT TELOMERE-BINDING PROTEIN

The ends of mammalian chromosomes consist of repetitive TTAGGG repeats that are maintained by telomerases and protected by the shelterin complex from recognition by the DNA damage repair machinery. Using SILAC-based quantitative mass spectrometry, we identified HOT1 (HMBOX1) as a telomere-binding protein in human and mouse. Despite the known significance of telomeres in ageing and cancer, this finding came as a surprise, as it is just the third direct double strand telomere-binding protein reported in 15 years. This exciting discovery opens up new avenues to study the maintenance of telomeres. HOT1 binds independently of the shelterin complex and exhibits distinct features. For example, HOT1 is a dynamic telomere binding protein with increased telomeric localization dependent on higher telomerase *in situ* activity. Indeed, we have shown that HOT1 is able to regulate telomere length in a positive manner and further experiments suggest its involvement in telomerase-dependent telomere homeostasis by a putative function in telomerase recruitment.

QUANTITATIVE INTERACTOME OF THE lncRNA TERRA

Recently, it was recognised that telomeric repeats are actively transcribed giving rise to telomeric repeat-containing RNA (TERRA). TERRA is involved in telomere homeostasis and telomere maintenance and contributes to chromatin organisation at the chromosome end. We reported the first quantitative interactome of TERRA using state-of-the-art high-resolution mass spectrometry and characterised over a dozen new TERRA-associated proteins for their effect on global TERRA levels and TERRA localization at telomeres using RNA interference. Nearly all candidates had an effect on at least one of the investigated features. We thus greatly increased the number of proteins known to influence this long non-coding RNA, providing a basis for further in-depth studies.

ULTRA-CONSERVED ELEMENTS AS TRANSCRIPTION FACTOR BINDING HUBS

Using quantitative mass spectrometry, we reported a large-scale DNA-protein interactome of 193 ultraconserved elements (UCE). This DNA-centric study identified more than 425 proteins able to bind UCE sequences in mouse embryonic stem cells. Comparison with existing *in vivo* data from ENCODE demonstrated that in the majority of cases, when bound factors are detected by chromatin immunoprecipitation, they are also detected by our mass spectrometry-based DNA-protein interaction screen. Based on this large number of sequences we were able to perform *de novo* motif discovery for transcription factors and chromatin-binding proteins, validating reported consensus binding motifs and suggesting new ones for factors that had not been previously investigated. Using these binding motifs we further demonstrated that ultraconservation in these sequences depends on binding of developmental transcription factors, providing firm evidence for the hypothesis that ultraconserved elements act as transcription factor binding hubs.

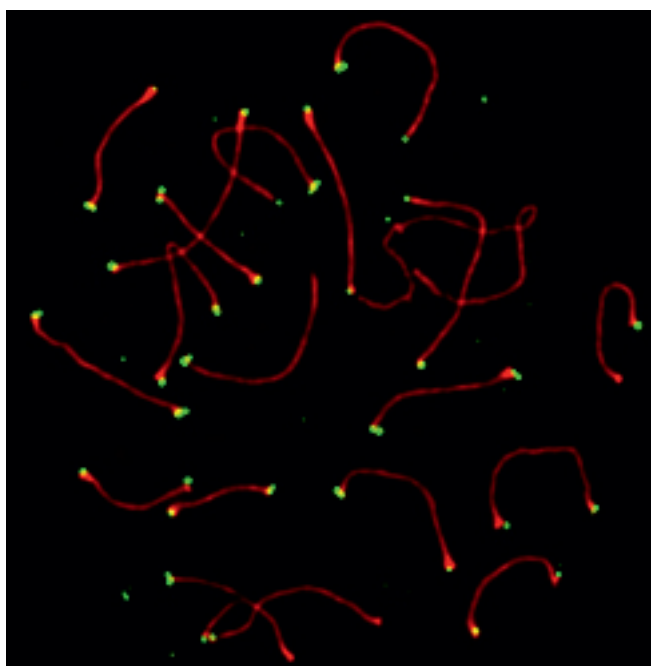


Figure 1. HOT1 localises prominently to telomeres in murine pachytene spreads. Immunofluorescence staining of HOT1 (green) at the chromosome ends with SYCP3 (red) marking the synaptonemal complex/chromosome axis.

FUTURE DIRECTIONS

Based on our previous investigations, we aim to further elucidate the molecular mechanism by which HOT1 executes its function in the telomerase recruitment pathway. Furthermore, we are continuing to use our expertise in quantitative mass spectrometry to study nucleic acid-protein interactions in diverse biological pathways and systems.

SELECTED PUBLICATIONS

Kappei D*, Butter F*, Benda C, Scheibe M, Draškovič I, Stevense M, Novo CL, Basquin C, Araki M, Araki K, Krastev DB, Kittler R, Jessberger R, Londoño-Vallejo JA, Mann M and Buchholz F (2013). HOT1 is a mammalian direct telomere repeat-binding protein contributing to telomerase recruitment. *EMBO J*, 32, 1681-1701. (* joint first authors)

Scheibe M, Arnoult N, Kappei D, Buchholz F, Decottignies A, Butter F* and Mann M* (2013). Quantitative interaction screen of telomeric repeat-containing RNA reveals novel TERRA regulators. *Genome Res*, doi: 10.1101/gr.151878.112. (* co-corresponding authors)

Vitrawong T, Meissner F, Butter F* and Mann M* (2013). A DNA-centric protein-interaction map of ultra-conserved elements reveals major contribution of transcription factor binding hubs to conservation. *Cell Rep*, 5, 531-545. (* co-corresponding authors)

CHRISTOPH CREMER

» *Seeing is knowing,
not just believing.* «



EDUCATION

- 1970 Diploma in Physics,
Ludwig Maximilian University 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, IMB
- Since 2013 Honorary Professor,
Johannes Gutenberg University (JGU) Mainz

RESEARCH OVERVIEW

In the age of superresolution light microscopy, novel "nano-imaging" techniques can be broadly applied, and their reach is constantly growing. However, not all advanced microscopy methods are equally suited for all biological settings. A bottleneck for various superresolution light microscopy techniques has been the use of very specific fluorophores followed by sophisticated sample preparation strategies. To overcome these challenges, we have developed and further improved a variety of superresolution "nanoscopy" approaches, making use of standard fluorophores. In 2013, we considerably enhanced the applicability of Spectrally Assigned Localization Microscopy (SALM), and have realised combinatorial measurements using SALM and Structured Illumination Microscopy (SIM).

GROUP MEMBERS

Alexander Al Saroori / Student Assistant; since 12/2013
 Sven Beichmanis / Student Assistant; 03/2013-08/2013
 Udo Birk / Postdoc; since 02/2012
 Mei-Yu Chen / Guest Researcher, DAAD Fellowship; 08/2013 – 09/2013
 Hyun-Keun Lee / Student Assistant; since 08/2013
 Kirti Prakash / PhD student; since 02/2013
 Aleksander Szczurek / PhD student; since 09/2013
 Sebastian Zeis / Student Assistant; 11/2012 – 03/2013

RESEARCH HIGHLIGHTS

In collaboration with the laboratory of Prof. René Ketting (IMB), we used the SALM technique of Spectral Precision Distance/Position Determination Microscopy (SPDM) with physically modified fluorophores to analyse the distribution of two important proteins of the mouse synaptonemal complex (SC), namely SCP1 (the central element of SC) and SCP3 (the lateral filament of the SC), which are critical for the synapsis of homologous chromosomes during the pachytene stage of meiotic prophase I. We are now using multi-colour SPDM to address questions related to the functional structure of the synaptonemal complex.

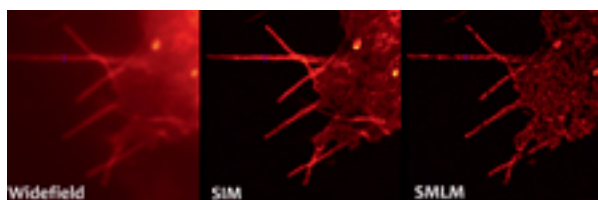
Recent studies have shown the feasibility of using YOYO-1 and cyanine derivatives for SALM imaging of lambda phage DNA stretches. However, DNA-binding molecules suitable for superresolution imaging of chromatin in nuclei are still elusive. Our preliminary data indicate that the experimental conditions of sample preparation and imaging have a substantial influence on the photophysics of appropriate DNA labelling. We are currently searching for further DNA dyes for the SPDM analysis of chromatin nanostructure.

We investigated HEK293 cells expressing Claudin3-YFP after stable transection. A comparative study of conventional and superresolution microscopy (SPDM and SIM) shows complementary information obtained using the different approaches to analyse the same region of interest. It also demonstrates the significant differences in resolution obtained in different advanced microscopy methods.

In order to further extend the application range of the novel super-resolution approaches developed in our group, several technical improvements of the instrumentation have been achieved in 2013, which are described below.

We expanded the SALM technique of Spectral Precision Distance Microscopy (SPDM) to four excitation wavelengths ranging from UV to far-red on the Vertico microscope setup. After a complete redesign of the microscope illumination subsystem, a significant enhancement in the localization accuracy was achieved (at best approximately 2 nm, which corresponds to about 1/250 of the exciting wavelength).

We combined the two complementary methods of Spectral Precision Distance Microscopy (SPDM) and Structured Illumination Microscopy (SIM) to achieve high-precision localization and enhanced structural information in the object plane in a single setup (SPDM-SMI nanoscope). The combination of a SIM image with SPDM data of the same object gives complementary information as the two imaging modalities rely on different contrasting mechanisms. This allows us to generate improved reconstructions of the SIM and SPDM data.



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.

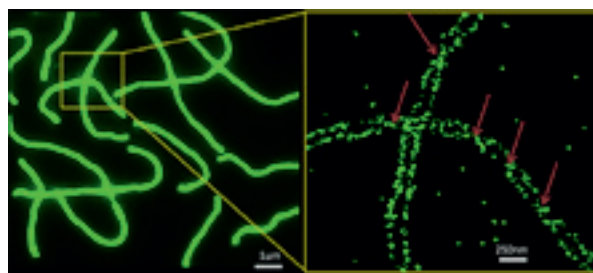


Figure 1. In the wide-field image (left, conventional resolution, scale bar 1 µm), the two strands of the synaptonemal complex (SC) are not resolved. In contrast, the SPDM image (right, scale bar 250 nm) not only shows a clear separation of the two lateral elements of the SC, but also indicates the twisting of the SCP3 protein complex (red arrows).

FUTURE DIRECTIONS

In addition to ongoing projects in the application of our methods, we plan to study the organisation of chromatin in the synaptonemal complex and how the components of the recombination machinery are organised on the nanoscale (in collaboration with René Ketting of IMB).

On the methodological side, we plan to improve the current prototype 3D SPDM superresolution microscope to image multiple types of molecules with different fluorescence emission characteristics. We aim to make the microscope compatible with conventional, commercially available markers and standard specimen preparation protocols. Based on the concept of astigmatism localization, we want to extract 3D information out of a single 2D slice spanning a depth of field of roughly 500nm with a single molecule 3D resolution in the range of a few tens of nanometres.

In 2014, we also plan to combine the methods of Spectral Precision Distance Microscopy (SPDM) and Spatially Modulated Illumination (SMI) microscopy in the Vertico microscope, i.e. create a single SPDM-SMI nanoscope. In addition to the existing SPDM mode, this will allow advanced structured illumination imaging even along the optical axis, thus providing novel options for advanced 3D analysis. Furthermore, we plan to adapt the SPDM-SMI nanoscope to also allow imaging of non-fixed cells with a temperature-regulated incubator.

Our additional ongoing collaborations include: superresolution analysis of (1) small molecules that provoke transcription from methylated promoters (Dr. G. Reid, IMB), (2) Piwi RNA pathway components in zebrafish gonads (Prof. R. Ketting, IMB) (3) DNA hypersensitive sites in cell nuclei (Prof. J. Stamatoyannopoulos, U Washington, and Prof. T. Cremer, LMU Munich) (4) tobacco mosaic viruses (Prof. C. Wege, U Stuttgart), (5) HER2 and HER3-membrane protein receptor clusters (Prof. M. Hausmann, U Heidelberg), (6) DNA damage sites before and after radiation exposure (Prof. J. Dobrucki, U Krakow; Prof. G. Dollinger, UniBw Munich; Prof. M. Hausmann, U Heidelberg; Prof. P. Huber, DKFZ Heidelberg).

Figure 2. Comparison of various fluorescence microscopy methods of the same region of a HEK293 cell expressing Claudin3-YFP (stable transection). A) Conventional wide-field, B) Structured Illumination Microscopy (SIM) and C) Localization Microscopy (SPDM). In (C) a triangulation algorithm was applied to visualise the SPDM data.

RENÉ KETTING

» *The complexity and flexibility of small RNA pathways is staggering.* «



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 Laboratory
2000 – 2004 Postdoctoral Researcher, Hubrecht Laboratory, Utrecht
2005 – 2012 Group Leader, Hubrecht Institute, Utrecht
Since 2010 Professor of Epigenetics in Development, University of Utrecht
Since 2012 Director, IMB Professor, Faculty of Biology, Johannes Gutenberg University (JGU) Mainz

GROUP MEMBERS

- Bruno Albuquerque / PhD student; since 10/2012
Miguel Almeida / PhD student; since 06/2013
Anke Ries / Lab Manager; since 12/2012
Yi-Yen Chen / Postdoc; since 03/2013
Holger Dill / Postdoc; since 02/2013
Sabine Dominitzki / Technician; since 10/2012
Lucas Kaaij / Postdoc; since 11/2013
Laura Krebs / Student Assistant; since 05/2013

RESEARCH OVERVIEW

Our genome encodes tens of thousands of genes, all of which must be properly regulated in order to sustain a healthy human being. In addition, the genome is home to a multitude of selfish elements that self-multiply, and actually make up most of the DNA we carry in our cells. We study how the presence of these endogenous parasites influences gene expression, and how their mobility is controlled. We discovered that a process called RNA interference, or RNAi, has an important role in this interplay. Our goals are to fully understand how RNAi affects the mobile elements in our genome and how this in turn affects gene expression. In addition to RNAi-related effects we are also picking up on more general epigenetic dynamics during early development. We make use of two model systems: the zebrafish and the nematode *C. elegans*.

- Svetlana Lebedeva / Postdoc; since 03/2013
Nail Deniz Pir / MSc student; 02/2013 – 12/2013
Maria Placentino / PhD student; since 11/2013
Stefan Redl / PhD student; since 04/2013
Ricardo Rodrigues / PhD student; since 12/2012
Elke Roovers / PhD student; since 05/2013
Saskia Weiß / Animal Caretaker; since 05/2012
Nadine Wittkopp / Postdoc; since 11/2012

RESEARCH HIGHLIGHTS

We have found that one of the small RNA pathways, the Piwi pathway, can induce an epigenetic switch that can be stably remembered across generations. We identified this phenomenon in *C. elegans*. While analysing transgenic strains for a GFP transgene that is silenced by the Piwi pathway, we noticed that transgene silencing can take place independently of the Piwi pathway, a state we named RNAe (RNA-induced epigenetic silencing). 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, this 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 transgenerational inheritance, we showed it is not required for silencing within one generation. These results indicate that in animals, extremely stable epigenetic states can be induced by small non-coding RNAs, and that these states can be inherited through the germline. Through mutagenesis we have identified novel mutants in which these processes are disrupted. We have identified mutations in several known genes, as well as in novel genes. One of these genes is required for piRNA biogenesis, while the rest are required during the actual silencing processes.

We have also developed a similar GFP-based piRNA silencing system in the zebrafish. This has been very successful, and we are now fully characterising the system and applying it to the study of piRNA-mediated gene silencing in the zebrafish. Ongoing studies of the piRNA silencing mechanism aim to characterise the gene mutants that affect the piRNA pathway. One such gene we have been studying during the past year is Tdrd9. We found this factor to be dispensable for global piRNA biogenesis, but essential for the efficient generation of piRNAs from specific retro-transposons.

