

Institute Report 2011–2013

Max Planck Institute of Immunobiology and Epigenetics





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Preface



THOMAS JENUWEIN MANAGING DIRECTOR (2012–2014)

➡ Consolidation and Change: what seems contradictory at first has been sensibly combined in our Institute during the last years. On the occasion of the 50th anniversary of the Institute in December 2011, Peter Gruss, President of the Max Planck Society, emphasized that "Future needs Ancestry" – both is lived at our Institute.

Since its foundation in 1961, the Institute has undergone substantial changes, while the continuing interest in the function of B and T cells provided a natural bridge for studies into hostpathogen interactions, signaling pathways, self vs. non-self discrimination, transcriptional control and lineage plasticity. During the last years, the Institute has adopted the strategy to focus on two key areas of modern biology: Immunobiology and Epigenetics. This strategy rests on but also expands the proven strengths of the Institute and aims at dissecting the molecular mechanisms that govern cell type identity and chromatin-dependent responses of the epigenome to changes in the environment.

The definition of these research areas was also recognized by the external

evaluation through the Scientific Advisory Board (SAB) in May 2013 that highlighted the productivity, innovation and scientific excellence of the Institute. To proceed even further, we continuously adopt measures to build a united vision of the Institute, to increase interactions among departments and to liaise with all peer groups of the Institute. There are also long-standing co-operations with the University of Freiburg that are manifested by a joint University/Max Planck department, several innovative research consortia and an international PhD program. This successful IMPRS-MCB PhD program ensures that the Institute can attract academic talent from all over the world.

The success of the recent developments is also demonstrated by the awards of two ERC Starting Grants to Andrew Pospisilik and Patrick Heun and of two ERC Advanced Grants to Thomas Boehm and Michael Reth. With the promotion of Asifa Akhtar to full directorship in April 2013, a major milestone for the consolidation of the Epigenetics focus was accomplished.

Initiated in December 2010, the Max Planck Freiburg Epigenetics Meeting was held again in December 2012, and has rapidly gained international reputation with lectures from many renowned scientists and poster presentations by Postdocs and PhD students. This successful meeting will be continued on a bi-annual basis.

The past three years have also seen some changes within the faculty of the Institute: with the retirement of Marina Freudenberg and the Emeritus transition of Rolf Kemler, two colleagues who have shaped the research profile of the Institute for many years have completed the active part of their scientific careers. Several group leaders (Tilman Borggrefe, Wolfgang Schamel, Robert Schneider) have been rewarded for their excellent work and were appointed to senior positions at other institutions both in Germany and abroad. At the same time, new group leaders (Ritwick Sawarkar, Eirini Trompouki) have joined the Institute, and more will follow soon. This testifies to the attractive career building opportunities for junior faculty of the Institute.

The success and attractiveness of our Institute would not be possible without the support of state-of-the-art research facilities and service units and the help of the administration. This enables us to focus on the research and to address the big questions with the most recent and advanced technologies. I would like to thank all colleagues, scientific and non-scientific staff for their commitment and dedication to build a coherent structure and to develop the full potential of the Max Planck Institute of Immunobiology and Epigenetics.

This Institute Report covers a three year period (2011–2013), is a continuation of the previous 'Annual Report' (2008–2010) and supplements the newly revised website (*www.ie-freiburg.mpg.de*) that is regularly curated and updated.

Enjoy reading our Institute Report 2011–2013.

Prof. Dr. Thomas Jenuwein Managing Director, November 2013

01 About us



History of the Institute







1976

2010

1961

The Max Planck Institute of Immunobiology (MPI-IB) was founded in 1961 on the premises of the former research institute of the pharmaceutical company Wander AG in Freiburg. Until the end of the 1970's, under the directorship of Otto Westphal, Herbert Fischer and Otto Lüderitz, the institute was primarily engaged in studying the interactions between infectious agents and the immune system, with special emphasis on the bacterial substance endotoxin.

1980

With the recruitment of Klaus Eichmann (1981) and Georges Köhler (1984), the thematic focus of the institute expanded to cellular and molecular mechanisms of B and T cells. Klaus Eichmann and colleagues were the first to describe the development of functional lymphoid tissue from embryonic stem cell lineages. In 1984, Niels Jerne, Georges Köhler and Cesar Milstein were awarded the Nobel Prize for their pioneering work on monoclonal antibodies using the hybridoma technique.

1990

Through a special funding by the State of Baden-Württemberg, Developmental Biology was added as another scientific focus, resulting in the recruitment of Davor Solter (1991) and Rolf Kemler (1992). Davor Solter was one of the first to identify genomic imprinting and his research focused on genetic and epigenetic mechanisms regulating mouse pre-implantation development. Rolf Kemler identified the first cellcell adhesion molecule (E-cadherin) in

mouse development and significantly advanced the understanding of mouse embryogenesis.

1998

With the appointment of Thomas Boehm (1998) as successor of George Köhler, Developmental Immunology was added as a new research focus. Efforts towards a stronger cooperation between MPI-IB and the Faculty of Biology at the University of Freiburg led to the establishment of the University Department of Molecular Immunology at the MPI-IB and recruitment of Michael Reth as its head (1998). In addition, the Spemann Laboratory, consisting of three independent junior research groups, was established with the aim of promoting early independence of junior scientists. With the appointment of Rudolf Grosschedl as successor of Klaus Eichmann (2004), the thematic connection between Immunology and Developmental Biology was further strengthened and the molecular mechanisms of lymphoid cell differentiation and the regulation of genes via extracellular signals were added as new research areas.

2006

In 2006, the International Max Planck Research School for Molecular and Cellular Biology (IMPRS-MCB) was intitiated by Rudolf Grosschedl, in collaboration with colleagues of the University of Freiburg. At the beginning of 2006, the President of the Max Planck Society launched a competition between all institutes of the Society to establish a new department with an innovative research theme. Among all proposals, "Epigenetics" was selected and

Thomas Jenuwein (2008) accepted an offer of the Max Planck Society to direct the new department "Epigenetics". To make a relevant impact in the field of epigenetic research, the Kollegium decided to additionally appoint an epigenetics researcher as successor of Davor Solter.

At the end of 2009, Asifa Akhtar was appointed as a Max Planck Investigator focusing on "Chromatin Regulation" and was promoted to Director in April 2013.

2010

In December 2010, the institute was renamed to "Max Planck Institute of Immunobiology and Epigenetics" (MPI-IE), reflecting the two key areas of modern biology being conducted at the institute. With the establishment of the "Epigenetic Focus" at the MPI-IE, an international biennial meeting on the broad area of epigenetics and chromatin was founded. In December 2011, more than 200 guests celebrated the 50th anniversary of the MPI-IE. "Future needs ancestry" emphasized Peter Gruss, President of the Max Planck Society, and honoured the achievements of the MPI-IE.

In February 2013, Rolf Kemler retired from the director position. He will continue research in an emeritus group until 2015. The search for a successor of the director position in the field of immunobiology is currently underway. As in the past, new junior group leader positions are continuously being established at the MPI-IE to ensure new input for exciting fields of research.

Previous Directors of the Institute















Otto Westphal Otto V (Director from establ 1961–1982) deterr charic ing Pr

Herbert Fischer (Director from 1964–1981)

Otto Lüderitz (Director from 1965–1988)

Klaus Eichmann (Director from 1981–2004)

Georges Köhler (Director from 1984–1995)

Davor Solter (Director from 1991–2006)

Rolf Kemler (Director from 1992–2013) Otto Westphal founded the Max Planck Institute of Immunobiology in 1961 and established it as a leading research facility. His scientific achievements include the determination of the primary structure of *E. coli* lipid A, an endotoxic lipopolysaccharide. He was the founder of the European Journal of Immunology and found-ing President of the German Society for Immunology.