Furthermore, we have analysed how the small RNAs associated with the Piwi pathway, piRNAs, are inherited across generations. Using zebrafish as a model, we have shown that genetically distant strains are characterised by distinct piRNA populations. Interestingly, progeny obtained from crosses between these strains show two types of piRNA inheritance. First, piRNA abundance is largely set by the zygotic genome. Second, the ratio between sense and anti-sense piRNAs from a given element has a significantly 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 expression changes in the female germline can be epigenetically transmitted to the progeny.

We also identified a protein that is required for the proper maternal inheritance of piRNAs. Interestingly, this factor is also required for proper assembly of the germ plasm, a cytoplasmic region in the embryo that induces germ cell fate in blastomeres, and associates with many mRNAs known to play a role in germ cell specification and maintenance. These results imply a mechanistic link between germ

cell specification in the embryo and their protection from harmful activities of mobile genetic elements.

Finally, we have described the DNA methylation dynamics during intestinal stem cell development in mice. We found that the DNA methylation state of the stem cell already largely reflects the pattern observed in differentiated daughter cells, implying that at the level of DNA methylation, adult stem cells are already fully primed for differentiation into only a limited set of cell types.

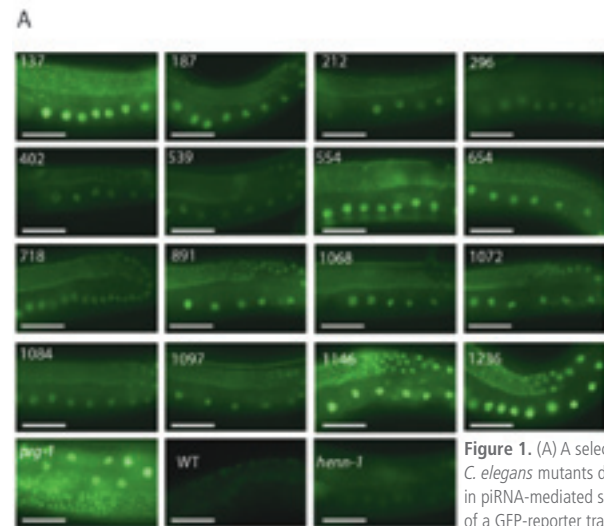


Figure 1. (A) A selection of *C. elegans* mutants defective in piRNA-mediated silencing of a GFP-reporter transgene.

FUTURE DIRECTIONS

The finding that a small RNA pathway can initiate an extremely stable form of epigenetic gene silencing is intriguing and prompts further investigation. Through novel genetic screens in *C. elegans* we will aim to identify the links between small RNA pathways and chromatin, and to decipher how these factors interact. In particular, we are characterising a factor required for piRNA biogenesis, both on a biochemical as well as on a genetic level. Furthermore, we plan to use the zebrafish piRNA sensor system we have developed in studies of the vertebrate silencing mechanism, from the characterisation of histone modifications to the genetic behaviour of silencing. Importantly, we will start to implement superresolution microscopy in order to address particular sub-cellular events during piRNA-mediated silencing.

Finally, since epigenetic effects in germ cells clearly affect the epigenetic state of the early embryo, we will extend our efforts to studying chromatin dynamics during early development. We will do this by investigating both DNA methylation and histone marks during early embryonic stages of zebrafish development. These studies will lead to the identification of a distinct set of enhancers that drives the expression of lineage specifying transcription factors. Further characterisation of these enhancers will be pursued.

SELECTED PUBLICATIONS

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van Wolfswinkel JC, Claycomb JM, Batista PJ, Mello CC, Berezikov E, 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, Cronenbold D, Girard A, van den Elst H, Filippov DV, Blaser H, Raz E, Moens CB, Plasterk RH, Hannon GJ, Draper BW, Ketting RF (2007). A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in Zebrafish. *Cell*, 129, 69-82.

JULIAN KÖNIG

» *Splicing is key for generating protein diversity in humans.* «



EDUCATION

- 2003 Diploma in Biology, Ludwig Maximilian University Munich
- 2008 PhD in Biology, Phillip University Marburg

POSITIONS HELD

- 2008 – 2013 Postdoctoral Researcher, MRC Laboratory of Molecular Biology, Cambridge
- Since 2013 Group Leader, IMB

GROUP MEMBERS

- Simon Braun / PhD student; since 11/2013
- Heike Hänel / Lab Manager; since 11/2013
- Reymond Sutandy / PhD student; since 12/2013

RESEARCH OVERVIEW

Pre-messenger RNA (pre-mRNA) splicing – the removal of introns and joining of exons – is a main pillar of gene expression in higher eukaryotes. Splicing is regulated by activators and repressors that recognise cis-elements at their target exons. It is clear that the recruitment of these RNA-binding proteins (RBPs) is not solely dependent on the underlying RNA sequence, but is strongly shaped by their interactive environment, including direct competition or cooperative recruitment of RBPs as well as modulations of secondary structure. However, the general rules that govern the interactive assembly of pre-messenger ribonucleoprotein (pre-mRNP) complexes remain to be established. Our lab unites a systematic analysis of pre-mRNP assembly during splicing with a molecular understanding of the underlying processes. We employ cutting-edge genomic RNA biology techniques, such as iCLIP and RNA-seq, in combination with biochemical and genetic tools.

RESEARCH HIGHLIGHTS

UNCOVERING hnRNP C AS A GUARDIAN OF THE TRANSCRIPTOME

A particularly interesting protein family to study in the context of splicing regulation and interactive pre-mRNP assembly are the heterogeneous nuclear ribonucleoproteins (hnRNPs). Rivaling histones in their abundance, hnRNP proteins have been described to form hnRNP particles, which have – in analogy to nucleosomes – been referred to as “ribonucleosomes”. Their high abundance and presence along most transcripts suggest them as major players in guiding the binding and function of other RBPs.

We have recently shown that hnRNP C, the core component of hnRNP particles, prevents the binding of the splicing factor U2AF65 to the uridine tracts of thousands of Alu elements that cut across the human genome. These highly abundant retrotransposons pose a great threat to transcriptome integrity as they can be erroneously recognised as exons by the splicing machinery in a process called Alu exonisation. Since U2AF65 is a major player in early 3' splice-site definition, hnRNP C's competition with this protein ensures that cryptic splice sites within the Alu elements are kept silent, which is a vital mechanism to preserve human health.

DEVELOPING iCLIP, A METHOD TO MAP PROTEIN-RNA INTERACTIONS WITH UNPRECEDENTED RESOLUTION

The development of quantitative individual nucleotide resolution CLIP (iCLIP) was key for the understanding of hnRNP C's role in the regulation of Alu exonisation, and enabled us to obtain the first genome-wide and quantitative data on the interaction between two RBPs (Figure 1). This innovative approach allows mapping of protein-RNA interactions at single-nucleotide resolution on a genome-wide scale. It overcomes a number of critical limitations of previous ribonomic methods and offers unprecedented insights into RBP function, as evidenced by the large number of studies already using the technique.

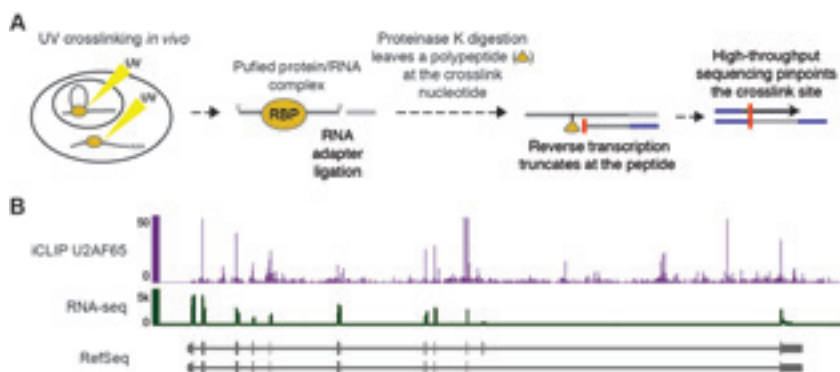


Figure 1. (A) Overview of the iCLIP protocol. Crosslinked protein/RNA complexes are purified from cells. Upon reverse transcription, high-throughput sequencing of truncated cDNA molecules allows the pinpointing of the nucleotide within the RNA that was crosslinked to the RBP. (B) Genome browser view showing U2AF65 iCLIP and RNA-seq data.

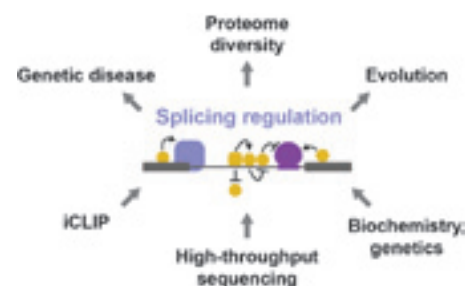


Figure 2. General overview of the research interests and key experimental techniques of the König group.

FUTURE DIRECTIONS

The quantitative description of protein-RNA interactions using iCLIP in combination with the clinically relevant model of Alu exonisation offer an attractive system for understanding the forces of competition and synergy that govern pre-mRNP complexes and splicing regulation. We will focus our efforts on elucidating the machinery for 3' splice-site definition, which has previously been identified as a hotspot for cancer-associated mutations. To achieve this, we will combine *in vivo* and biochemical approaches on a genome-wide scale, which will yield a systemic understanding of pre-mRNP function in splicing regulation (Figure 2). Our group's core competencies are the qualitative, quanti-

tative, and comparative description of RBP binding, which will provide knowledge on how RBPs behave in their complex and interactive environment. These aspects will be addressed in the context of intronic cryptic splice sites as they are present in Alu elements, which will be an important contribution to the understanding of genetic disease.

SELECTED PUBLICATIONS

Zarnack K*, König J*, Tajnik M, Martincorena I, Eustermann S, Stévant I, Reyes A, Anders S, Luscombe NM, Ule J (2013). Direct Competition between hnRNP C and U2AF65 Protects the Transcriptome from the Exonization of Alu Elements. *Cell*, 152, 453-466.

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(* joint first authors)

STEFAN LEGEWIE

» *Modelling approaches provide quantitative insights into cellular heterogeneity.* «



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 Centre (DKFZ), Heidelberg
- Since 2010 Group Leader, IMB

GROUP MEMBERS

- Stephan Baumgärtner / PhD student; since 11/2011
- Kolja Becker / PhD student; since 08/2013
- Mihaela Enculescu / Postdoc; since 10/2013
- Lu Huang / Postdoc; since 10/2012
- Matthias Jeschke / Postdoc; 07/2011 - 03/2013

RESEARCH OVERVIEW

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

- Monika Kuban / Research Technician; since 10/2012
- Tamara Milhaljev / Postdoc; since 11/2010
- Shankarrao Patil / PhD student; since 10/2013
- Uddipan Sarma / Postdoc; since 07/2013
- Marcel Schnieder mann / Intern; since 09/2013

RESEARCH HIGHLIGHTS

MODELLING GENE REGULATORY NETWORKS

One of the major focuses of our research group is the quantitative modelling of gene regulation. We use various modelling approaches to describe different aspects of this process. Network models derived from large-scale data sets take into account the wiring of many genes. We further study small genetic modules, and these models describe mechanistic details at the single promoter level. An example of this is the control of the peptide hepcidin, whose expression level reflects the blood iron levels and controls iron resorption.

Mammals maintain iron homeostasis by a negative feedback loop through hepcidin expression. Molecular perturbations in this homeostasis loop lead to iron-related diseases such as hemochromatosis or anemia of inflammation. To understand the dynamics of the iron homeostasis circuitry in health and disease, we collaborated with Martina Muckenthaler (Heidelberg) to investigate how the expression level of hepcidin is regulated by combining experiments with mathematical modelling. We derived a multi-scale model that describes the signalling network and the gene promoter controlling hepcidin expression. Possible scenarios of hepcidin regulation were tested experimentally using reporter gene assays in human hepatoma cells. The physiological relevance of these findings was interpreted using a network model of iron metabolism *in vivo*. The analysis showed that the presence of multiple redundant regulatory elements in the hepcidin gene promoter facilitates homeostasis, because changes in iron blood levels are sensed with high sensitivity. Our analyses also explain why inflammatory stimuli like IL6 strongly perturb iron homeostasis in a clinically relevant disease, anemia of inflammation: IL6 stimulation establishes molecular competition at the hepcidin promoter, implying that hepcidin expression no longer appropriately responds to changes in plasma iron levels. Taken together, our simulations reveal why the iron homeostasis circuit is sensitive to perturbations implicated in disease. We are currently testing these hypotheses *in vivo* using mouse models.

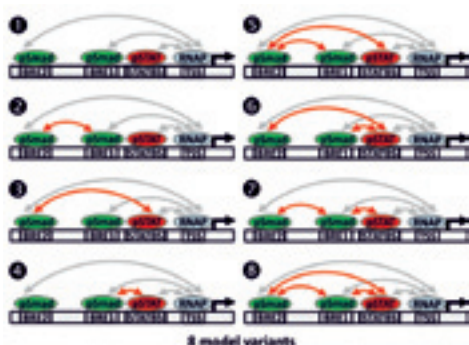


Figure 1. Mathematical models of hepcidin transcription. Phospho-STAT/SMAD transcription factors (TFs) bind to cognate DNA sequence elements (STATBS, BRE1/2). Transcription occurs if STAT/SMAD recruit RNA polymerase (RNAP) to the transcription start site (TSS; grey arrows). Promoter occupancy is modulated by TF-TF and TF-RNAP protein-protein interactions (red arrows).

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 experiences certain outcomes. It is thus important to understand how the heterogeneity of cellular signalling can be modulated. We study how fluctuations in signal transduction arise from cell-to-cell variability in the concentration of signalling proteins.

In cooperation with the Niehrs group at IMB, we previously showed that negative feedback loops suppress fluctuations in the BMP signalling cascade. In our current research efforts, we analyse how variability can be modulated in more complex signalling networks. In particular, we use analytical theory to characterise how noise is amplified or dampened in multi-step signalling cascades like the mammalian and yeast MAPK pathways. We found that complex MAPK signalling systems can be inherently robust to protein concentration fluctuations. We further defined how the kinetics and site of action of feedforward and negative feedback loops in the cascade determine the variability of the signalling output. This has helped us to identify the design principles of signalling cascades that promote robustness. Our results may explain why certain signalling cascades like the yeast pheromone pathway show switch-like decision-making with little cell-to-cell variability. We apply theory to interpret live-cell imaging data of yeast MAPK signalling networks.

The heterogeneity of signalling contains valuable information about the underlying molecular network. However, comprehensive single-cell measurements are typically restricted to certain signalling intermediates, while less informative, population-based assays can be done on a higher throughput. We try to integrate these complementary data sources by simultaneously describing single-cell and population data with an ensemble of mathematical models, each describing a single cell. This approach allowed us to discriminate mechanisms of caspase-8 activation at the plasma membrane, and showed that clustering of death receptors is an important step in the initiation of apoptosis.

FUTURE DIRECTIONS

We are currently studying gene expression in response to extracellular stimulation in cooperation with other IMB groups. We investigate the temporal dynamics of epigenetic events and transcription at oestrogen-sensitive promoters in collaboration with the Reid group. Together with the Tiwari group, we systematically perturb the levels of transcription factors controlling the epithelial-to-mesenchymal transition, and use mathematical modelling to characterise the dynamics of the transcription factor network and its impact on target gene expression responses.

SELECTED PUBLICATIONS

Stelnic 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.

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 signaling range and canalises development. *PNAS*, 108, 10202-10207.

Legewie S, Herzel H, Westerhoff HV and Blüthgen N (2008). Recurrent design patterns in the feedback regulation of the mammalian signalling network. *Mol Syst Biol*, 4, 190.