Herbert Fischer had an ardent interest in macrophages at a time when the interest in the field of immunology was universally focused on lymphocytes. His group studied the role of phospholipid metabolism in the activation of macrophages and lymphocytes and its subsequent effects on the activation of the innate and adaptive immune systems.

Otto Lüderitz and his group showed that lipopolysaccharides (LPS) of Gram-negative bacteria are built up according to a common architecture, consisting of the O-polysaccharide chain, the core and lipid A. In chemical and biological studies they brought the final evidence that lipid A is the toxic and biologically active part of LPS which led to the total chemical synthesis of biologically active lipid A.

Klaus Eichmann and coworkers were involved in research on T cell development, T cell activation, and antigen processing in cell-mediated immunity. They discovered the autonomous signaling function of the pre-T cell receptor in the development of the alpha/beta T cell lineage. They were the first to describe the development of functional lymphoid tissue from ES cell lines.

ler In the year Georges Köhler joined the Max Planck Institute of Immunobiology he was awarded the Nobel prize in Physiology or Medicine, together with Cesar Milstein and Niels Jerne for their pioneering work on the immune system and the generation of monoclonal antibodies using the hybridoma technique. His untimely death in 1995 was a great loss to the institute and the scientific community.

In seminal experiments, Davor Solter studied the developmental potential of maternal and paternal genomes by nuclear transplantation. He was one of the first t o identify genomic imprinting. His research focused on genetic and epigenetic mechanisms regulating mouse pre-implantation development. Solter made significant contributions to mammalian development, including differentiation of germ layers, biology and genetics of teratocarcinoma, biology of embryonic stem cells, cloning, and reprogramming.

Rolf Kemler identified the first cell-cell adhesion molecule in mouse development, E-cadherin. He discovered catenins as cytoplasmic anchorage proteins. Particularly β -catenin is well-known because of its dual function in cell adhesion and Wnt signaling. Kemler was the first to establish mouse embryonic stem (ES) cells in Germany. He studied their differentiation potential and used gene targeting to investigate the function of cadherin and catenins in development, genomic maintenance and stem cell vs. oncogenic potential.

Institute Highlights



October 2012: Ground-breaking ceremony for the child care facility.

2011

NOVEMBER

The first Max Planck Day, initiated by the Max Planck Society and hosted by the individual institutes, takes place. The event at the MPI-IE aims at bringing school classes in contact with research, work and life at the institute.

DECEMBER

More than 200 guests celebrate the 50th Anniversary of the MPI-IE with festivities including commemorative speeches and a scientific symposium with notable speakers from all over the world. Peter Gruss, President of the Max Planck Society honours the accomplishments of the institute.



2012

AUGUST

On the occasion of the 60th birthday of Rudolf Grosschedl, a one-day symposium takes place with 20 scientists, presenting their research within four sessions: gene control, long-range chromatin interactions, B lymphopoiesis, signaling and development.

Patrick Heun is awarded an ERC Starting Grant for "Dissection of centromeric chromatin and components: A biosynthetic approach"

J. Andrew Pospisilik is awarded an ERC Starting Grant for "Metabolic Polycombics".

OCTOBER

The scientific members of the MPI-IE attend the institute retreat in Saint Hippolyte, Alsace, France. The event aims at strengthening scientific interactions and supporting the collaborative atmosphere at the institute.



With a ground-breaking ceremony the construction of the new child care facility is initiated. From December 2013 on, it will provide space for children at the age of three months to six years in close vicinity to the institute.

NOVEMBER

Michael Reth is awarded an Advanced ERC Grant for his project "Nanoscale analysis of protein islands on lymphocytes".

Thomas Boehm is awarded an Advanced ERC Grant for the project "Thymopoiesis: From evolutionary origins to future therapies".

DECEMBER

The 2nd Max Planck Epigenetics Meeting takes place. Talks are given by 20 invited speakers plus 15 speakers that are selected from the abstracts. About 120 scientists participate in the meeting and about 60 posters are presented.





July 2013: The new cafeteria is opened after extensive renovation.

2013

APRIL

Asifa Akhtar is promoted to full Max Planck director position after four years of highly successful research at the MPI-IE. She leads the department of Chromatin Regulation.



MAY

The Scientific Advisory Board (SAB) conducts the external evaluation of the MPI-IE. In its feedback, the SAB highlights the excellence and innovation of the institute and encourages towards continuous growth and the development of a strong identity to exploit the full potential of the institute.

EMBO announces Asifa Akhtar as a new member.

JUNE

The Max Planck – The University of Tokyo Center of Integrative Inflammology is established under codirectorships of Rudolf Grosschedl from the MPI-IE and Tadatsugu Taniguchi from the University of Tokyo. It aims at bundling research projects of both institutions and facilitating the exchange of knowledge and experience.

Kickoff meeting for the construction of a new animal service building. It will house core infrastructure, isolator units and personnel rooms, ensuring highest scientific and animal care standards.

JULY

Ritwick Sawarkar starts as a Guest Scientist in the Epigenetic focus.

Building component BT IV is reopened after extensive renovation. It provides room for the new cafeteria, offices, seminar rooms and a centralized IT and server area.



MPI-IE contributes to the Freiburg Science Fair, an event for the general public.



SEPTEMBER

Andrew Pospisilik is awarded the Rising Star Award of the EASD for a new concept about the emergence of diabetes.

Eirini Trompouki starts as Group Leader in the Department of Cellular and Molecular Immunology.

Organization of the MPI-IE



The Management Board of the MPI-IE: (from left) T. Boehm, T. Jenuwein, A. Akhtar, B. Tarkan, R. Grosschedl.

→ The Max Planck Institute of Immunobiology and Epigenetics (MPI-IE) is organized in five departments, plus one joint appointment between the University of Freiburg and the MPI-IE. Each department of the MPI-IE is headed by a director, also named 'senior group leader' (at present one director position vacant).

In addition, currently nine junior group leaders conduct research at the MPI-IE. They are either department-associated or department-independent. All junior groups are considered equivalent. They have their own budget and pursue within the framework of the MPI-IE their research fully independently. Junior group leader positions are established for five years (with possibility of extension) and are attractive career-building appointments. Central scientific infrastructure units and the administration complement the MPI-IE.

The central decision-making body of the MPI-IE is the management board

("Kollegium"), comprising the directors and the senior executive manager. It meets on a bi-weekly basis. The managing directorship rotates every three years among the department heads of the MPI-IE. The management board establishes the general scientific and administrative policies and promotes long-term developments of the MPI-IE. Both the management board and the administration interact closely with the Max Planck Society in Munich regarding budgetary, personnel, and policy issues.

In coordination with all group leaders and heads of infrastructure, the management board initiates the establishment of new scientific facilities, and ensures a collaborative atmosphere at the institute. Monthly team leader meetings ('Faculty lunches') facilitate internal communication, identification of solutions, and dissemination of information.

Managing Director (2012-2014) - Thomas Jenuwein

Scientific Members of the Institute

- Asifa Akhtar
- Thomas Boehm
- Rudolf Grosschedl
- Thomas Jenuwein
- Senior Executive Manager
- Bülent Tarkan

Adjunct External Scientific Member

Michael Reth: since 2002 Department of Molecular Immunology, University of Freiburg / MPI-IE, Freiburg, Germany

External Scientific Members

- Michael Sela: since 1967 Department of Immunology, Weizmann Institute of Science Rehovot. Israel
- Barbara B. Knowles: since 2002 Institute of Medical Biology, Singapore
- Paolo Sassone-Corsi: since 2011 Center for Epigenetics and Metabolism, University of California, Irvine, USA

Management Board

Asifa Akhtar, Thomas Boehm, Rudolf Grosschedl, Thomas Jenuwein, Bülent Tarkan represented by Thomas Jenuwein, Managing Director (2012-2014)



Scientific Advisory Board and Board of Trustees

Scientific Advisory Board

To ensure the high quality and productivity of research, the Max Planck Institute of Immunobiology and Epigenetics (MPI-IE) routinely undergoes evaluations by independent scientific advisors - the 'Scientific Advisory Board' (SAB).