(*joint first authors)

CHRISTOF NIEHRS

» *The dynamic nature of DNA methylation has finally been recognised.* «

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 Centre (DKFZ), Heidelberg
- 2000 Professor of Molecular Embryology, DKFZ, Heidelberg
- Since 2010 Founding Director, IMB
Professor, Faculty of Biology, Johannes Gutenberg University (JGU) Mainz

GROUP MEMBERS

- Khelifa Arab / Postdoc; since 11/2011
- Carolin Brandscheid / Student Assistant; 06/2013–12/2013
- Tamara Dehn / Animal Caretaker; since 06/2011
- Mathias Gierl / Postdoc; since 07/2011
- Wolfram Gruhn / PhD Student; 09/2010 - 06/2013
- Dandan Han / PhD Student; since 12/2013
- Sabine Karl / Postdoc; since 07/2011
- Manuel Leichsenring / PhD Student; since 04/2011
- Medhavi Mallick / PhD Student; since 08/2012
- Konstantina Marinoglou / Postdoc; 09/2011–08/2013

RESEARCH OVERVIEW

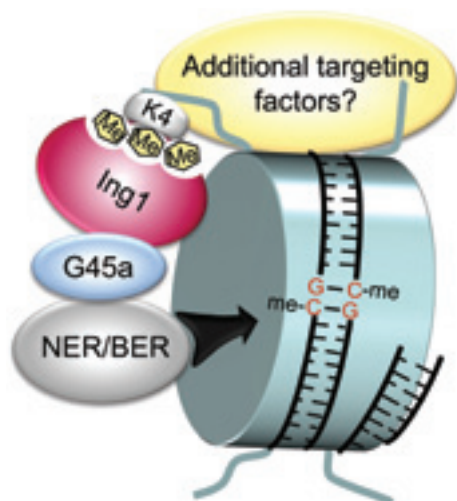
In the DNA of many multicellular organisms, DNA methylation is a common epigenetic mark associated with gene silencing. DNA methylation is a dynamic process and can be reversed by enzymatic demethylation, a process that is still incompletely understood. DNA demethylation is a widespread phenomenon and occurs in plants as well as in animals, during development, in the adult, and during somatic cell reprogramming of pluripotency genes. We have shown 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 using biochemical, molecular biological as well as cell biological approaches, and employ the mouse model as a genetic system.

- Bernadette Mekker / PhD Student; since 07/2010
- Svetlana Melcea / PhD Student; since 07/2013
- Michael Musheev / Postdoc; since 07/2011
- Sandra Rölle / Research Technician; since 04/2011
- Andrea Schäfer / Postdoc; since 09/2010
- Lars Schomacher / Postdoc; since 07/2011
- Dominik Sebastian / PhD Student; 07/2011 - 03/2013
- Susanne Spengler / Lab Manager; since 09/2013
- Ulrike Stapf / Technician; since 05/2011
- Annika von Seggern / Technician; since 06/2011

RESEARCH HIGHLIGHTS

DNA methylation at 5-methylcytosine (5mC) of CpGs is a common epigenetic mark in metazoa, and plays important roles in regulating gene expression, genomic imprinting, X chromosome inactivation, genomic instability, embryonic development, and cancer. It has become clear that DNA methylation is reversible by enzymatic active DNA demethylation, with examples in plants, animal development, cancer, and immune cells, yet the molecular mechanisms underlying active demethylation are only beginning to be understood. We have shown that growth arrest and DNA damage protein 45a (Gadd45a) mediates active DNA demethylation. Gadd45 is member of a small gene family of stress response genes encoding histone fold proteins. Gadd45 proteins are multifunctional and regulate a range of cellular processes, including DNA repair, proliferation, apoptosis, and differentiation. Gadd45a-mediated demethylation involves recruitment of the nucleotide excision repair and/or base excision repair machineries.

DNA demethylation of target genes by Gadd45 proteins 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 are the cofactors involved?



We discovered that p33ING1b (ING1b) serves as a cofactor for Gadd45. ING1b shares many properties with Gadd45a: it is a tumor suppressor, is induced by stress or UV irradiation, inhibits cell growth by interacting with p21waf1, and promotes nucleotide excision repair. Furthermore, Ing1 mouse mutants are radiation-sensitive and tumor-prone, like Gadd45a mutants. Ing1 contains a PHD finger domain, which specifically binds to histone H3 trimethylated at lysine 4 (H3K4me3). We found that ING1b is a novel factor required for gene-specific DNA demethylation. Gain and loss-of-function experiments showed that Ing1 functions during Gadd45a-mediated DNA demethylation. ChIP experiments and manipulation of cellular H3K4me3 levels demonstrate that ING1b recruits Gadd45a to the promoter of the cancer testis antigen MAGEB2. Genome-wide gain and loss-of function experiments identified additional target genes regulated synergistically by Gadd45a and ING1b in a H3K4me3-dependent fashion. Thus, ING1b is a novel factor essential for targeting DNA demethylation. Specifically, the results show that histone methylation is required to direct DNA demethylation. The fact that H3K4me3 regulates DNA demethylation is in line with the previous observation of a reciprocal relationship between DNA methylation and histone modifications.

Figure 1. Model for Gadd45a targeting by Ing1 during DNA demethylation. Chromatin at the transcription start site is occupied by H3K4me3. Ing1 acts as adapter between H3K4me3 and Gadd45a, likely requiring additional unknown targeting factors. The nucleotide excision (NER) - or base excision (BER) repair machinery is engaged by Gadd45a and excises 5mC, which is replaced by unmethylated cytosine via DNA repair synthesis (not shown), thereby promoting demethylation.

FUTURE DIRECTIONS

Our discovery of ING1b as a targeting factor for Gadd45 has raised new questions. What are the genome-wide target genes of Gadd45/Ing1? How does their function in DNA demethylation relate to the biological processes in which Gadd45/Ing1 have been implicated, such as autoimmunity, ageing, and cancer? These questions can now be addressed in mutant mice. Moreover, H3K4me3 is present at many more loci than are regulated by Gadd45-mediated DNA methylation, suggesting additional targeting mechanisms beyond ING1b. Indeed, we had previously shown that Gadd45 is an RNA-binding protein, and we are analyzing which RNAs are physiologically bound to Gadd45 and what their role is. Is the role of Gadd45a-bound RNAs purely structural or is RNA involved in e.g. specific targeting of demethylated DNA regions? We plan to study the significance of ncRNAs in Gadd45-mediated DNA methylation.

SELECTED PUBLICATIONS

Schäfer A, Karaulanov E, Stapf U, Döderlein G and Niehrs C (2013). Ing1 functions in DNA demethylation by directing Gadd45a to H3K4me3. *Genes Dev*, 27, 261-73.

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

GEORGE REID

» *“We dance round in a ring and suppose, but the Secret sits in the middle and knows.”* «

Robert Frost (1874-1963)



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, IMB

GROUP MEMBERS

- Iryna Charapitsa / Postdoc; since 07/2011
- Christoph Fritsch / PhD student; since 09/2012
- Wolf Gebhardt / Postdoc; since 08/2011
- Ina Kirmes / PhD student; since 06/2012
- Monika Kuban / Research Technician; since 10/2012
- Martha Paluschinski / MSc student; 01/2013-11/2013

RESEARCH OVERVIEW

The functional template of gene expression is chromatin, which generates multiple regulatory barriers that have to be overcome prior to the initiation of RNA synthesis. Our group has demonstrated that dynamic methylation of DNA is an inherent process in the expression of tightly regulated genes. Through synchronizing gene expression in cell populations and then describing, on a genome-wide scale, the methylation status of single CpG dinucleotides, we have discovered several thousand genomic regions that undergo dynamic changes in methylation. Additionally, we have discovered novel small molecules that can overcome the repressive effect of DNA methylation through inducing phosphorylation of methyl-binding proteins. We have also applied these methodologies to investigate the effect of oxygen deprivation in the heart and have found that profound epigenetic changes occur in response to reperfusion following oxygen deprivation.

RESEARCH HIGHLIGHTS

As a partner within a pan-European consortium of experimental and computational biologists (SYNERGY), we have generated global profiles of chromatin in oestrogen-dependent breast cancer cells following synchronous release from oestrogen starvation. Bayesian interpretation of these global datasets has generated new bioinformatic tools, characterised the dynamics of oestrogen early response genes and suggested clinical markers of poor prognosis in breast cancer. Moreover, genome-wide bisulphite sequencing at multiple time points after release from transcriptional blockade indicates several thousand genomic regions undergo dynamic changes in DNA methylation (Figure 1). The majority of dynamic regions are associated with known transcriptional start sites. This result confirms and extends our previous work on cyclical DNA methylation. We are at present performing analyses at shorter time intervals and investigating the role that TET-mediated sequential oxidation of 5-methyl-cytosine and the base excision repair process may have in these processes.

We previously described how the proximal promoter DNA of certain genes could undergo rapid DNA demethylation, mediated by base excision repair, which suggested that it should be possible to discover small molecules that act to overcome DNA methylation. We performed a phenotypic screen where fully methylated plasmid DNA was transfected into cells that were then incubated with test compounds in the hopes of discovering small molecules that could reactivate expression of a reporter gene. Two chemically distinct series of compounds were discovered that overcome the repressive effect of DNA methylation, although this was not associated with active demethylation of plasmid DNA. Subsequent mechanistic analysis indicated that the compounds activate phosphoinositol signalling, which in turn results in phosphorylation of the 5-methyl CpG-binding protein MeCP2. Recent reports have described how phosphorylation of MeCP2 can block the subsequent recruitment of repressive complexes, providing an alternative mechanism to overcome DNA methylation on a global scale. Additionally, these compounds induce a profound, global increase in permissive histone marks. We have initiated a global siRNA screen to identify components of the pathway that is perturbed by our compounds, with the expectation that this may also identify candidate targets that directly interact with our small molecules. This avenue of research may provide a first-in-class epigenetic intervention applicable to cancer, inflammation, and stem cell biology.

Clinical evidence implicates epigenetic processes as having a significant role in the heart. For example, ischaemia preconditioning protects against subsequent myocardial infarction and the widely used anti-tumour agent doxorubicin induces cardiomyopathy that often presents many years after exposure. To investigate these phenomena, we performed ischaemia reperfusion on mouse hearts, followed by immunocytochemistry of heart sections using a panel of antibodies against histone marks. Upon reperfusion the affected area

of the heart acquires activating histone modifications (acetylation of histone H3 and H3K4 trimethylation) and loses the repressive mark of H3K27 trimethylation. Moreover, these effects can be recapitulated in cultured myofibroblasts following transient hypoxia. Massively parallel RNA sequencing of heart tissue at 0, 4, and 24 hours following reperfusion indicates that the transcription factors AP1, EGR1, and hypoxia inducible factor 1a are likely to mediate the extensive changes in gene expression profiles that occur following recovery from local oxygen deprivation. Intriguingly, this set of transcription factors are down-regulated throughout the heart upon exposure to doxorubicin.

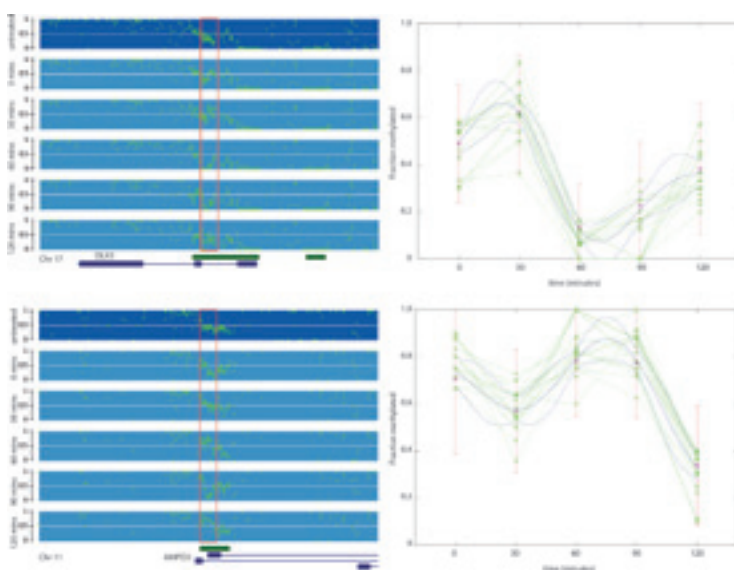


Figure 1. Sequential whole genome bisulphite sequencing of DNA from transcriptionally synchronised cells identifies several thousand loci that undergo dynamic methylation. Two examples (top and bottom) are shown. The panels (left) and graphs (right) show methylation at genomic loci and in unsynchronised cells (top lane) and at 0, 30, 60, 90, and 120 minutes after release.

FUTURE DIRECTIONS

We will continue to investigate the mechanisms of dynamic DNA methylation, through describing the involvement of TET-mediated sequential oxidation of 5-methyl cytosine and base excision repair at specific loci. The role of epigenetic marks in normal and pathological cardiology is almost entirely unexplored. Further describing these processes in cardiac preconditioning, and additionally in doxorubicin mediated cardiac hypertrophy, will provide a novel conceptual framework through which rational intervention strategies can be formulated.

SELECTED PUBLICATIONS

Reid G, Gallais R and Métivier R. (2009). Marking time: the dynamic role of chromatin and covalent modification in transcription. *Intl J Biochem Cell Biol*, 41, 155-163.

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.



HOLGER RICHLY

» *The environment impacts epigenetic regulation via chromatin signalling pathways.* «

EDUCATION

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

POSITIONS HELD

- 2005 - 2011 Postdoctoral Researcher, Centre for Genomic Regulation (CRG), Barcelona
- Since 2011 Group Leader, IMB

GROUP MEMBERS

- Jonathan Byrne / PhD Student; since 08/2011
- Shalaka Chitale / PhD Student; since 02/2013
- Ekaterina Gracheva / PhD Student; since 08/2011
- Fabian Jung / Research Assistant; 09/2012-09/2013
- Aysegül Kaymak / PhD Student; since 02/2013
- Rebeca Medina / Research Technician; since 08/2013
- Thaleia Papadopoulou / PhD Student; since 10/2011
- Stephanos Papaefstathiou / Research Technician; 10/2011-07/2013
- 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 still puzzling how the environment impacts epigenetic regulation and we therefore study how signalling pathways crosstalk with chromatin. Central to our research are the questions of how epigenetic components are recruited to chromatin and how epigenetic marks are specifically read. The capacity of epigenetic components to read and interpret histone marks gives rise to molecular pathways that we intend to decipher with our research. We make use of next-generation sequencing, chromatin biochemistry, high-resolution microscopy, and *C. elegans* biology and genetics to understand the molecular orchestration of epigenetic events.

RESEARCH HIGHLIGHTS

EPIGENETIC MECHANISMS OF EMBRYONIC STEM CELL DIFFERENTIATION

Non-coding RNAs have been called the dark matter of the genome because of their abundance but still 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 found that Polycomb complexes and other epigenetic players team up with non-coding RNAs to fix either a pluripotent or differentiating state in mouse embryonic stem cells. In brief, we have identified novel RNA-protein complexes and have gained insight into their functional roles and their impact on the genomic architecture. Our research suggests an essential function for ncRNAs as a structural and functional player in dynamically changing the conformation and the three-dimensional structure of chromatin in the course of gene activation.

IMPACT OF EPIGENETIC COMPONENTS DURING UV-TYPE DNA REPAIR

Research on DNA repair has traditionally 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 in chromatin especially in an epigenetic context. 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 ubiquitination signals govern the regulation of epigenetic components and repair factors. The main goals of this project are to understand the complex interplay of chromatin and DNA repair factors and to decode the meaning of differently linked ubiquitin chains on epigenetic components and DNA repair proteins.

EPIGENETIC DETERMINANTS OF AGEING

Ageing is certainly one of the most interesting but least understood processes of multicellular 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 are a model organism for ageing research and have been used to reveal genes that regulate the lifespan of the animal. We have set out to study the epigenetic landscape of *C. elegans* during ageing utilizing a host of next-generation sequencing techniques and sophisticated proteomics. We have developed an siRNA-based screening method to isolate novel epigenetic players affecting organismal ageing, which has already provided insight into potential epigenetic ageing markers. We will continue to employ this descriptive approach to better understand the molecular mechanisms of selected epigenetic components.

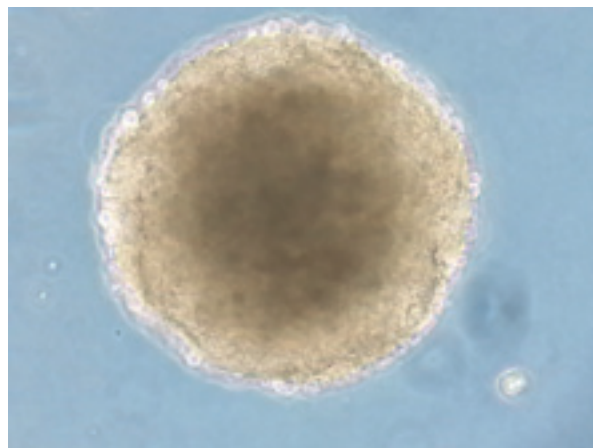


Figure 1. Mouse ES cells undergoing differentiation can be converted into embryoid bodies, which allows the study of the development of the three germ layers *in vitro*.

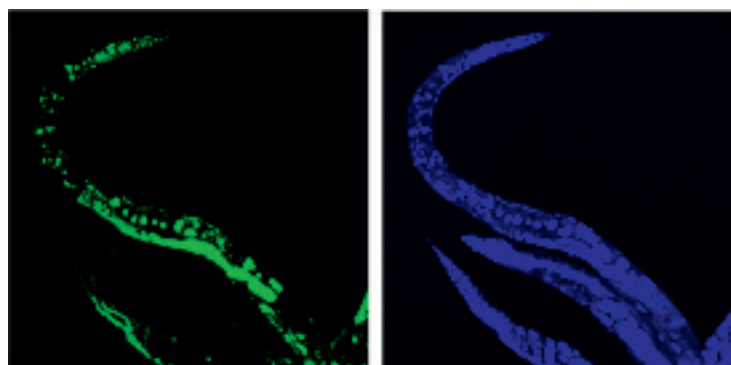


Figure 2. Specific methylation pattern of ageing worms. Immunofluorescence of ageing nematodes with a specific histone-methyl antibody (green). DNA is stained with DAPI (blue).

FUTURE DIRECTIONS

In addition to our findings concerning the impact of ncRNAs on stem cell differentiation, we and others have discovered a role for RNA in chromatin during DNA repair. The process of ageing also seems to be accompanied by specific functions of various classes of RNA. We plan to further investigate the 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 Dis*, 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.

JEAN-YVES ROIGNANT

» *We study how chromatin and non-coding RNA impact alternative splicing during development.* «

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
 Since 2012 Group Leader, IMB

GROUP MEMBERS

- Junaid Akhtar / Postdoc; since 02/2012
 Laura Götzinger / Student Assistant; since 08/2013
 Giriram Kumar / Research Assistant; since 07/2013
 Anna Lena Leifke / Research Technician; since 07/2013
 Tina Lence / PhD student; since 07/2013
 Cristiana Lungu / PhD student; 07/2012-02/2013
 Claire Mestdagh / PhD student; since 01/2012

RESEARCH OVERVIEW

Pre-mRNA splicing is a fundamental process by which introns are removed from primary transcripts and exons are joined together to form the mature transcripts. This process plays an essential role in constitutive and regulated gene expression in eukaryotes, and flawed splicing is associated with a large number of human diseases and cancer. Intriguingly, despite its relative early discovery, the precision and complexity of intron removal is not fully understood yet. The research in our lab aims to understand the roles and mechanisms of pre-mRNA splicing during development in *Drosophila*. We are particularly interested in investigating the influence of chromatin structure in this process. To study these questions we focus on the mechanistic function of the exon junction complex during splicing, as well as on the effect of epigenetic marks on alternative splicing during embryonic development. We expect our research will have a major impact on the understanding of this recently uncovered connection between chromatin and pre-mRNA splicing.

RESEARCH HIGHLIGHTS

ROLE OF THE EJC IN SPLICING HETEROCHROMATIC TRANSCRIPTS

The exon junction complex (EJC) is a highly conserved ribonucleo-protein complex, which binds RNAs during their splicing and remains strongly associated with them following export to the cytoplasm. This complex is involved in several cellular post-transcriptional processes during development, including mRNA localization, translation, and degradation. Our work recently uncovered a role for the EJC in photoreceptor differentiation in *Drosophila melanogaster*. Investigation of the mechanisms has revealed a novel nuclear function for the EJC in the splicing of MAPK transcripts and other similar transcripts containing long introns preferentially expressed from heterochromatic loci. We are particularly interested in understanding the importance of these features with respect to the EJC requirements. Introns of heterochromatic genes are rich in repetitive DNA composed of transposons, retrotransposons, and satellite sequences. Furthermore, heterochromatin genes are embedded in a compact chromatin structure, which can also directly or indirectly influence pre-mRNA splicing. Recently, we have shown that depletion of the EJC impaired heterochromatin integrity at the MAPK locus, suggesting that the loss of the EJC might indirectly affect pre-mRNA splicing of heterochromatic transcripts by altering the structure of the chromatin. Additional experiments are currently in progress to validate this hypothesis. We have also identified two proteins that co-immunoprecipitate with the EJC and which have previously been shown to modulate the structure of heterochromatin. We are currently validating these interactions and examining their functional roles *in vivo*.

ROLE OF THE EJC IN THE piRNA PATHWAY

To gain more insight into the mechanism of the EJC in the splicing process, we set out to look for other developmental targets. The EJC has previously been shown to control axis polarity in *Drosophila* ovaries. We have discovered that this function might be mediated through the regulation of the small piRNA pathway. Indeed, we have found that the EJC is required for preventing transposon accumulation in *Drosophila* ovaries. This function is mediated in part through its requirement in the splicing of the *piwi* transcript, which encodes a member of the Argonaute family. Intriguingly, *piwi* is a euchromatic gene that does not contain large introns. However, we have found that the retained intron contains degenerated transposon sequences, which is a hallmark of heterochromatic introns. Therefore, our data demonstrate a role for the EJC in the piRNA pathway through the control of the splicing of an essential effector. We have further observed that the retained intron is already incompletely spliced in the wild type and requires the flanking introns together with the EJC to increase its removal. We propose a model in which the EJC is rapidly deposited to exon junction after the splicing of *bona fide* introns and subsequently facilitates the splicing of flanking introns containing divergent canonical cis-acting sequences (Figure 1). This model nicely explains how the EJC controls intron removal despite its late recruitment during the splicing process.

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, 273-81.

Roignant JY and Treisman JE (2010). Exon junction complex subunits are required to splice *Drosophila* MAP kinase, a large heterochromatic gene. *Cell*, 143, 238-50.

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, 1913-22.

ROLE OF CHROMATIN IN ALTERNATIVE SPLICING REGULATION DURING CELL FATE DECISION

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, have been shown to be linked to exon-intron architecture in many species. However, their causal role in alternative splicing remains to be clearly demonstrated, and we still lack a clear genome-wide picture of this relationship *in vivo*. We have implemented a system to isolate a pure population of cells from *Drosophila* embryos in order to investigate the contribution of histone marks to splicing events in several tissues in a genome-wide manner. Computational analysis is used to establish initial correlations, which are subsequently validated *in vivo* using the power of *Drosophila* genetics. The use of the fly model, as opposed to vertebrates, to answer these questions should simplify the interpretation of this relationship, due to the absence of DNA methylation and the reduced number of alternative splicing events.

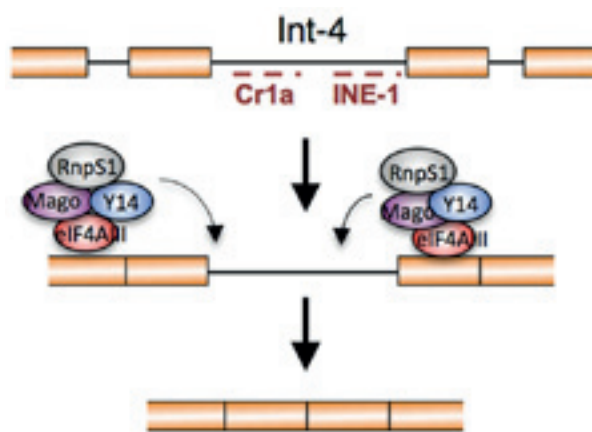


Figure 1. Model for rnpS1 function in the splicing of *piwi* transcript. The presence of degenerated transposon in intron 4 render its splicing challenging. The rapid splicing of introns 3 and 5 leads to the deposition of the EJC at exon junctions, which in turn facilitates the recruitment of the spliceosome for the removal of intron 4.

FUTURE DIRECTIONS

The specificity of the EJC in the splicing process remains poorly understood. The reduction in heterochromatin marks at the MAPK locus could provide one part of the explanation of this effect. But how general is this decrease? Is there a correlation between the loci that require the EJC to maintain their histone modifications and those that require this complex for their splicing? Genome-wide expression studies should answer these questions. Furthermore, what is the role of transposon sequences in the EJC's requirement? How do they affect intron removal? Since half of the genome in mammals is comprised of transposons or remnants of transposons, it is crucial to understand their influence in gene expression and RNA processing.

NATALIA SOSHNIKOVA

» *We focus on functional and molecular characterisation of stem cells in mouse embryonic intestine.* «

EDUCATION

- 1998 Diploma with honours 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
- Since 2012 Group Leader, IMB

GROUP MEMBERS

- Stefanie Grimm / Research Technician; since 01/2012
- Juri Kazakevych / PhD student; since 09/2012
- Christina Krienke / PhD student; since 02/2013
- Liron-Mark Lavitas / Postdoc; since 07/2013
- Lira Nigmatullina / PhD student; since 04/2013
- Valerio Del Vescovo / Postdoc (Marie Curie Fellowship); 12/2012-11/2013

RESEARCH OVERVIEW

We are interested in the regulatory mechanisms underlying vertebrate pattern formation during embryogenesis and tissue homeostasis in adults. Our current focus is mouse small intestine. Homeostasis of the intestinal epithelium requires rapid and continuous regeneration. The intestinal stem cells (ISCs) constantly replenish absorptive and secretory cell populations. The generation of specific cell types from the ISCs is determined by the combinatorial actions of diverse signals that impose developmental restrictions on progenitor cells. Transcription factors and histone-modifying complexes are instrumental for the maintenance of stem cell potential and for a stable determination of cell fate. We are investigating when and how the ISCs are defined and integrated into the future stem cells compartment during embryogenesis. To functionally characterise different epithelial cell populations within the embryonic small intestine we are using both mouse genetics and genomics tools.

RESEARCH HIGHLIGHTS

The adult small intestine is a highly organised structure consisting of two compartments: villi and crypts. The intestinal stem cells (ISCs) residing in the crypts give rise to differentiated enterocytes, goblet and entero-endocrine cells, which move upward along the villi structure. The other differentiated cells, Paneth cells, are located in the crypts and form the stem cell niche. In contrast to postnatal and adult periods, the embryonic small intestine is a simple tube containing cytologically identical epithelial cells. Little is known about which and how many cell types are within the embryonic intestinal epithelium. We have defined the whole transcriptome of both embryonic intestinal endoderm at different developmental stages and adult ISCs using RNA sequencing. We have found that the embryonic endoderm and adult stem cells have different transcriptional programs. Based on the correlation between transcriptional activity and chromatin states, including DNA and histone methylomes, we have learned

about potential functions of chromatin-modifying complexes in the regulation of gene expression during ISC maintenance and differentiation. To further elucidate the molecular pathways regulating formation, maintenance and differentiation of the ISCs we have established crypt-villus organoid cultures for conducting *ex vivo* shRNA screens (Figure 1A). Use of various fluorescent markers expressed under the control of the endogenous gene promoters at the specific stages allows us to monitor the switch between embryonic and adult stem cell states. Moreover, we have identified the number of endodermal cell populations within the embryonic intestine. We are applying tamoxifen-inducible genetic tools to evaluate how these specific embryonic cell populations contribute to the adult intestine, as well as to test their potential function in patterning this tissue *in vivo* (Figure 1B).

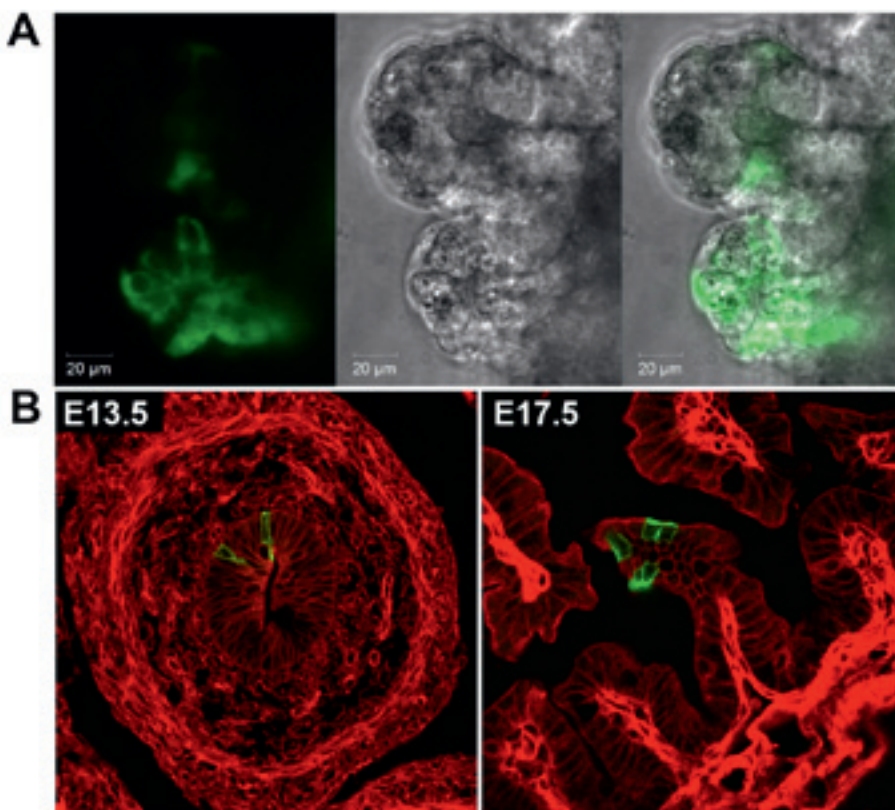


Figure 1. (A) An example of the crypt-villi organoid culture from *Lgr5-GFP-ires-CreERT* mice. The ISCs are GFP positive (green). (B) An example of a lineage tracing analysis. Confocal images of the small intestine from mouse embryos administered tamoxifen at E11.5 and analysed at E13.5 (left panel) or E17.5 (right panel). A specific cell population is labelled by EGFP (green), whereas the rest of the cells are expressing tdTomato (red).

FUTURE DIRECTIONS

We will characterise the novel cell populations identified within the embryonic endoderm on a molecular level. Using organoid mini-gut cultures we will address the mechanistic bases for the transcriptional differences between embryonic and adult intestinal stem cells. We are also interested in how environmental cues (microbiota) may influence

the maintenance and differentiation of the adult ISCs in health and after induced injury. A better understanding of these processes may lead to development of novel stem cell and regenerative medicine therapies.

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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.