Members of the MPI-IE SAB are internationally renowned scientists who are appointed by the president of the Max Planck Society for a period of six years and who are not affiliated with the Max Planck Society.

The SAB reviews the activities of the institute every three years and issues a report to the President of the Max Planck Society. This evaluation serves as an important basis for the planning of further scientific developments as well as for the distribution of resources by the Max Planck Society. The SAB also supports the MPI-IE in recruiting new directors and group leaders.



Markus Affolter Universität Basel Basel, Switzerland

Prof. Dr.



Prof. Dr. Susan Gasser Friedrich Miescher Institute, Basel,

Switzerland



Prof. Dr. Adrian Hayday King's College London London, UK

Prof. Dr.

tondo

Philip Avner

EMBL Montero-



Prof. Dr. Max D. Cooper Emory University School of Medicine, Atlanta, USA

Prof. Dr.

Dan Littman

Langone Medical

New York, USA

Center, Skirball Inst.



Prof. Dr. Amanda Fisher (Chair person) Imperial College London London, UK

Board of Trustees

The Board of Trustees provides the institute with valuable advice in social and science-political issues and supports further developments of the institute. The board meets once



Prof. Dr. Dr. h.c. mult. Hubert E. Blum Dean of the Medical Faculty, University Medical Center Freiburg, Germany



Michael Kleiner

Ministerialdirigent

Ministry of Science,

Research and Art

Stuttgart, Germany



Pfizer Deutschland GmbH Freiburg, Germany

Dr. Axel Glatz

Dr. Nicola von Lutterotti (Chair person) Free Journalist Zurich, Switzerland



Society.

Prof. Dr. Martin Haag Mayor of the City of Freiburg Freiburg, Germany



a year (November) to interact with the management board of the MPI-IE and with other members of the Max Planck



Research University Freiburg, Germany



Dr. Christian Hodeige Publisher and Managing Director Badische Zeitung Group Freiburg, Germany



Dr. Dieter Salomon Lord Mayor of the City of Freiburg Freiburg, Germany

Facts and Collaborations



→ Facts about the institute

In November 2013, a total of 325 persons from 41 nationalities were members of the institute, including 26 guests.

Senior Group Leaders
 Group Leaders
 Postdocs
 PhD Students

In addition to the institutional funding through the Max Planck Society, the MPI-IE received about 9 Million Euros Third Party Funds in the period of 2011–2013.

Collaborations with the University of Freiburg

Traditionally, the Max Planck Institute of Immunobiology and Epigenetics (MPI-IE) has long-standing interactions and collaborations with the University of Freiburg and its associated University Medical Center. One of the best examples of this successful cooperation is the University/MPI-IE Department of Molecular Immunology that has conducted research at the MPI-IE since 1996.

Furthermore, there are numerous research consortia that build on the collaborations between the MPI-IE and the University. For example, MPI-IE research groups are integrated in the cluster of Excellence "BIOSS 2" and the Center of Chronic Immunodeficiency "CCI". Also, several SFBs (collaborative research consortia) show strong participation by MPI-IE scientists: SFB592 "Signalling pathways in thymus development", SFB620 "Immunodeficiency – Clinical manifestations and animal models", SFB746 "Functional specificity by coupling and modifications of proteins", SFB850 "Control of Cell Motility in Morphogenesis, Cancer Invasion and Metastasis", CRC992 "Medical Epigenetics – From basic mechanisms to clinical applications".

Most group leaders including directors, who are usually affiliated with the Faculties of Biology or Medicine, are involved in active teaching, mainly at the graduate level. All MPI-IE research groups also participate in the International Max Planck Research School for Molecular and Cellular Biology (IMPRS-MCB). This joint international PhD Program of the MPI-IE and the University of Freiburg provides an outstanding scientific education in Molecular and Cellular Biology, Immunobiology and Epigenetics.



CLINIKUM

Administration and Service

The administration of the Max Planck Institute of Immunobiology and Epigenetics (MPI-IE) supports the research groups and scientific infrastructure at the institute. It seeks to ensure optimal working conditions with a special emphasis on meeting the needs of the international community of scientists and guests.



Administration and ...



... service teams of the MPI-IE.

Senior Executive Manager: Bülent Tarkan Assistant to Senior Executive Manager: Nathalie Schulz

Administration and Service

Finances

- Head: Martina Enderlein
- Jenny Barabasch
- Manuela Mattmüller
 Saskia Moos
- Birgit Poitz

- Human Resources
- *Head: Daniela Moll* – Susanne Demme
- Corinna Kanisch
- Veronika Klank
- Sabine Stallone

Purchasing

- Head: Michele Fieber
- Regina Burger
- Claudia Höferlin
- Sven Mußmann
- Head: Bülent Tarkan – Rose Black

Reception/Switch Board

Head: Daniela Moll

Susanne Demme

Sabrina Fögele

- Gabriele Prosch

Trainee office

IT Service

Head: Daniela Moll

Florian Stiegeler

Daniel Andris

Head: Christoph Gartmann

Wolfgang Arndt

- Wolfgang Burger

- Christian Pagel

Rose Black

Library

Public Relations

- *Head: Bülent Tarkan* – Johannes Faber

Safety

- Head: Bernadette Lippok – Dietmar Borowiak
- Pouria Dasmeh
- Sabine Fietzeck
- Andreas Rolke

Washing & Media Kitchen

Head: Bernadette Lippok

- Doris Hoppe
- Ute Lenz
- Yan-Qing Li
- Petra Lüderitz
- Patricia Mayer
- Roswitha Mellert
 Madalena Moreira
- Andrea Reinecke
- Maria Will

Workshop

- Head: René Volz – Uwe Bachstein
- Adalbert Bleichner
- Roland Kaiser
- Steffen Papenfuß
- Raimund Schelb
- Kurt Willaredt

Cleaning

- Head: Raimund Schelb
- Andrea Aukthun
- Irmgard Bregenhorn
- Michael BreithauptMarlies Ferch
- Olga Lai
- Beata Zipfel

Staff Restaurant and Lounge

- Head: Bülent Tarkan – Martin Haberstroh
 - (Tenant)

Focus Immunobiology

Owing to immunological research many infectious diseases have lost their grip on humankind.

→ Immunobiology is concerned with the ways multicellular organisms defend themselves against the onslaught of pathogens. They have evolved a plethora of strategies to guard their bodily integrity, and to promote survival and reproduction.

Also for humans, a properly functioning immune system is of central importance. Indeed, of all branches of medicine, the translation of results from immunological research to medical treatments over the last two centuries has probably had the most significant impact on human life expectancy.

Many devastating infectious diseases have lost their grip on humankind, thanks to preventive strategies such as vaccination and general hygiene. Furthermore, immunology provides us with critical information for the treatment of inflammatory diseases and cancer that can afflict many organ systems and are a substantial burden to patients and modern health care systems.

Our current research focuses on the molecular mechanisms underlying lymphocyte generation from hematopoietic stem cells. Of exceptional interest are factors within lymphocytes and those emanating from the microenvironment in lymphoid organs that foster the emergence of mature effector cells. Apart from sophisticated in vitro systems, we use a wide range of animal model systems to study various aspects of the immune system in a physiological context.