VIJAY TIWARI

» *We study how cell fates are specified during development and misspecified in disease.* «

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 (FMI) for Biomedical Research, Basel
- Since 2012 Group Leader, IMB

GROUP MEMBERS

- Sarbashis Das / Postdoc; since 02/2013
- Angela Garding / Postdoc; since 04/2012
- Johannes Jung / PhD student; since 05/2013
- Federico Marini / PhD Student; 01/2012–07/2013
- Sanjeeb Sahu / PhD student; since 05/2013
- 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 our lab is aimed at understanding the mechanisms by which epigenetic machinery and transcription factors contribute to transcriptional reprogramming that defines cell fate during development, and how this communication is altered in diseases such as cancer. We employ a multidisciplinary approach combining cutting-edge epigenetics and genomics 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 and carcinogenesis
- Transcription factors and lineage specification
- Epigenomics of cell type specification
- Systems biology of gene regulatory networks

RESEARCH HIGHLIGHTS

GENE REGULATION BY TOPOISOMERASES DURING CELLULAR DIFFERENTIATION

Topoisomerases resolve superhelical strains in DNA. However, their role in gene regulation, especially during development, remains poorly understood. We find that the expression of Topo II isoforms, TOP2 α and TOP2 β , is characteristic of dividing and postmitotic tissues, respectively. TOP2 α and β preferentially bind to active gene promoters in ES cells and neurons respectively. Common targets of TOP2 α and TOP2 β are housekeeping genes, while unique targets are involved in proliferation/pluripotency and neurogenesis, respectively. TOP2 α activity further confers a set of developmental genes an accessible chromatin state in ES cells as a prerequisite for their activation upon differentiation. Absence of TOP2 α activity affects pluripotency and differentiation potential of ES cells while TOP2 β deficiency causes premature death of postmitotic neurons. These defects result primarily from misregulation in the expression of Topo II target genes. These results uncover a distinct division of labour between the two topoisomerase II isoforms during differentiation (Figure 1).

CROSSTALK BETWEEN TRANSCRIPTION FACTORS AND EPIGENETIC MACHINERY IN EPITHELIAL TO MESENCHYMAL TRANSITION

Epithelial to mesenchymal transition (EMT) plays crucial roles in generating the body plan by contributing to the morphogenesis of multiple tissues and organs during embryonic development. It further contributes to wound healing and tissue regeneration in adults. However, its aberrant activation is known to cause organ fibrosis and promote carcinoma progression through a variety of mechanisms. This cell fate remodeling capacity involves loss of epithelial markers such as E-cadherin, cytoskeletal reorganization, morphology changes, loss of cell polarity and acquisition of mesenchymal markers like N-cadherin and vimentin. Using genome-wide transcriptome profiling we discovered a large number of coding and noncoding RNAs that are modulated during stepwise progression of TGF- β -induced EMT (unpublished observations). Computational analysis revealed motifs for certain novel transcription factors and epigenetic regulators at the promoters of genes that are induced during EMT. Interestingly, depletion of these proteins perturbs EMT. Furthermore, these factors are also highly expressed in invasive cancers. We next plan to gain mechanistic insights into how these novel transcription factors and epigenetic regulators contribute to the transcriptional reprogramming underlying cell fate changes during EMT (Figure 2).

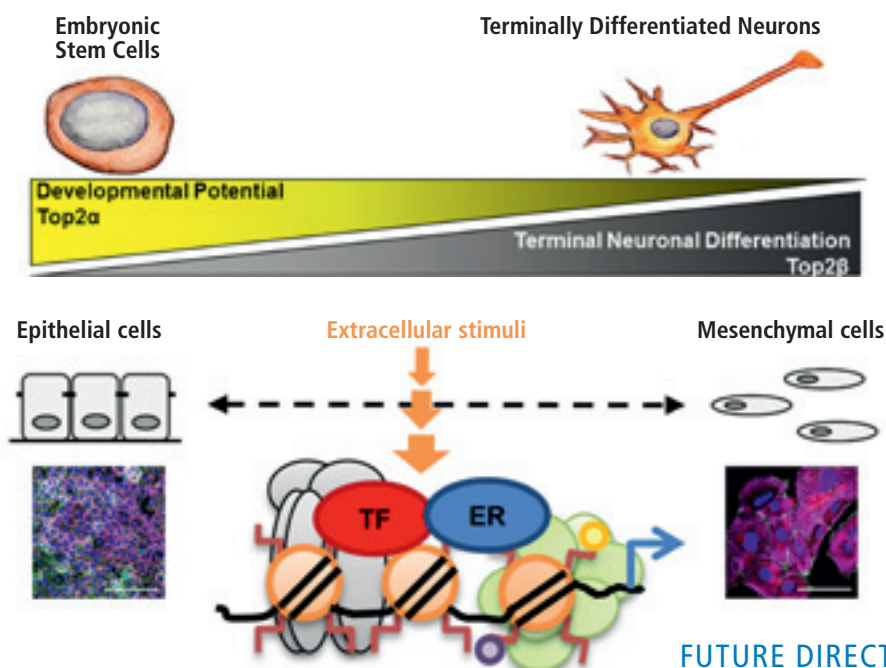


Figure 1. Distinct division of labour between the topoisomerase II isoforms during development. By contributing to the gene regulatory program, TOP2 α and TOP2 β define the identity of proliferating, pluripotent and postmitotic, terminally differentiated cells, respectively. TOP2 α also primes developmental genes in ES cells for later activation.

Figure 2. In response to certain extracellular cues, a defined set of transcription factors (TFs) and epigenetic regulators (ERs) modulate transcription of key EMT genes to drive phenotypic changes underlying epithelial to mesenchymal transition. Scale bars for immunofluorescence images are 100 μ m.

FUTURE DIRECTIONS

Our work has identified novel proteins and pathways critically involved in gene regulation during development and cancer progression. Using a multidisciplinary approach, we next aim to attain an integrated molecular and systems-level understanding of the mechanisms by which they contribute in defining cell fate.

SELECTED PUBLICATIONS

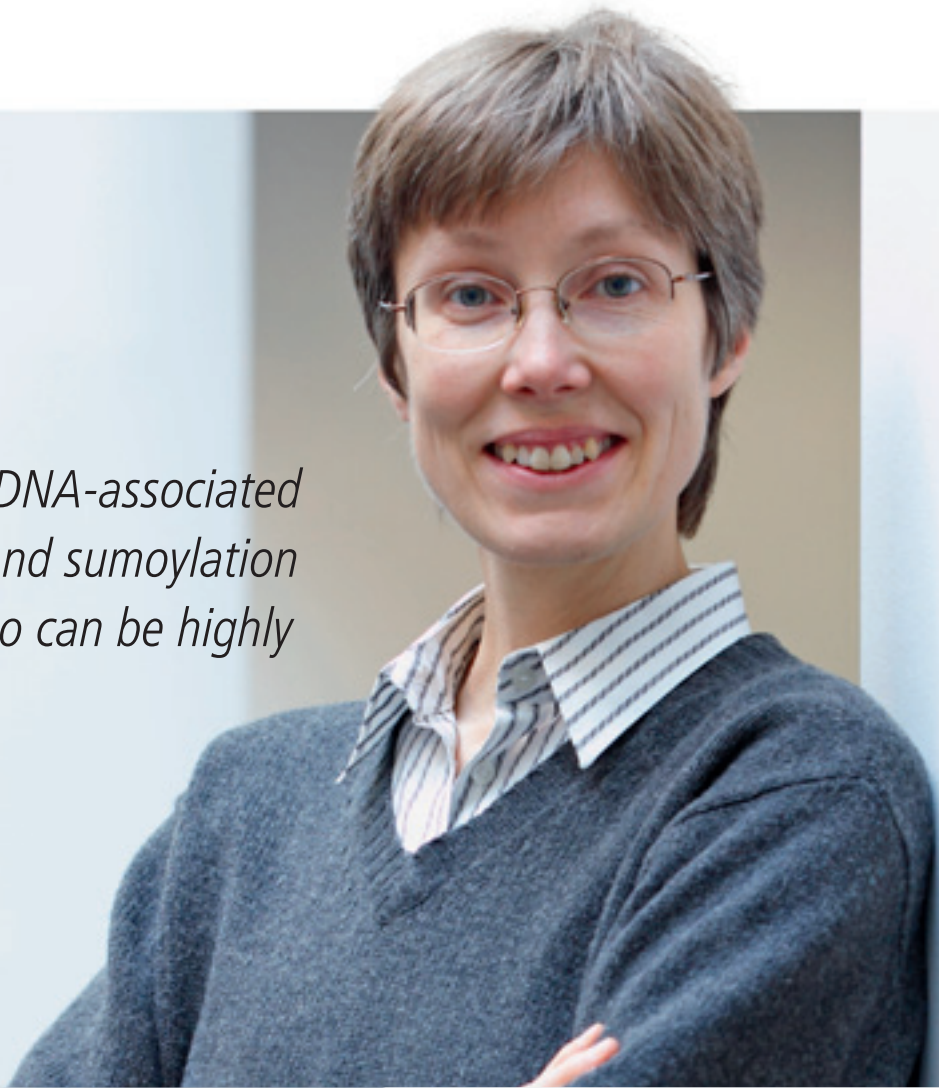
Thakurela S*, Garding A*, Jung J, Schübeler D, Burger L, Tiwari VK (2013). Gene regulation and priming by topoisomerase II α in embryonic stem cells. *Nat Commun*, 4, 2478. (* joint first authors)

Tiwari VK, Stadler M, Wirbelauer C, Paro R, Beisel C, 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, Schübeler D (2011). Transcription factor binding dynamically shapes the mouse methylome at distal regulatory regions. *Nature*, 480, 490-495.

HELLE ULRICH

» *Reconstituting DNA-associated ubiquitylation and sumoylation reactions in vitro can be highly instructive.* «



EDUCATION

- 1992 Diploma in Biology, University of Göttingen
- 1996 PhD in Chemistry, University of California, Berkeley
- 2004 Habilitation, Faculty of Biology (Genetics), Philipp 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
- Since 2013 Director, IMB
Professor, Faculty of Biology,
Johannes Gutenberg University (JGU) Mainz

RESEARCH OVERVIEW

Ubiquitin and SUMO are small proteins that act as posttranslational modifiers. When attached to a target, they modulate its properties and interactions, thus serving as a rapid and reversible means of regulating protein function. Our research aims at understanding the mechanisms by which ubiquitin and SUMO contribute to the maintenance of genome stability. To this end we are investigating the modifications of selected chromatin-associated proteins, the consequences of their association with DNA and other interaction partners, and the biological impact of these modifications. One of the main focuses of our lab is the system of DNA damage tolerance, which promotes the replication of damaged DNA and thereby ensures that cells can proliferate even in the presence of genotoxic agents. This pathway contributes to the cell's overall resistance to DNA damage, but as it is often associated with mutations, it is also a potential source of genome instability in itself and therefore needs to be tightly controlled by ubiquitin and SUMO.

GROUP MEMBERS

- Liliana Batista / Postdoc; since 02/2013
- Sabrina Batke / PhD Student; since 11/2013
- Heike Brinkman / Lab Manager; since 02/2013
- Heike Duda / Research Assistant; 10/2013-12/2013
- Néstor García-Rodríguez / Postdoc; since May 2013

- Laure Gonzalez / Postdoc; since 04/2013
- Magdalena Morawska / Postdoc; 8-10/2013
- Ilaria Ugolini / Summer Student; 07-08/2013
- Hanna Windecker / Postdoc; since 05/2013
- Ronald Wong / Postdoc; since 04/2013

RESEARCH HIGHLIGHTS

UBIQUITIN LIGASE RAD18 CAN FUNCTION AS A SUMO LIGASE *IN VITRO*

DNA damage bypass is initiated by the ubiquitylation of the replicative sliding clamp protein, PCNA. During undisturbed replication, budding yeast PCNA is modified by SUMO, which prevents unscheduled recombination events. Monoubiquitylation is catalysed by the ubiquitin-conjugating enzyme (E2) Rad6 in complex with the ubiquitin ligase (E3) Rad18, whereas sumoylation requires SUMO-E2 Ubc9 and SUMO-E3 Siz1. We previously showed that budding yeast Rad18 harbours a SUMO interaction motif (SIM) that promotes the recognition of sumoylated PCNA as a substrate. This has provided a useful model for the transition from one to the other modifier upon exposure of the cell to DNA damage. We now found that the SIM in Rad18 endows the ubiquitin-E3 with the ability to also transfer SUMO to PCNA in an *in vitro* conjugation assay (Figure 1). Surprisingly, this reaction proceeds with similar characteristics as Siz1-dependent sumoylation, yet there is no evidence that Rad18 contributes to PCNA sumoylation *in vivo*. Mechanistic analysis has revealed that Rad18's SUMO-E3 activity is independent of its catalytic RING finger and most likely proceeds via the recruitment of SUMO-loaded Ubc9 by means of the SIM. Taken together, these phenomena illustrate the intimate crosstalk between ubiquitin and SUMO in the control of damage bypass and at the same time highlight the danger involved in extrapolating from biochemical assays to physiological situations.

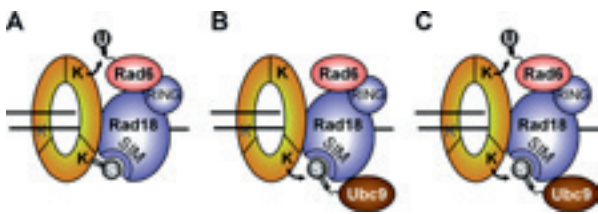


Figure 1. Budding yeast RAD18 can act as a SIM-dependent SUMO ligase *in vitro*. (A) Interaction with SUMO normally promotes ubiquitylation of sumoylated PCNA. (B) *In vitro*, high concentrations of Ubc9~SUMO can drive PCNA sumoylation. (C) Ubiquitylation and sumoylation are mutually independent reactions. K: lysine; S: SUMO; U: ubiquitin.

FUTURE DIRECTIONS

Analysis of the crosstalk between ubiquitin and SUMO in the regulation of PCNA function in DNA replication and repair will remain a major focus of our research. We are currently aiming for a better understanding of pathway choice within this system that determines the accuracy of damaged DNA replication. As polyubiquitylation of PCNA plays a dominant role in this process, we are using genetic and biochemical approaches to investigate the features and interaction partners of the ubiquitin chains that are attached to PCNA in response to DNA damage. The function of PCNA sumoylation in vertebrates is another open question, which we are addressing in mammalian

SELECTED PUBLICATIONS

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

Zhao S and Ulrich HD (2010). Distinct consequences of post-translational modification by linear versus K63-linked polyubiquitin chains. *Proc Natl Acad Sci USA*, 107, 7704-7709.

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.

SUMO MODIFICATION OF PARP-1 IS CONTROLLED BY DNA

Another posttranslational protein modification that plays an important role in genome maintenance is poly(ADP-ribosyl)ation, catalysed by the enzyme Poly(ADP-ribose) polymerase 1 (PARP-1). Beyond DNA repair, PARP-1 also contributes to other aspects of nucleic acid metabolism, such as transcriptional regulation. In this context, PARP-1 had previously been shown to be modified by SUMO. A possible contribution of PARP-1 sumoylation to DNA repair, however, had not been reported. As we had identified PARP-1 as a chromatin-associated SUMO target in a proteomic approach, we set out to investigate the potential impact of DNA on the modification reaction. We found that PARP-1 sumoylation is indeed strongly influenced by DNA. Consistent with a function in transcription, we were able to show that sumoylation *in vitro* is enhanced by the binding of PARP-1 to intact, but not to damaged DNA, in a manner clearly distinct from the mechanism by which DNA lesions stimulate PARP-1's catalytic activity. An enhanced affinity of PARP-1 for the SUMO-conjugating enzyme Ubc9 upon binding to DNA is likely responsible for this effect. Sumoylation does not interfere with the catalytic or DNA-binding properties of PARP-1, and structural analysis by NMR spectroscopy revealed no significant impact of SUMO on the conformation of PARP-1's DNA-binding domain. In cells, sumoylated PARP-1 was found associated with chromatin, but the modification is not responsive to DNA damage and is unaffected by PARP-1 catalytic activity. Our results therefore suggest that PARP-1 follows two alternative modes of DNA recognition, which serve as a means to differentiate between distinct aspects of the enzyme's function (Figure 2). While binding to damaged DNA stimulates the enzyme's automodification with poly(ADP-ribose) as part of the DNA damage response, association with intact DNA is accompanied by the damage-independent sumoylation in the context of transcriptional regulation.

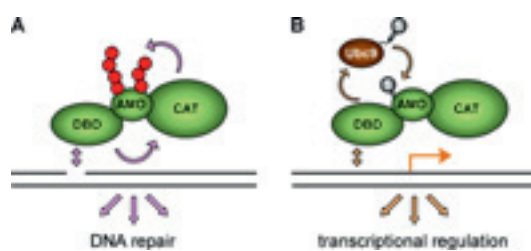
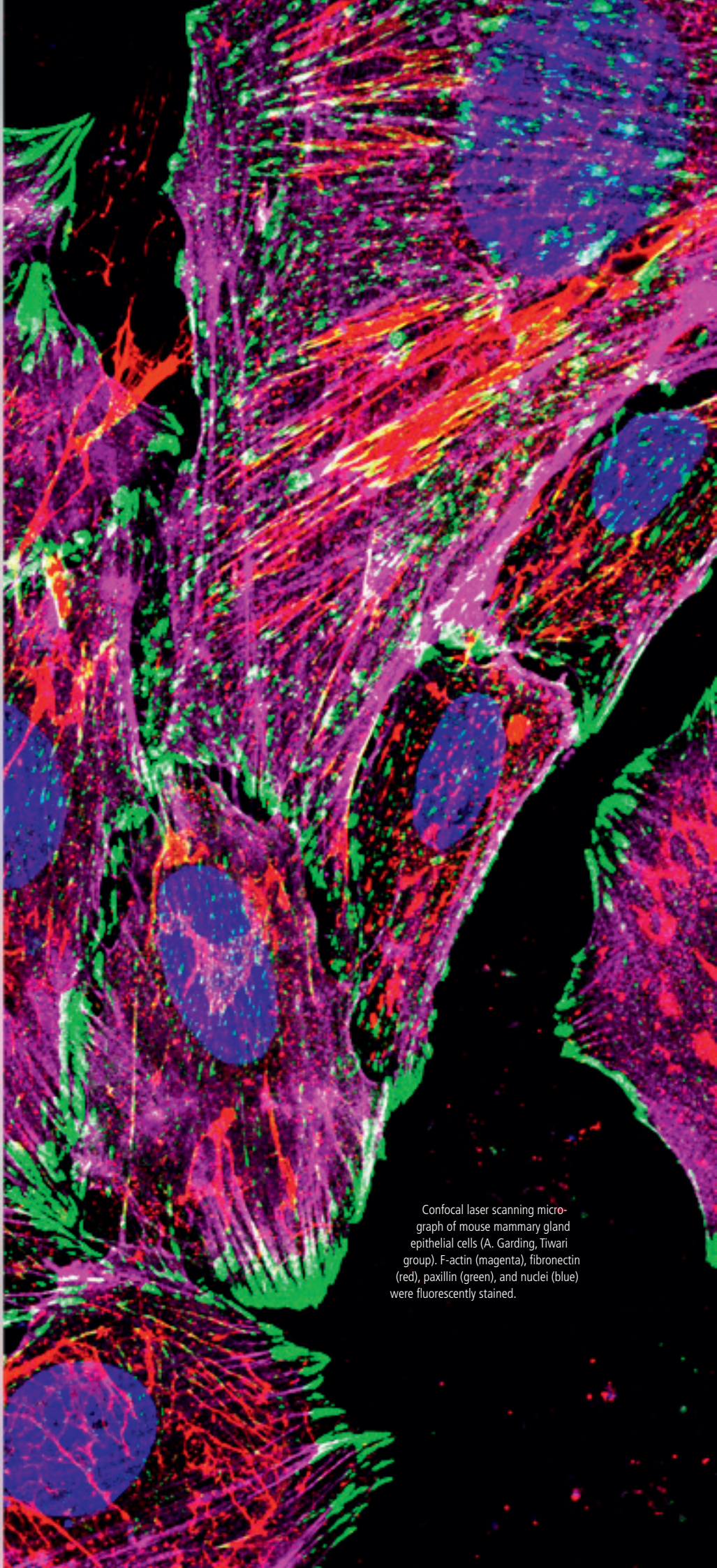


Figure 2. PARP-1 modifications are controlled by DNA. (A) Interaction with damaged DNA promotes PARP-1 auto-modification. (B) Interaction with intact DNA stimulates PARP-1 sumoylation. AMD: automodification domain; CAT: catalytic domain; DBD: DNA-binding domain; S: SUMO; red balls: poly(ADP-ribose).

cell culture as well as *Xenopus laevis* egg extracts. Finally, we are exploring different systems for the realtime analysis of DNA damage bypass in live cells, based on the introduction of sequence-specific damage into the yeast genome. With these investigations we hope to better understand how the processing of DNA lesions during replication affects genome stability and replication fidelity.

CORRE FACILITIES



Confocal laser scanning micrograph of mouse mammary gland epithelial cells (A. Garding, Tiwari group). F-actin (magenta), fibronectin (red), paxillin (green), and nuclei (blue) were fluorescently stained.

A portrait of Andreas Vonderheit, a man with glasses and a checkered shirt, standing in a laboratory setting. He is looking towards the camera with a slight smile.

ANDREAS VONDERHEIT

DIRECTOR OF CORE FACILITIES
AND TECHNOLOGY

OVERVIEW

The IMB Core Facilities (CF) consist of separately managed sub-units that provide either full service (Media Kitchen, Genomics, Bioinformatics, Proteomics core facilities) or assisted service (Cytometry, Histology, Microscopy) to IMB users and the broader campus scientific community. In addition to these units, the core facilities collectively are responsible for further services, such as the hot lab, the S2 lab, and the infrastructure of the animal facilities.

Researchers can consult each core facility's dedicated staff before planning an experiment. For Genomics, initial meetings take place with the CF head and a bioinformatician who can give appropriate input to the experimental design. In Cytometry and Microscopy personnel assist in choosing the correct fluorophores and in sample preparation. The Core Facilities not only provide access to up-to-date technology and hands-on-training or service, but also organise lectures and courses to train researchers in new techniques and instrumentation, proper experimental setup, and data processing.

The Core Facilities purchase and update equipment based on user demand. In 2013, DFG funding enabled the Cytometry Core Facility to acquire a large particle sorter, which allows *Drosophila* larvae, *C. elegans*, and zebrafish to be sorted into 96-well plates. The Genomics core now has a MiSeq for fast sequencing of small libraries and quality control, and the Microscopy core coordinated the purchase and implementation of deconvolution software for image processing, which will be accessible to the entire campus community. Our lectures, courses, and facilities are also open to users outside IMB. Around 40 percent of the Cytometry and Microscopy users are non-IMB.

BIOINFORMATICS CORE FACILITY



CORE FACILITY MEMBERS

Holger Klein / Head of Bioinformatics; since 05/2011
Eugen Eirich / LIMS Programmer; 03/2012-04/2013
Emil Karaulanov / Bioinformatician; since 07/2011
Matthias Koch / System Administrator; since 05/2011
Nastasja Kreim / Bioinformatician; since 04/2012
Sergi Sayols Puig / Bioinformatician; since 10/2013
Denise Scherzinger / Bachelor Student; 03-12/2013
Joern Toedling / Bioinformatician; 06/2011-07/2013
Sebastian Uhrig / System Administrator; since 11/2011

CORE FACILITY OVERVIEW

The number and diversity of projects carried out by the Bioinformatics Core Facility increased significantly in 2013, as many research groups at IMB reached their full productivity after a warm-up phase. The core facility continues to build up and extend its IT and bioinformatics infrastructure, which speeds up standard analyses and also enables researchers to analyse experimental data on their own. Together with the Genomics Core Facility, our bioinformaticians offer consultation on experimental design and are engaged in the development of novel quality control methods in the field of next-generation sequencing (NGS). Our ongoing projects include the development of a laboratory information management system (LIMS) together with Johannes Gutenberg University Mainz ZDV Data Centre, and the creation of standard protocols for a robotic system for the preparation of genomics libraries with the Genomics Core Facility.

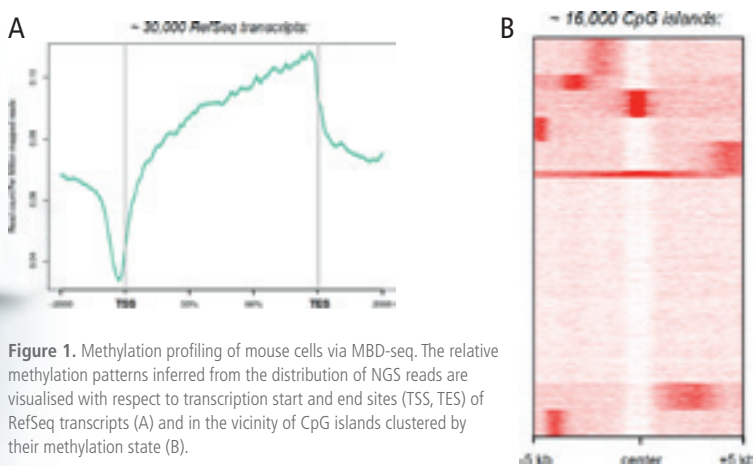


Figure 1. Methylation profiling of mouse cells via MBD-seq. The relative methylation patterns inferred from the distribution of NGS reads are visualised with respect to transcription start and end sites (TSS, TES) of RefSeq transcripts (A) and in the vicinity of CpG islands clustered by their methylation state (B).

SERVICES OFFERED

The Bioinformatics Core Facility provides both standard and more in-depth consulting services.

- General services for IMB users:
 - Assistance with experimental design of high-throughput assays such as ChIP-Seq, RNA-Seq, or Bisulfite-Seq, and advice on proper methods for statistical analysis.
 - Implementation and adaptation of open-source tools and commercial software solutions for “omics” data interpretation; maintenance and customization of analysis platforms for data analysis (Galaxy + Genomatix (NGS), Chipster (Microarrays), Omero (Microscopy), and R-Studio (R)).
 - In-house training and tutorials on bioinformatics tools and databases to facilitate data access and analysis (R, Galaxy, Chipster, Genomatix); lectures on bioinformatics topics.
- Setup and development of analysis pipelines and databases for sequencing, microarray, cytometry, and microscopy data.
- Quality assessment, read mapping, and basic analyses of raw data coming from the sequencing instruments.
- In-depth collaboration on individual projects:
 - Comprehensive analysis of high-throughput data; interpretation and presentation of results.
 - Development of custom tools to address specific bioinformatics needs.
 - Exploration and implementation of novel analysis methods and pipelines.
 - Data mining of “omics” databases and published high-throughput data sets.

CYTOMETRY CORE FACILITY

CORE FACILITY MEMBERS

Malte Paulsen / Head of Cytometry; 05/2011-08/2013

Jens Hartwig / Head of Cytometry; since 10/2013

Heinz Eipel / Senior Scientist; since 06/2011

Ina Schäfer / Technical Assistant; since 08/2011

CORE FACILITY OVERVIEW

The Cytometry Core Facility provides high-end FACS sorters and analysers to give IMB users and collaborators a great deal of flexibility and choice for applications and experimental design. Dedicated staff provide measurement assistance, individual training, and specific support for every step of an experiment.

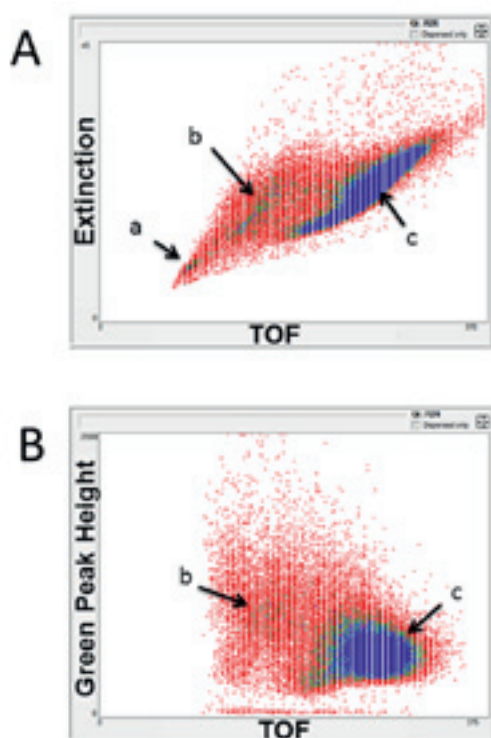


Figure 1. A novel method for removing offspring from a liquid culture of *C. elegans*. (A) Population of larval worms (GFP-containing strain PD4251); TOF= time of flight: a) Eggs, b) L1 larvae, c) L2 larvae (B) Separation and sort scheme.

SERVICES OFFERED

The Cytometry Core Facility is equipped with two FACS Sorters (Becton Dickinson (BD) FACS Aria SORP and Union Biometrica BioSorter) and one analyser, BD LSR Fortessa SORP. The BD machines are optically identical and harbour a five-laser excitation suite and an 18-parameter detector bench (excitation lasers: 355/405/488/561/640 nm; emission detection (number of PMTs per laser): 2/5/3/5/3).

In May 2013 the core facility acquired the Union Biometrica BioSorter, which is equipped with a 488 and a 561 nm laser and 12 PMTs. This allows the sorting of very large particles (up to 1500 μm), such as *C. elegans* or *Drosophila* embryos, which is crucial for projects at IMB and JGU. The BioSorter was funded by DFG.

Routine checks and daily quality control along with sufficient repair capabilities ensure the stability of our cytometer as well as the reliability, accuracy, and consistency of collected data.

We provide regular training for the cytometry core facility. Individual support and assistance at all relevant experimental stages – planning, designing, and finding antibody panels, and measuring, troubleshooting, and analysing data – round out our core facility services.

Currently the Cytometry Core Facility serves 50 different users, with very short wait times. 59 percent of users are internal (IMB) and 41 percent are external; 68 percent make use of the FACS sorter.



GENOMICS CORE FACILITY

CORE FACILITY MEMBERS

Bernhard Korn / Head of Genomics; 07/2010 - 02/2013
Chung-Ting (Tina) Han / Head of Genomics; since 04/2013
Jasmin Cartano / Research Technician; since 07/2012
Andrea Haese-Corbit / Research Technician; since 02/2013
Hanna Lukas / Research Technician; since 01/2013
Julia Müller / Research Technician; since 10/2010

CORE FACILITY OVERVIEW

The Genomics Core Facility provides full-service next-generation sequencing (NGS) and microarray technologies for in-house researchers and their collaborators.

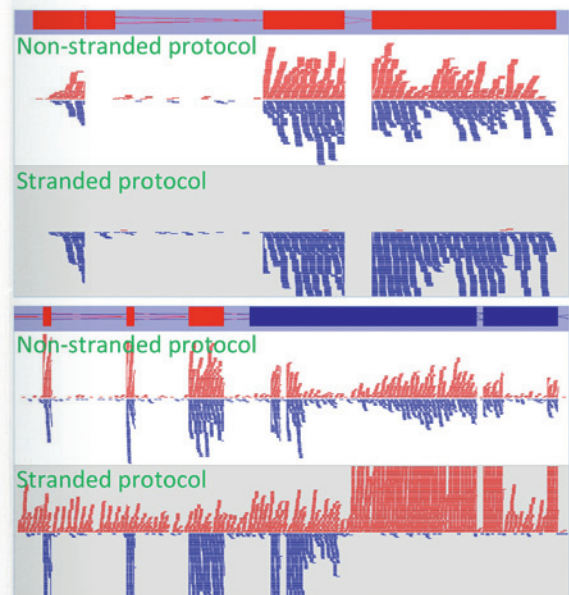


Figure 1. Comparison between non-stranded and stranded RNA-Seq. Strand-specific RNA-Seq protocol was introduced to allow discrimination between sense and antisense strands. The SeqMonk browser shots show the coverage of two gene loci with sense (red) and antisense (blue) strand mapped reads in samples from non-stranded and stranded libraries.

SERVICES OFFERED

For microarrays, we provide access to the Agilent platform. Our NGS service is mainly based on the Illumina platform. At the end of 2012, an Illumina MiSeq system was acquired. Through Johannes Gutenberg University Mainz we have access to their recently upgraded Illumina HiSeq 2500 system. With respect to library preparation, we support 15 different applications for mRNA-Seq, total RNA-Seq, strand-specific RNA-Seq, small RNA-Seq, CHIP-Seq, MBD-Seq, RIP-Seq, amplicon-Seq, and whole genome sequencing. The development of both whole genome bisulfite sequencing and ribosome profiling are currently ongoing.