Additionally, immunobiology serves as a paradigmatic research field for key questions in modern biology, such as cellular identity, cell-cell interactions, the structure of protein complexes and signal transduction in cells.



life-time development of hematopoetic cells can serve as a model system for fundamental questions such as cellular identity, cell-cell interactions, the structure of protein complexes and signal transduction in cells. At the same time, it allows a better understanding of many diseases.

Focus Epigenetics

Epigenetics describes the inheritance of acquired traits that are not based on alterations of DNA sequence.

■ "Are we more than the sum of our genes and how can environmental cues alter gene expression?" While almost all cell types (ca. 200) within a human body share an identical DNA sequence, its utilization will differ significantly according to the designated function of a cell. The DNA template within the cell nucleus is wrapped and protected by specific proteins (histones). This DNA-histone polymer is called chromatin. Stable chromatin alterations that do not affect the DNA sequence, are summarized under the term 'Epigenetics'.

Due to the plasticity of chromatin states a genome has a variety of epigenetic variants (epigenomes). Establishment and maintenance of these epigenomes is critical for embryonic development, cell type identity and cell differentiation.

Although many diseases (e.g. cancer, neurodegenerative and metabolic disorders) are primarily caused by DNA mutations, epigenetic disregulation can significantly contribute to disease progression. Thus, epigenetic research promises far-reaching implications for new forms of therapy and diagnosis. Epigenetic changes also allow responses to environmental influences such as nutrition, stress and hormones. Intriguingly, there is growing evidence that epigenetic alterations might even be inheritable over a few generations. The research groups of the Epigenetic Focus combine topics addressing dosage compensation, heterochromatin formation, and posttranslational modifications of histones. A variety of model organisms and experimental approaches (biochemistry, cell biology, *Drosophila* and mouse genetics, genome-wide profiling) are used to dissect the epigenome of distinct cell types.



The DNA template within the cell nucleus is not naked, but wrapped and protected by specific proteins (histones). This DNA-histone polymer is called chromatin. Histone modifications and other chromatin alterations are important elements of epigenetic gene control. This is critical for embryonic development, cell type identity and cell differentiation.

The International Max Planck Research School

The International Max Planck Research School for Molecular and Cellular Biology (IMPRS-MCB) was started in 2006 upon an initiative of scientists from the Max Planck Institute of Immunobiology and Epigenetics (MPI-IE) and the University of Freiburg. The idea of the research school is to provide a broad scientific education to young researchers interested in molecular and cellular biology, immunobiology and epigenetics.

Since its initiation, the PhD program has been growing steadily and cur-

rently has 63 students, coming from 31 countries, and 23 faculty members (15 from MPI-IE and 8 from the University of Freiburg).

Our PhD students are given an opportunity to carry out research on specific projects of their choice. In addition they participate in the education program covering:

- experimental methods in molecular and cellular biology;
- theoretical knowledge and in-depth analysis of scientific literature;

 complementary skills in presenting scientific data in oral and written form and in applying for research funding.

Besides this mandatory program, we organize additional scientific courses (e.g. statistics, bioinformatics) and support participation of students in external workshops and scientific conferences. Students also have the possibility to learn German and to obtain advice in planning their future career.

KEVIN DALGAARD

from Denmark IMPRS Student since 2010, Group Pospisilik



After being an IMPRS associated PhD student for three years, I can honestly say that moving to Freiburg was one of the best decisions I have made in my career so far. From the beginning I have felt very welcomed by everyone, particularly the

IMPRS office. After a three months' rotation period in different laboratories, a strong relationship with other research groups was established, which is essential for constructive feedback and advice during your studies. I believe having experts in different fields next door is one of the hallmarks of MPI-IE, leading us to conduct cutting-edge research. I am very grateful for being an IMPRS associated student and have gotten the chance to work in a young and dynamic group, where we investigate epigenetic regulation of complex metabolic diseases.

FRIEDERIKE DÜNDAR

from Germany

IMPRS Student since 2011, joint PhD at Bioinformatics Facility and Senior Group Akhtar



From the beginning, I have been positively surprised by the trust and support I received from everyone – from fellow PhD students to group leaders and the IMPRS. Even though I did not have any formal training and very little experience, I was allowed to dive into the bioinformatic analysis of high-through-

put sequencing data. I am ever so grateful for this chance as I am now involved in a very young, very dynamic, quickly developing field that is directly at the interface between cutting edge molecular biology and state of the art analysis methods.

Against my initial skepticism, Freiburg, as a city, has also completely won me over. Despite its modest size, it is a very friendly, even international city with a lively arts and music scene and its surroundings are amazingly beautiful. The proximity to France and Switzerland is an additional plus - not only regarding culinary aspects: it is also the basis for an active exchange between the MPI-IE in Freiburg, the FMI and ETH in Basel and the IGBMC in Strasbourg.

	1st year		2nd year		3rd year		4th year	
Pre-PhD Phase	O OOO Lab Rota	tions						
Thesis Committee Meeting	Introduction week	0		0		0		
Institute Seminar			0		0		0	
Obligatory Courses	Scienti	ific Present	ation	SMLC				
Retreat	0		0		0			
Course of Choice		0	0		0	0		
Conferences				Regional	0	International		
Teaching Activities		0		0		& submission		
Final Phase							0	• Defence

Schedule for IMPRS students (SMLC: Scientific Method & Logic Course)

One of the distinctive aspects of the program is a rotation period. Students spend three months in three different laboratories before starting the PhD project. In this way, they become familiar with research themes and methodology of different groups and concurrently can identify which laboratory is the most suitable for their PhD work. The rotations are also a chance for group leaders to verify which student will be the best fit for their groups. In addition, this period enhances communication and networking within the institute.

Carrying out their PhD studies at IMPRS-MCB enables students to work on exciting projects in a first class research environment, providing them with broad training in different areas and support for their scientific development. → IMPRS COORDINATOR



Magdalena Baer Rademacher

More information can be found at www.imprs-mcb.mpg.de

After acclimatizing to life

in the institute, I really

started to appreciate the

scientific environment we

work in. The institute in-

vites amazing guest speakers, has highly equipped

labs and secure funding,

TANYA KAPOOR

from India IMPRS Student since 2011, Senior Group Grosschedl



I decided to apply to the IMPRS programme in Freiburg mainly because I was interested in the research topics on offer. I also liked the fact that there was a rotation period that gave me more confidence in choosing my final lab and supervisor. Another plus point is the size of the institute: it is not a big impersonal environment and this provides an opportunity to have discussions with

people from different fields and get a different perspective on your work which is highly beneficial.

OSKAR SCHNAPPAUF

from Germany IMPRS Student since 2010, Group Saccani



and has the advantage of great collaborations between different labs and departments. I personally also like the feeling of flat hierarchies. You can sit next to a PI in the lounge and start asking questions, and the group leaders' doors are always open to you. All these factors allow you to perform cutting-edge science and to become an expert in your field quickly. The IMPRS coordinators are also very helpful and friendly, and helped us to settle down in Freiburg and feel at home faster.

Minerva



Minerva is the Roman goddess of science and wisdom and the emblem of the Max Planck Society. This bust was a gift of Peter Gruss, President of the Max Planck Society, on the occasion of the 50th anniversary of the Institute.

Research groups



⇒ SENIOR GROUP ASIFA AKHTAR

Epigenetic mechanisms underlying X chromosomal regulation



Dosage compensation as a paradigm to study transcriptional complexity

Figure 1: The MSL complex in *Drosophila*, consisting of two non-coding RNAs and five proteins, is a key factor in male dosage compensation.