The facility carries out each NGS project in this order:

- 1) Consultation in the planning and sample preparation
- 2) Quality control of submitted samples

- 3) Preparation of sequencing library
- 4) Quality control of sequencing library
- 5) High-throughput sequencing

The Genomics Core Facility works closely with the Bioinformatics Core Facility (BCF) at the project planning phase for each request. This joint effort is to ensure optimal experimental design and data output for the analysis that will be carried out by the BCF. All submitted samples must pass pre-defined quality measures before the start of library preparation and before loading onto the sequencer. In 2013, BCF implemented a laboratory information management system (LIMS) that helps both GCF and BCF track more than 750 samples submitted from 60 different projects.

HISTOLOGY CORE FACILITY

CORE FACILITY MEMBERS

Mathias Gierl / Head of Histology; since 07/2011

CORE FACILITY OVERVIEW

The Histology Core Facility is a self-service unit that is supervised by Mathias Gierl. In 2013 the CF moved to a dedicated space in the newly completed southern half of the IMB building. Current instrumentation allows automated tissue processing and paraffin embedding and sectioning with a cryotome, a microtome, and a vibratome.

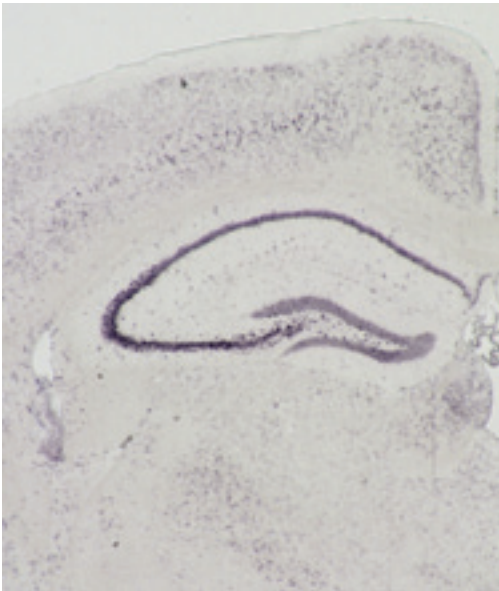


Figure 1. Gadd45a *in situ* hybridization on coronal adult brain cryo-sections. Prominent Gadd45a expression is observed in the hippocampus, weaker expression in cortical and thalamic regions. Sections were generated on the Leica CS3050 S cryostat by A. von Seggern, staining was performed in the Niehrs lab by M. Gierl and images were taken in the Microscopy Core Facility with the Leica DM2500 microscope by M. Gierl.



SERVICES OFFERED

The Histology Core Facility can be used by the whole scientific community on campus. All machines are operated on a self-service basis and are accessible 24/7 for trained users.

Currently the facility is equipped with machines for paraffin tissue processing, embedding and sectioning, vibratome sectioning and cryo-sectioning:

- Leica TP1020: Automated tissue processor for tissue fixation, dehydration, and infiltration
- Leica EG1150: Tissue embedding centre for paraffin embedding
- Leica RM2255: Rotary microtome for motorised paraffin sectioning
- Leica CS3050 S: Cryostat for routine cryo-sectioning
- Leica VT1000 S: Vibratome to generate slices/sections of fresh (living) and fixed/stained samples

MICROSCOPY CORE FACILITY

CORE FACILITY MEMBERS

Andreas Vonderheit / Head of Microscopy; since 04/2011

Katharina Böse / Microscopy Assistant; since 01/2013

Sandra Ritz / Microscopy Assistant; since 10/2013

CORE FACILITY OVERVIEW

In the Microscopy Core Facility two full-time imaging specialists provide hands-on training to all available microscopes and to a variety of image processing software. Over one hundred users take advantage of our facility and services; 64 percent of them are IMB-affiliated and 36 percent are from the broader scientific community in Mainz (JGU, UMC, and MPI-P).

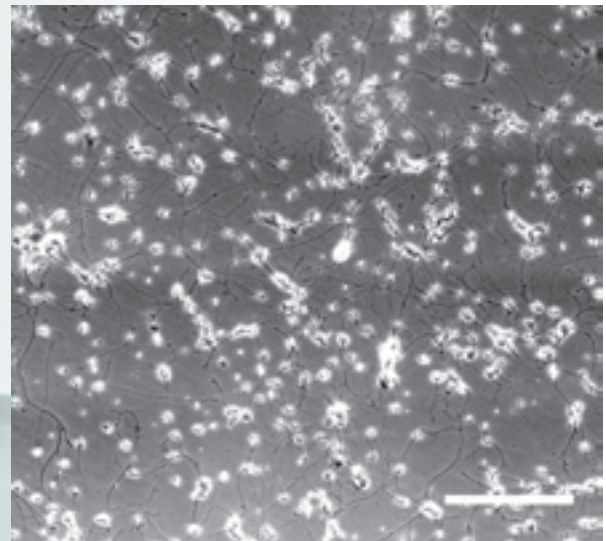


Figure 1. High-resolution imaging of large areas for screening processes and statistical analysis in bright-field or fluorescence mode. Example shows a bright-field image (1.2 mm x 1.1 mm) of primary neuronal mouse cells (J. Jung and A. Garding, Tiwari group) reconstructed from sixteen single 3D stacks (z-projection of 4x4x18 images, 20x, AF7000, Leica). Scale bar length is 250 μ m.

SERVICES OFFERED

The facility provides assisted access to eight principal microscopes and various smaller ones, and a diverse array of microscopic techniques. After a complimentary introduction, users can book and use the microscopes on their own. We also give microscopy courses and lectures throughout the year, and provide image processing software

for deconvolution and 3D reconstruction, and an image database (OMERO). The trained core facility staff help in the choice and use of the right software and give introductions into the different data analysis options available.

PROTEOMICS CORE FACILITY

CORE FACILITY MEMBERS

Falk Butter / Head of Proteomics; since 05/2013

Anja Freiwald / Engineer; since 04/2013

CORE FACILITY OVERVIEW

The Proteomics Core Facility was established in 2013, with its first staff engineer starting in April. We are currently setting up an EASY nLC 1000 ultraHPLC chromatographer coupled online to a recently launched Q Exactive Plus mass spectrometer to perform basic proteomic experiments.



Figure 1. Overview of mass spectrometry sample workflow. Proteins are visualised in a Coomassie Brilliant Blue-stained SDS polyacrylamide gel and subsequently digested to peptides for measurement using a high-resolution mass spectrometer platform (Q Exactive Plus). The chromatogram depicts the usual analysis of a highly complex protein sample.

SERVICES OFFERED

A room within the IMB Core Facility has been adapted to satisfy the infrastructure needs of state-of-the-art mass spectrometry and to house the high-resolution mass spectrometer. This included installation of a powerful cooling system to guarantee stable room temperature, new electrical wiring backed up by an online uninterrupted power supply, and a cabinet for pressurised gas cylinders. After setup

of the mass spectrometry equipment in early 2014, the facility will be able to provide 1500 measurement-hours per year to the IMB research community. In the coming year, we will focus on establishing standard proteomic workflows such as protein band identification and analysis of SILAC-labelled samples.



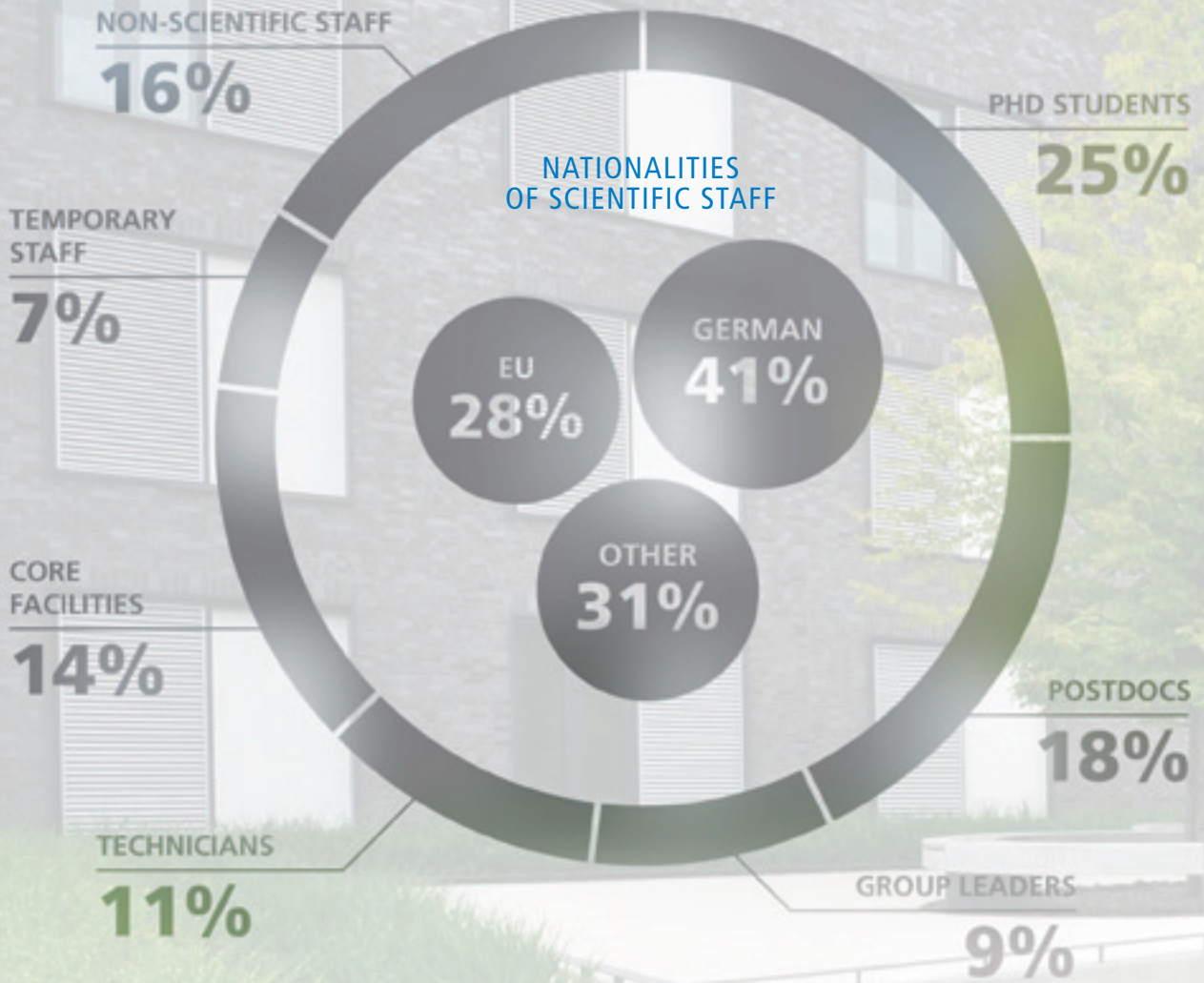




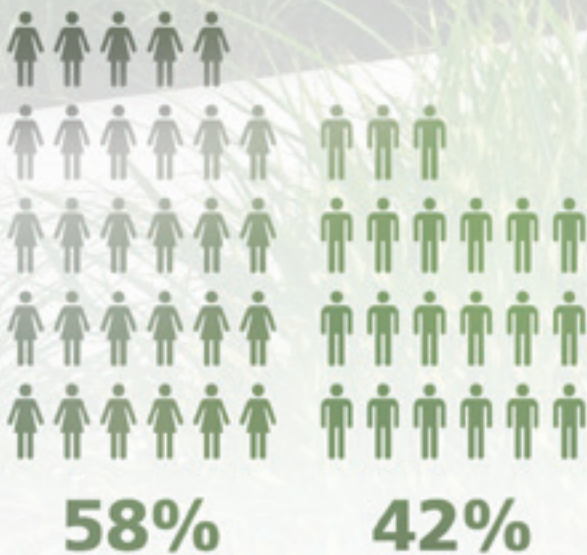
FACTS & FIGURES

STAFF

EMPLOYEES BY STAFF CATEGORY



GENDER DISTRIBUTION

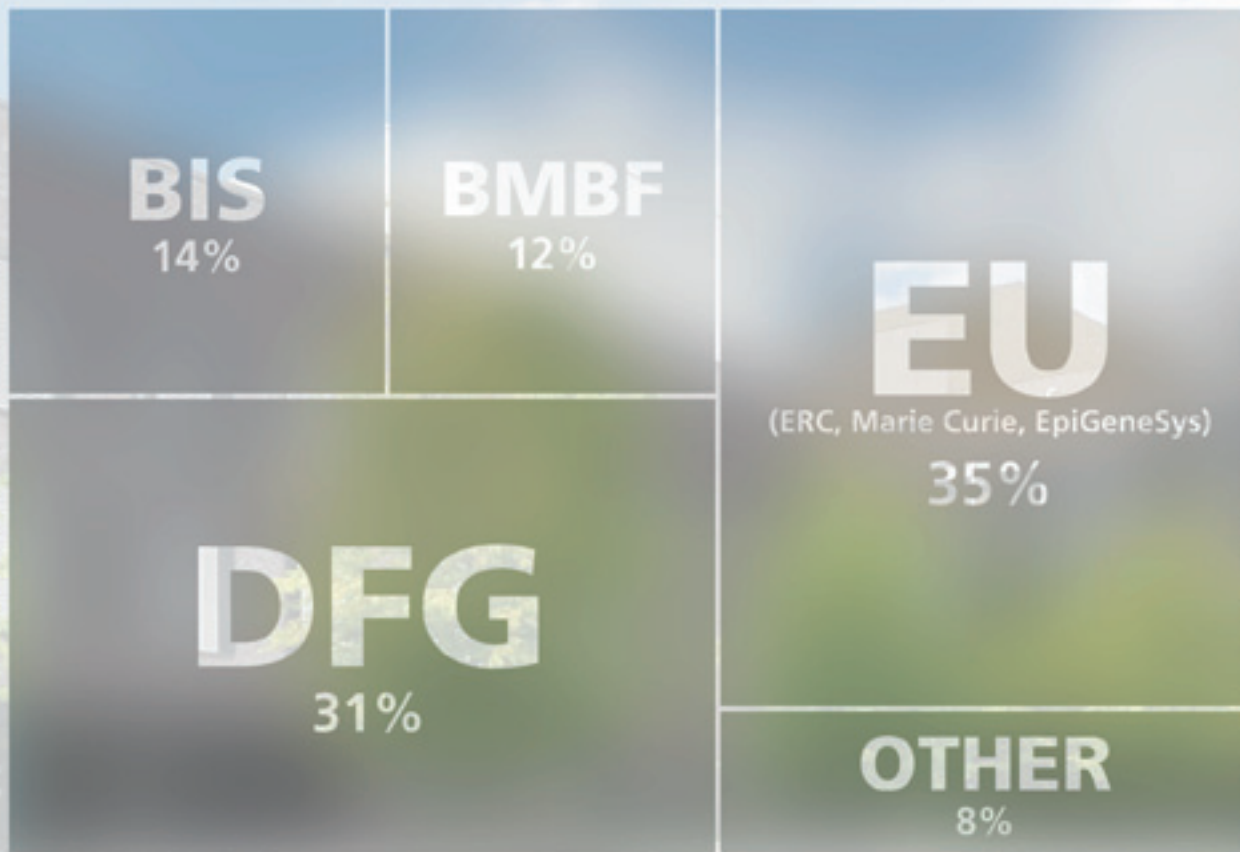


STAFF GROWTH



EXTERNAL FUNDING

RESEARCH GRANTS



MAJOR FUNDERS

European Research Council



European Research Council (ERC)



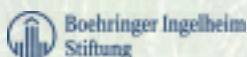
Marie Curie Actions



EpiGeneSys



Deutsche Forschungsgemeinschaft (DFG)



Boehringer Ingelheim Stiftung (BIS)



Federal Ministry of Education and Research (BMBF)



Naturwissenschaftlich-Medizinisches Forschungszentrum (NMFZ)