DNA tightly packed together with ■ histones into nucleosomes is not easily accessible to the enzymes that use it as a template for transcription or replication. Consequently, remodelling of chromatin structure may play an essential role in the regulation of gene expression. Structural changes in chromatin may also form the basis for dosage compensation mechanisms that have evolved to equalise levels of X-linked gene products between males and females. In humans, one of the two X chromosomes in females is randomly inactivated by condensation of the chromosome into a Barr body, a process known as X-inactivation. In contrast, in Drosophila this is achieved by a twofold hyper-transcription of the genes on the male X chromosome. Genetic studies have identified a number



Figure 2:

a) In male *Drosophila*, gene expression of the single X chromosome is upregulated to get equal levels with female individuals.
b) In female mammals, one of the two X chromosomes is silenced to compensate for differences in X chromosome dosage between male and female.

of factors that are important for dosage compensation in *Drosophila*, including five proteins [MSL1, MSL2, MSL3, MLE, MOF] and two non-coding RNAs [roX1 and roX2], known as the Male-Specific-Lethal (MSL) complex. The hyperactive X is also specifically hyper-acetylated at histone H4, which is achieved by the MOF histone acetyl transferase.

Our major goal is to study the epigenetic mechanisms underlying X-chromosome specific gene regulation using *Drosophila* dosage compensation as a model system. More specifically, we are interested in addressing how the dosage compensation complex, composed of RNA and proteins [the MSL complex], gets targeted to the X chromosome. In addition, we are studying the mechanism by which the MSL complex modulates X chromosomal transcriptional output.

The role of non-coding RNA in dosagecompensation

Long non-coding RNAs (lncRNAs) are emerging as important regulators of chromatin state and transcription in eukaryotic cells. They can contribute to the regulation of single genes or whole chromosomes and can influence the 3-D structure of large genomic regions. Due to their length, which typically is in the range of kilobases, it has been difficult to understand their exact contributions to transcriptional regu-



Figure 3: The protein MLE grabs the RNA strand like a monkey grabs a liana. One site serves as a simple anchor (feet), while the other is able to mould the strand. This consumes energy (banana). The moulded RNA allows other proteins to bind and thus to activate the X chromosome in male flies. (Highlight from Ilik et al, Mol Cell 2013)

lation. Interestingly, the dosage compensation complex includes two long non-coding roX RNAs. However, the mechanism by which these RNAs function is poorly understood. Our recent work has shown that roX RNAs harbor several binding sites for MSL complex members, thus providing a platform for complex assembly. One of our future aims will be to elucidate how these RNA-protein interactions in the MSL complex influence transcription activation of the male X chromosome.

Chromosome dynamics and gene expression

It is becoming increasingly clear that



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chromosomal organization as well as gene positioning has the potential to influence gene expression. The X chromosome provides a nice example of a chromosome that is decorated with a ribonucleoprotein complex and is transcriptionally upregulated. We are interested in understanding how X chromosomal genes are organized within the X chromosomal territory but also within the nucleus with respect to the nuclear periphery to study whether and how this influences X-linked gene expression. We employ a multifaceted approach combining cell biology, biochemistry and genetics to gain novel insights into the role of genome organization and gene expression.



Figure 4: The NSL complex binds to all chromosomes. It is enriched on promoter regions and is involved in the regulation of many housekeeping genes in *Drosophila*.

The role of the NSL complex in gene regulation

Our earlier work identified that in addition to the MSL complex the MOF histone acetyltransferase is part of an evolutionary conserved Non-Specific Lethal (NSL) complex in Drosophila and mammals. Members of this complex are essential for male and female Drosophila. We have subsequently shown that the NSL complex is a chromatin bound complex that is enriched on promoters of target genes. Furthermore, it appears to be a major regulator of expression of housekeeping genes in Drosophila. We are currently exploring how this complex alone or in association with MOF regulates gene expression. We are also interested in studying how MOF activity is regulated in the NSL complex.

The function of the mammalian MSL and NSL complexes

There is a remarkable evolutionary conservation of the Drosophila and mammalian MSL and NSL complexes at the biochemical level, implying a functional role for these proteins in gene regulation. Interestingly, loss of MOF leads to early embryonic lethality indicating that this protein is essential during mouse development. Furthermore, MOF and H4K16ac are frequently mis-regulated in cancer suggesting that it is critical for cellular homeostasis of mammalian cells to maintain appropriate levels of this histone modification. We are interested in exploring what aspect of MOF mediated regulation is conserved in mammals and how is the division of labor between the MSL and the NSL complexes achieved in mammalian cells.

→ ASIFA AKHTAR

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Thymopoiesis: From evolutionary origins to future therapies

Evolution of adaptive immunity in vertebrates



Figure 1: A histological section of thymus tissue of a young shark shows that the organ structure has remained essentially unchanged for about 500 million years.

■ Our goal is to contribute to the understanding of the genetic basis of immune system function with a view to explaining human disease and to developing targeted therapies for correcting failing immune function.

Studying animals as diverse as lampreys and mice, we aim to understand the mechanism(s) by which adaptive immune systems achieve an effective quality control to eliminate and/ or control the function of potentially self-reactive receptors that are generated by a somatic and essentially random assembly process. Because this selection process takes place in primary lymphoid organs such as the thymus, we are investigating the genetic basis of the development and function of organs. In an iterative process, we combine forward genetic screens and methods of precise genetic interference in two model systems – zebrafish and mouse – to examine the role of single genes or combinations thereof in the formation of the thymic anlage and the development of T cells. Our aim is to use this information to reconstruct ancient forms of thymopoietic tissue and to build artificial equivalents for potential therapeutic use.

Thymus and T cell development in the mouse

The thymus is a primary lymphoid organ whose function is to provide mature and self-tolerant T lymphocytes that are required to fight infection and maintain tissue integrity. Thymopoiesis depends on the provision of a dedicated epithelial microenvironment that attracts, maintains and specifies T cell progenitors and supports their differentiation into mature, self-tolerant T cells. We are interested in the molecular basis of thymic epithelial development and the characterization of the epithelial progenitor cell. To this end, we interfere with the function of various signalling pathways, such as BMP, Wnt, Fgf, etc. in thymic epithelial cells (TEC) in vivo in order to study their roles in the regulation of TEC specification, proliferation and differentiation. We have previously shown that the function of the stromal niche required for the attraction and specification of lymphoid progenitor cells depends on the Foxn1 transcription factor. We have rebuilt this niche function in vivo in



Figure 2: Thymocyte development in wild-type (left panel) and mutant (right panel) fish, visualized by RNA *in situ* hybridization. The thymus is marked with red arrowheads.



Figure 3: Macroscopic view of an embryonic mouse thymus. The fluorescence originates from the epithelial compartment.



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transgenic mice nullizygous for *Foxn1* by re-expression of individual target genes of the Foxn1 transcription factor, singly or in combination. To date, we have achieved the reconstitution of T cell development until the CD4⁺CD8⁺-double-positive stage of $\alpha\beta$ T cells using just two factors, Cxcl12 and Dll4. Ultimately, we wish to use this information to engineer artificial thymus stroma at ectopic sites as a potential means of countering the ill-effects of diseased thymic tissue.

Genetics of thymopoiesis and T cell development in vertebrates

A forward genetic screen in zebrafish was undertaken in order to establish the genetic basis of thymopoiesis and T cell development in vertebrates and about 40 mutant lines have been established. The mutant genes so far identified by positional cloning show that the zebrafish model is an excellent tool to define novel genetic pathways important for T cell development. For instance, we have identified an evolutionarily conserved function of the ikaros transcription factor in zebrafish lymphopoiesis and defined the key requirement of the c-myb transcription factor for definitive hematopoiesis. The molecular nature of other genes identified in this screen also support the notion that the overall mechanism of thymopoiesis is well conserved in vertebrates, and we are working towards the application of these findings to explain previously uncharacterized

immunodeficiency syndromes in humans. We also use long-term live imaging analysis with our mutants and novel transgenic fish lines to examine the genetic basis of essential steps during thymopoiesis, i.e. migration and specification, and to establish their spatial and temporal characteristics. Here, we are exploiting the unique possibility in fish of interfering in vivo with single and multiple gene functions through sequence-specific genetic interference in order to examine the structure of genetic networks controlling key steps in the thymopoietic process, such as homing and T lineage specification.

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Evolution of adaptive immune systems

Most species in the animal kingdom lack an adaptive immune system and instead rely on innate immune functions for immune defence. By contrast, vertebrates additionally employ an adaptive immune system. Based on a broad-ranging analysis of chordate species, we are examining the structure, function and evolutionary trajectories of genetic networks underlying the emergence of mechanistic and morphological features of adaptive immune systems. This work encompasses the development of gene inventories for species occupying key phylogenetic positions, such as cephalochordates, lamprey and shark, and the functional probing of gene functions in genetically tractable animals such as teleosts and mouse, with a particular focus on the thymus.

THOMAS BOEHM

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The role of micro-RNAs in hematopoietic and immune system



Figure 1: Background: Heat map of miRNA microarray. Expression level of each miRNA is compared from mouse tissue samples. Front: ChIP-Seq result of wild-type and miRNA knockout mouse tissue samples.

Functional genomics and reverse genetic approaches to uncover mechanisms of malignant hematopoiesis

The aim of our research group is to understand the biology of functional RNAs in the hematopoietic-immune system. Currently, we are focusing on the role of microRNAs in the mammalian hematopoietic-immune system.

MicroRNAs are a class of non-coding RNAs that bind to the complementary mRNAs and thereby regulate their expression. They are found in animals, plants and viruses with their sequences conserved even among relatively distant species. Various reports have

Plasmacytoid

DC (pDC)

shown the involvement of microR-NAs in a broad range of physiological events such as development, differentiation, proliferation, morphogenesis, apoptosis and metabolism.

At present, little is known about the role of microRNAs in hematopoiesis and immunity. However, microRNAs are considered to be critical regulators for development and functions of immune cells. Studying the biology of micro-RNAs in the immune system may directly contribute to understanding the molecular mechanism of immune system integrity. Furthermore, the knowledge of microRNAs in immunology would provide clues to elucidate the molecular pathogenesis of infection and immune diseases such as autoimmune and inflammatory diseases. In our lab, we are studying the roles of microRNAs in the hematopoietic-immune system by using various reverse genetics approaches (Knock-out and Knock-in) and transgenic technologies in the mouse system with highthroughput multi-(prote-/gen-/etc.) Omics-based bioinformatic analyses.



maturation

conventional

DC (cDC)

Current model for Flt3L-dependent steady-state DC

Trend analysis of miR-transcriptome dynamics



Figure 2: Ligand (Flt3L) stimulation induces the differentiation of both conventional dendritic cells (cDC) and plasmacytoid DC (pDC). The term cDC summarizes all DC subsets with "professional antigen presenting" function, while pDC represent the "professional interferon-alpha producers".

Figure 3: Conventional dendritic cells (cDC) and plasmacytoid DC (pDC) were generated and subjected to transcriptomic analysis (on the Agilent micro array platform) and miRNome analysis (on miRxplorer platform).



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→ TARO FUKAO

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Mechanisms of malignant hematopoiesis through functional genomics and reverse genetic approaches

In this project my group is focusing on the biology of functional RNAs in the hematopoietic-immune system. Taking advantage of various reverse genetics approaches and transgenic technologies in mouse system with high-throughput multi-(prote-/gen-/etc.) Omics-based bioinformatics, we investigate the roles of microRNAs in the hematopoietic and immune system.

MicroRNA profiling in hematopoietic cells

Profiling of microRNA expression patterns in different tumor tissues has been extensively performed to understand the role of microRNAs in malignant hematopoiesis. As we have substantial information on microRNA genes and strong bioinformatics tools to analyse the microRNA functional genomics, we would like to perform microRNA functional genomics in multiple myeloid leukemia types. This challenge would give insights into pathogenesis of many types of myeloid leukemia. Furthermore, characterizing the microRNome with the functional profile in each myeloid leukemia type might provide clues to establish novel therapeutic and diagnostic approaches.

Exploiting a bioinformatics-based approach, we recently demonstrated that some microRNAs are expressed in hematopoietic cells and regulated by well-known hematopoietic transcription factors such as PU.1 and C/EBPs. Furthermore, our preliminary study predicted that such microRNAs might

target several molecules involved in hematopoiesis. These observations suggest a role for microRNAs in hematopoiesis. Based on the genomic information, we developed several mouse models carrying genetically modified microRNA genes. Using these mouse models, we would like to uncover the role of microRNAs in nomal hematopoiesis. We expect that these genetically modified mice might show some abnormal hematopoietic phenotypes and give insights to understand the role of microRNAs in normal hematopoiesis. We would like to understand how the microRNA system is influencing other molecular systems (transcriptome, proteome, etc.) during hematopoietic lineage commitment. Our preliminary results showed that the microRNA expression pattern (microRNome) is different between distinct hematopoietic lineages.

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Identification of microRNAs differentially regulated during DC differentiation

Multiple subsets have been identified so far in the dendritic cell (DC) lineage and shown to play distinct roles in various physiological circumstances. The identity of each DC subset should be defined by the unique gene expression pattern, associated with the specialized functions. We explored the role of microRNAs in the control of gene expression in DC subsets by exploiting a comprehensive systems biology approach. Concomitant profiling of the microRNome and transcriptome in plasmacytoid DCs, convectional DCs and their common progenitor (common DC progenitor) revealed a dynamic change of microRNA expression signatures during differentiation into each subset. The alteration in transcriptome patterns during the subset specification was significantly correlated with such dynamic regulation of microRNAs. Thus, we identified several microRNAs differentially regulated during DC differentiation and observed intriguing trends of the target gene expression consistent with the level of corresponding microRNAs in DC subsets. Indeed, aberrant transcriptome patterns were observed in DC subsets isolated from several knockout mice lacking some of those differentially regulated microRNAs, indicating that dynamic regulation of microRNA expression during DC differentiation would give significant impacts on DC functions. On the other hand, it is now well accepted that microRNAs shape multiple -Omes patterns and thus confer the cellular identity to specific cell lineages. Given the distinct microRNome patterns, it is expected that cellular identities of different blood cells would be also maintained by microRNAs through modulation of other molecular systems.

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Regulatory circuitries of B lymphopoiesis



Role of transcription factors in signal integration and higher-order chromatin structure

Figure 1: Structure of the Ebf1 : DNA complex reveals structural similarities with NF- κ B and NFAT proteins (DBD; DNA-binding domain; TIG transcription factor/immunoglobulin domain; HLH; helix-loop-helix domain).

≥ Lymphocytes are generated from multipotential hematopoietic stem cells in an ordered process of terminal differentiation. Several transcription factors regulate distinct steps of lymphocyte differentiation, including the specification and commitment of progenitor cells to a particular cell lineage and the maturation of these cells into functionally distinct subsets. We address questions of which genes regulate functional differences between conventional B cells and innate-like B cells, which signals from stromal niches regulate the expression of transcriptional determinants of lymphopoiesis, and how specific signals and transcription factors coordinate the complex differentiation process. Many regulators of specific cell lineages are also expressed in other cell types and therefore, it is important to understand the combinatorial code of these proteins and to elucidate the regulatory network of transcription factors and cis-regulatory sequences. Other questions include the role of epigenetic modifications in cell differentiation, the influence of higher-order chromatin structure and the function of transcription networks in the regulation of stem cell pluripotency.