Wilhelm Sander-Stiftung



Netherlands Organisation for Scientific Research



Stiftung Rheinland-Pfalz für Innovation



International Society of Differentiation



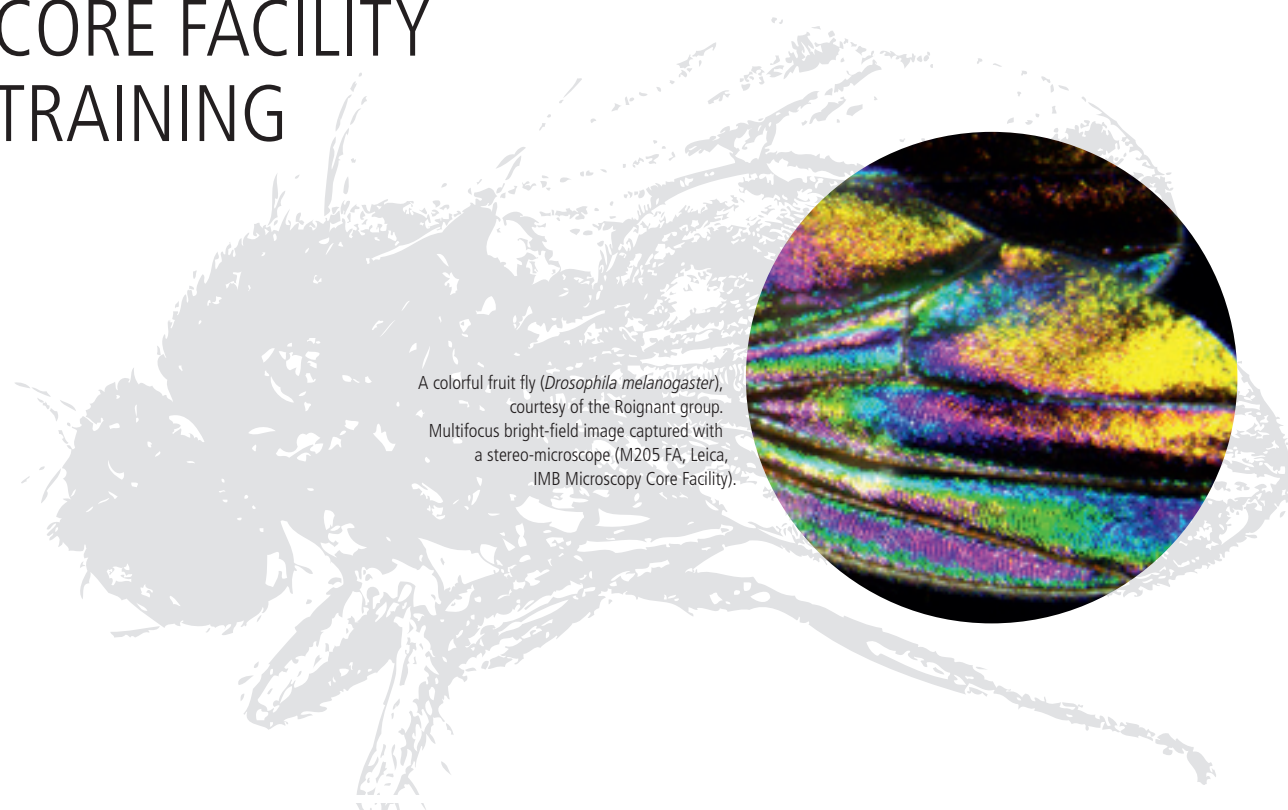
German National Academy of Sciences Leopoldina

EXTERNAL SPEAKERS 2013

Date	Event	Speaker	Institution	Title	JANUARY
24	Seminar	Hannah Mischo	Cancer Research UK London Research Institute	Wear and tear – how transcriptional processes impact on genome stability and cell cycle progression	
31	Seminar	Michael Boutros	DKFZ, Heidelberg	Extracellular space travel by Wnt proteins	
MARCH					
5	TechTalk	Mike Schutkowski	Martin Luther University Halle-Wittenberg	Profiling of epigenetic targets using peptide microarrays	
7	Seminar	Thomas Jenuwein	Max Planck Institute of Immunobiology and Epigenetics, Freiburg	Making and breaking the heterochromatin	
19	TechTalk	Illip Burmester	Silicon Biosystems S.p.A, Bologna	DEP array - Image based sorting of viable, 100% pure single cells	
28	Seminar	Till Bartke	MRC Clinical Sciences Centre, London	Using chemical biology and proteomics to understand how DNA and histone modifications regulate the activity of chromatin	
APRIL					
4	Seminar	Noel Buckley	King's College London	Transcriptional and epigenetic regulation of neural stem cells	
11	Seminar	Axel Imhof	Ludwig Maximilian University Munich	Heterochromatin and the formation of species	
16	TechTalk	Christoph Merten	EMBL, Heidelberg	Living droplets: biology on a drastically miniaturised scale	
25	Seminar	Ernst Stelzer	Buchmann Institute for Molecular Life Sciences, Frankfurt	Light sheet-based fluorescence microscopy (LSFM) reduces phototoxic effects providing new means for modern life sciences	
MAY					
7	TechTalk	Alexander Seitz	Lexogen GmbH, Vienna	Enabling complete transcriptome sequencing through SENSE™ strand-specific mRNA-Seq and SQUARE™ full-length transcript amplification technologies	
13	Seminar	Jason Lieb	University of North Carolina, Chapel Hill	The nuclear envelope: a nexus for gene regulation	
28	TechTalk	Soeren Alsheimer	Carl Zeiss Microscopy, Göttingen	Light sheet microscopy – next generation live imaging	
JUNE					
4	TechTalk	Falk Schladraff	Leica Microsystems CMS, Wetzlar	Laser microdissection and Parkinson's disease: quantitative gene expression analysis of individual human dopaminergic neurons from post-mortem midbrain tissue	
11	TechTalk	Carsten Sönnichsen	Johannes Gutenberg University Mainz	Plasmonic nanoparticles as sensor for single molecules	
13	Seminar	Alain Prochiantz	Collège de France, Paris	Homeoprotein signalling in development, health and disease	

Date	Event	Speaker	Institution	Title	J U N E
20	Seminar	Eric Miska	Gurdon Institute, University of Cambridge	Non-coding RNA: from genome stability to viral immunity	
27	Seminar	Peter Becker	Adolf Butenandt Institute, Munich	Chromosome-wide tuning of transcription – how male flies get away with just one X chromosome	
J U L Y					
30	TechTalk	Mark D. Lynch	Fluidigm Europe B.V., Amsterdam	Exploring single-cell genomics with the C1™ Single-Cell Auto Prep System	
A U G U S T					
29	Seminar	Wolf-Dieter Heyer	University of California, Davis	Mechanisms and regulation of recombinational DNA repair	
S E P T E M B E R					
3	TechTalk	Markus Goddemeier	ProteinSimple, Germany	Gel-free, blot-free, hands-free: Quantitative data by fully automated western systems	
5	Seminar	Gunter Meister	University of Regensburg	Biochemical dissection of miRNA biogenesis and function	
18	Seminar	Abraham Fainsod	Hebrew University, Jerusalem	Fetal alcohol spectrum disorder: How alcohol modifies gene regulation by retinoic acid	
26	Seminar	Christian Speck	MRC Clinical Sciences Centre, London	Loading of the MCM2-7 helicase onto DNA – one hexamer at a time!	
O C T O B E R					
24	Seminar	Alexander Loewer	Max Delbrück Centre for Molecular Medicine, Berlin	The guardian on the move: dynamic regulation of p53 in individual cells	
N O V E M B E R					
11	Seminar	Luiz Penalva	University of Texas, San Antonio	Post-transcriptional networks: interplay between RNA binding proteins and miRNAs in brain tumors	
14	Seminar	Frank Buchholz	Technical University Dresden	From high-throughput screens to biomedical knowledge	
21	Seminar	Wouter de Laat	Hubrecht Institute, Utrecht	Gene regulation in the 3D genome	
26	Seminar	Yoshiko Takahashi	Kyoto University	Morphogenesis in development, cell communications and tubulogenesis	
26	Seminar	Florian Steiner	Fred Hutchinson Cancer Research Center, Seattle	Holocentromeres are dispersed point centromeres localised at transcription factor hotspots	
28	Lecture	Marco Foiani	IFOM, Milan	Checkpoint-mediated mechanisms controlling chromosome integrity	
D E C E M B E R					
12	Seminar	Asifa Akhtar	Max Planck Institute of Immunobiology and Epigenetics, Freiburg	X chromosome dosage compensation: A paradigm to study epigenetic regulation	

CORE FACILITY TRAINING



A colorful fruit fly (*Drosophila melanogaster*), courtesy of the Roignant group. Multifocus bright-field image captured with a stereo-microscope (M205 FA, Leica, IMB Microscopy Core Facility).

LECTURES

PROVIDER	TITLE
BIOINFORMATICS CORE FACILITY	Bioinformatics for ChIP Sequencing
	Bioinformatics for NGS
	Bioinformatics for RNA Sequencing
	DNA Methylation Profiling: Methods, Applications & Data Analysis
	Sequencing
CYTOMETRY CORE FACILITY	Advanced Cytometry
	Basics of FACS
MICROSCOPY CORE FACILITY	Introduction to Microscopy I
	Introduction to Microscopy II
	Pitfalls in Image Acquisition
	Image Processing
	Imaging Ethics

PRACTICAL COURSES

PROVIDER	TITLE
BIOINFORMATICS CORE FACILITY	Genomatix
	Introduction to R
MICROSCOPY CORE FACILITY	Hands-on ImageJ

PUBLICATIONS BY IMB MEMBERS IN 2013



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CORE FACILITIES

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AWARDS 2013

PETRA BELI

Emmy Noether Programme Award,
Deutsche Forschungsgemeinschaft DFG

For outstanding young researchers with international experience to establish independent research groups early in their scientific careers. Petra Beli's group will investigate ubiquitin signals in DNA repair and chromatin organisation.

VIJAY TIWARI

Bruno Speck Award, Swiss Foundation of Haematological Research

For research into stem cell differentiation and the role of direct histone modification by the signalling molecule JNK in this process.

Tiwari VK, Stadler MB, Wirbelauer C, Paro R, Schübeler D and Beisel C (2011). A chromatin-modifying function of JNK during stem cell differentiation. *Nat Genet*, 44, 94-100.



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 scientists 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 teams 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, microscopy, and proteomics, 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 offers instruction in 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 next-generation sequencing) and advanced live and superresolution microscopy. We also make sure our junior scientists learn the transferable 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 superresolution 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 Centre
- Max Planck Institute for Polymer Research

www.imb.de/PhD

INTERNATIONAL SUMMER SCHOOL

IMB's 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's University Medical Centre.

The ISS 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. The lectures give students comprehensive insights 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.de/ISS

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 38,000 students, 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's 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 25 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

German Cancer Research Centre DKFZ
Heidelberg, Germany



PROF. DR JOSEF JIRICNY

Institute of Molecular Cancer Research
University of Zurich
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PROF. DR RUDOLF JAENISCH

The Whitehead Institute for
Biomedical Research
Cambridge, USA



PROF. DR JÖRG MICHAELIS

Scientific Advisor to the Executive Committee
of the Boehringer Ingelheim Foundation



PROF. DR RENATO PARO

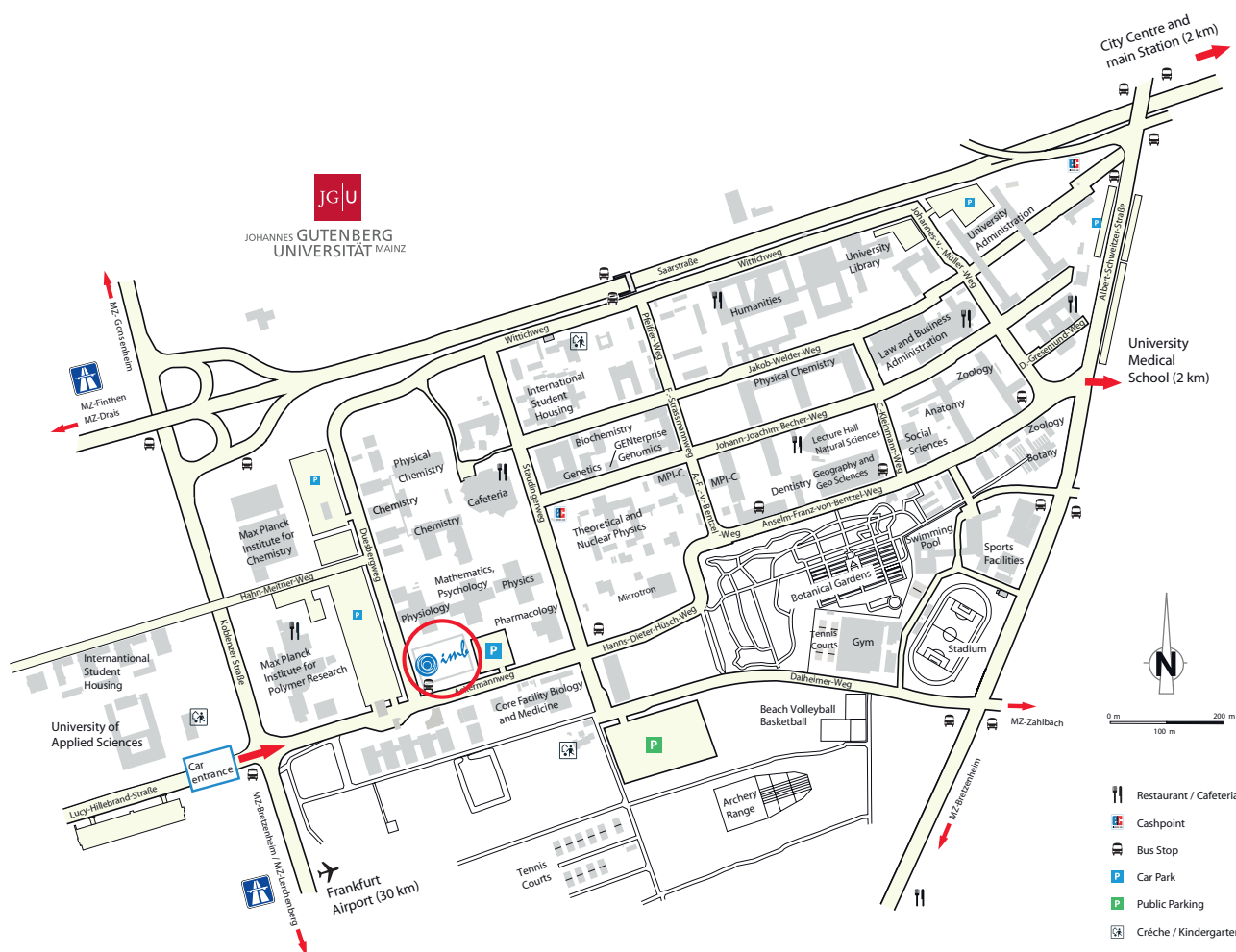
Department of Biosystems Science and Engineering
Swiss Federal Institute of Technology
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PROF. DR ERNST-LUDWIG WINNACKER

Secretary General, Human Frontier Science Program Organization
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CAMPUS MAP OF THE JOHANNES GUTENBERG UNIVERSITY MAINZ



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IMPRINT

PUBLISHED BY

Institute of Molecular Biology gGmbH

EXECUTIVE EDITORS

Christof Niehrs and Ralf Dahm

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Amanda Alvarez

LAYOUT & DESIGN

cala media

SCIENTIFIC FIGURES

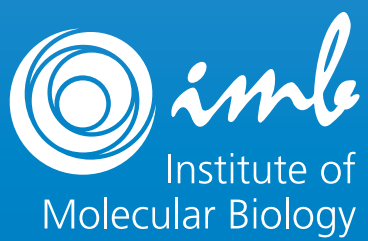
Courtesy of the respective Research Groups and Core Facilities

STAFF AND FUNDING DATA

Courtesy of IMB Human Resources and Grants Office

IMAGE CREDITS

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