Regulatory circuitries of B lymphopoiesis

B lymphopoiesis depends on the integration of extracellular signals by transcription factors that specify hematopoietic progenitors and allow for differentiation into highly-specialized effector cells. We are interested in understanding the molecular basis of B cell differentiation by dissecting the regulatory circuitries in which celltype-specific transcription factors operate. Toward this goal, we are studying the function of Early B cell Factor-1, EBF1, which is expressed in the early stages of the B cell lineage and in the stromal cells of the bone marrow. Lossand gain-of-function experiments indicated that EBF1 functions in a complex regulatory network with other tran-

scription factors, in which positive feedback loops and cross-antagonism stabilize the establishment of the B cell program. Genome-wide analysis of EBF1-bound regions by functional targets of EBF1 chromatin immunoprecipitations with anti-EBF1 antibodies and deep sequencing (ChIPseq analysis) allowed for the identification of genes that are bound and transcriptionally regulated by EBF1. Among the targets, we also identified genes at which EBF1 induces chromatin changes, defined by H3K4 dimethylation, that

poise the genes for expression at subsequent stages of differentiation. These and other data on B cell-specific EBF1 targets suggested that EBF1 can act as a "pioneer" factor in a hematopoietic chromatin context.

Stem cell pluripotency and higherorder chromatin structure

We found that the nuclear proteins Satb1 and Satb2, which function as determinants of higher-order chromatin structure, have opposing roles in the regulation of the pluripotency gene Nanog. In particular, Satb1 represses Nanog, whereas the closely



Figure 2: Schematic representation of the regulatory network in which the transcription factor $\mathsf{EBF1}$ acts.



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related Satb2 protein activates Nanog. Moreover, both Satb1-deficient ES cells and wild-type ES cells in which Satb2 is overexpressed are more efficient in reprogramming human B lymphocytes in heterokaryon fusion experiments. Currently, we are examining whether or not the balance of Satb1 and Satb2 expression contributes to the heterogeneity of ES cells in the expression of pluripotency genes. Satb 2 also plays a role in B lymphocytes by determining the higher-order chromatin structure of the immunoglobulin heavy chain (IgH) locus. We found that Satb2 is bound in vivo to AT-rich sequences that flank the intronic IgH enhancer. By studying the subnuclear localization and higher-order chromatin structure of the 3.2 Mb IgH locus in Satb2-deficient pro-B cells, we anticipate to unravel the molecular basis of Satb2 function in the regulation of higher-order



Figure 3: Subnuclear localization of Satb2, a regulator of higher-order chromatin structure



Figure 4: Regulatory circuitry of Satb2 in ES cells.

chromatin structure. By combining biochemical, imaging and genetic approaches, we are interested in elucidating how Satb proteins functionally organize chromatin via looping and how these proteins contribute to changes in epigenetic marks during stem cell differentiation.

Role of Mzb1 in peripheral B cell subsets

Peripheral B lymphocytes consist of multiple cell populations that differ in their phenotype, functional properties and anatomic locations. In addition to the vast majority of conventional B cells, also termed follicular B cells, which resides in lymph nodes and follicles of the spleen, marginal zone B cells occupy the marginal sinus of the spleen, and B1 cells are predominantly found in the peritoneal pleural cavities. B1 cells and marginal zone B cells have been termed 'innate-like B cells' because these cells quickly differentiate into antibody-secreting cells that produce 'natural', polyreactive antibodies. Therefore, these cells are considered to bridge the innate and adaptive immune responses. To gain insight into the regulation of functional differences between innate-like and conventional B cells, we have previously identified and cloned a gene, termed Mzb1, which is abundantly expressed in marginal zone B cells and B1 cells. We have shown that Mzb1 protein is an endoplasmic reticulum-localized protein that regulates antibody secretion, calcium homeostasis and integrin-mediated cell adhesion. Mzb1 interacts with a substrate-specific chaperone Grp94 and current efforts focus on the mechanism by which Mzb1 regulates functions specific to innate-like B cells.

→ RUDOLF GROSSCHEDL

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→ GROUP PATRICK HEUN

Epigenetic regulation of chromosome and nuclear organization



Unravelling centromere identity

Figure 1: Centromeres cluster at the periphery of the nucleolus. AntidCENP-A^{cenH3} antibodies reveal that the 13 centromeres of *Drosophila* Schneider S2 cells cluster in about 4–5 foci around the nucleolus marked by anti-fibrillarin antibody.

■ All inheritable chromosome conditions not encoded by the DNA sequence itself are called epigenetic, including gene expression and for most eukaryotes also centromere and telomere identity. The epigenetic transmission of these states through many cell generations is highly relevant for proper genome regulation and when perturbed can lead to genome instability and cellular malfunction. The goal of my lab is to understand how chromosome and nuclear organization affects genome function.

Centromeres are found at the primary constriction of chromosomes in mitosis where they remain connected before cell division. This structure is essential for an equivalent chromosomes distribution to the daughter cells. Using the fruit fly *Drosophila melanogas*- *ter* as a model organism my lab is particularly interested in the epigenetic regulation of centromere identity with a focus on neocentromere formation in flies and human tissue culture. We are further interested in the nuclear organization of centromeres and a third project investigates the role of the protein SUMO-E3 ligase dPIAS in chromatin organization and gene expression. In addition to genetic, developmental, biochemical, and cytological analysis, the lab uses live imaging and timelapse microscopy as major tools in these studies.

Epigenetic regulation of centromere identity

The human centromere specific histone H3-variant CENP-A is essential for kinetochore formation and centromere function. We have recently shown that overexpression of dCENP-AcenH3 (CID in Drosophila) results in its incorporation into chromosomes arms to form functional ectopic kinetochores in tissue culture and the fly. As a consequence mitotic arrest and chromosome fragmentation are observed. Following cells after a pulsechase induction of ectopic dCENP-A^{cenH3} expression, we could identify "dCENP-AcenH3 islands", representing functional kinetochores. Importantly, these sites are not randomly distributed along the chromosome, but are preferentially localized to hotspot regions at telomeres and pericentric heterochromatin. This suggests that heterochromatin boundaries contribute to the choice of ectopic kinetochore position and contribute to centromere identity. Independently of the approach outlined above the possibility remained





Figure 3: Scheme how PIAS might effect heterochromatin organization by SUMOylating one of its components or regulators.



From left: Heun, Patrick (Group Leader), Schade, Georg (PhD Student), Padeken, Jan (PhD Student, until 8/2013), Roure, Virginie (Postdoctoral Fellow), Schwarz, Hans-Jürgen (Technician), Kyriacou, Eftychia (PhD Student). Not present: Anselm, Eduard (PhD Student), Barrey, Evelyne (PhD Student)

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that dCENP-AcenH3 alone may be sufficient for centromere formation. To address this question we have established a biosynthetic approach to target dCENP-AcenH3 to specific noncentromeric sequences such as the Lac Operator and follow the formation of functional neocentromeres. Using this approach we were able to directly demonstrate that a dCENP-A^{cenH3} -LacI fusion can induce centromere formation at Lac Operator sites as well as self-propagation and inheritance of the epigenetic centromere mark. Using our LacO/LacI tethering system, we are now interested in exploring the formation of human artificial chromosomes (HACs) in human cells. In addition we are dissecting the function of dCENP-AcenH3 in Drosophila and human cells for its centromere targeting, kinetochore formation and self-propagation properties.

Nuclear organization of centromeres

The compartmentalization of the eukaryotic cell helps regulating proper genome function and gene expression. Using live imaging of stable cell lines as well as fluorescence microscopy in fixed cells we can show that in D. melanogaster centromeres and the pericentric heterochromatin are not randomly positioned in the nucleus, but are tightly tethered to the heterochromatin surrounding the nucleolus. There they form only 3 to 5 bigger clusters throughout interphase, although 13 centromeres are present in these cells in G1 (figure 1). We are currently investigating the factors involved in this particular organization. Recent findings have shown that the

protein NLP (Nucleoplasmin Like Protein) a member of the Nucleophosmin protein family, plays a major role for centromere positioning. It binds specifically to the centromere region of the chromosomes and causes their clustering near the nucleolus. The nucleolus protein Modulo anchors the complex consisting of the centromere and NLP to the nucleolus, and the protein CTCF supports NLP in the clustering of the centromeres.

We now investigate whether this effect can also be observed in mammalian cells and its potential contribution to mis-regulated gene expression in cancer.

The role of PIAS in heterochromatin organization

In Drosophila, PIAS (Protein Inhibitor of Activated STAT) has previously been identified as a suppressor of Position Effect Variegation (PEV). PEV is observed when a normally active gene is artificially placed close to silent heterochromatin leading to variegated gene expression. Homozygous mutants in dPIAS die as 3rd instar larvae and display melanotic tumors, hypocondensed chromosomes in mitosis, and disorganized chromosomes and telomeres in polytene chromosomes, suggesting roles also in chromosome function and nuclear organization. The diverse roles of dPIAS are consistent with the demonstration that PIAS proteins encode a SUMO E3 Ligase. The small ubiquitin-like modifier (SUMO) is a versatile modification, which serves a variety of functions, including transcriptional regulation, protein stability, nuclear transport and chromosome

and nuclear organization. SUMO E3 ligases are part of the SUMO conjugating system and together with the SUMO conjugating enzyme Ubc9 provide target specificity for SUMOylation of substrate proteins.

The aim of this project is to find out how PIAS mediated SUMOylation is involved in regulating heterochromatin organization. Currently we are determining SUMOylation targets in Drosophila tissue culture and whether these are SUMOylated in a PIAS dependent manner. The target proteins are being verified by SUMOylation assays in vitro and in bacteria.

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→ GROUP ANA IZCUE

Mechanisms of gut immune tolerance

CD4⁺ Foxp3⁺ Treg and tolerance to self- and exogenous antigens

Figure 1: Foxp3+ T cells (Treg) in a section of mouse colon. Blue: cell nuclei, red: E-cadherin as marker for epithelial cells, green: Foxp3 as marker for Treg cells

The immune system has evolved to fight pathogens while remaining tolerant to self and harmless antigens. The need for simultaneous immune vigilance and tolerance is particularly evident in the intestine, which is confronted with antigens coming not only from pathogens, but also from food and harmless commensals. Since the intestine is the largest barrier surface of the body with the outside world, it needs to be able to mount quick, efficient immune responses against potential pathogens. However, most of the intestinal antigens come from the food and the commensal flora, which need to be tolerated. Hence, the intestinal immune system combines a high number of inflammatory cells with a strong population of immune regulators that prevent detrimental reactions against harmless antigens. How the balance

between inflammation and tolerance is achieved is still not well understood. Our group focuses on the induction and maintenance of immune tolerance, especially through the generation and control of CD4⁺ Foxp3⁺ regulatory T cells (Treg). Treg can be produced in the thymus and the periphery, and prevent misdirected immune responses. They are key to avoid autoimmunity and inflammatory responses against exogenous antigens as the ones from food or the commensal microbiota, as shown in mouse models and immunodeficient patients. We want to dissect the factors that control the activity of antigen-specific Treg. Due to the specific requirements for tolerance in the gut, we are especially interested in the interactions of intestinal epithelial cells with the immune system.



Role of the intestinal epithelium in shaping the immune response

The complex intestinal microbiota is separated from the immune system by a single layer of epithelial cells. In the thymus, epithelial cells play a crucial role in T cell development through antigen presentation and provision of survival, apoptosis and differentiation signals. Intestinal epithelial cells can also produce signals that modulate lymphoid activity; however, the role of the epithelium in intestinal immune responses is not yet completely understood.

We are trying to identify in which way communication between epithelial cells and the immune system shapes inflammation and tolerance in the gut.

Intestinal epithelial cells can produce signals that modulate lymphoid activity, for example IL-7 and TSLP. On the other hand, the immune system harbours receptors capable of recognizing molecules on epithelial cells, and it also produces cytokines that affect epithelial activity. The outcome of immune responses depends on this crosstalk. We want to identify how intestinal epithelial cells interact with the immune system to establish and abrogate tolerance. By analysing systemic and intestinal immune populations and their activity, our lab aims to understand how tolerance is tailored to the different needs of each environment.



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CD4⁺ T cell subsets in the regulation of immune responses

The intestine has a strong requirement for specific immune tolerance. One population playing an important role in intestinal immune homeostasis are CD4+ Foxp3+ T cells (Treg). Treg are found not only in the lymphoid organs, but also in tissues such as the intestinal lamina propria, where they account for a larger proportion of the T cells than in the spleen or lymph nodes. There are two different pathways for Treg generation. On one hand, Foxp3 expression and regulatory activity can be induced in the thymus during T cell development. On the other hand, Foxp3 can be induced in naive T cells in the periphery after antigen stimulation, through a mechanism relying on the cytokine TGF-β and modulated by positive and negative factors such as IL-2 and IL-6. We and others have shown that Foxp3 induction in the intestine can be sufficient to inhibit local inflammation. We want to define the factors controlling Treg induction and activity in the periphery, and to find out if intestinal Tregs have specific requirements in this respect. We are especially interested in Treg subsets expressing specific receptors for molecules on intestinal epithelial cells and their role in local tolerance. The analysis of factors controlling local tolerance in the tissues could lead to new therapeutical treatments for intestinal inflammatory diseases such as Crohn's disease and ulcerative colitis.



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Figure 3: Intestinal immune environment. The immune homeostasis relies on the integration of various signals from intestinal immune and non-immune cells.

Figure reproduced from Izcue A. Coombes J. and Powrie F. (2009) Regulatory lymphocytes and intestinal inflammation. Annu Rev Immunol 27, 313-338.

⇒ SENIOR GROUP THOMAS JENUWEIN

Epigenetic control by histone lysine methylation



Investigating the basic composition of mammalian heterochromatin

Figure 1: Impaired Prdm3/Prdm16 function disintegrates heterochromatic foci. DNA-FISH for major satellite repeats in wild-type iMEF cells upon control and Prdm3/Prdm16 double knock-down. DNA is shown in blue and DNA FISH signals in green.

≥ Epigenetic mechanisms control eukaryotic development beyond DNAstored information. There are many pathways, such as DNA methylation, nucleosome remodelling, histone modifications, exchange of histone variants and non-coding RNAs that together contribute to differences of the chromatin template. In particular, the great diversity of covalent histone tail modifications has been proposed to reflect an index (,histone code') that can stabilize distinct proliferative and developmental options. We discovered the first histone lysine methyltransferase and then showed that histone lysine methylation represents a central epigenetic modification in eukaryotic chromatin. We are continuing with our analyses on histone lysine methylation to further dissect epigenetic gene regulation and to identify molecular pathways that initiate and maintain heterochromatic domains in mammalian chromatin.

How to make heterochromatin

Heterochromatin is important to protect genome integrity and to stabilize gene expression programs. Mammalian heterochromatin is characterized by its underlying repetitive DNA sequence, several epigenetic hallmarks such as H3K9me3 and H4K20me3, DNA methylation and non-coding heterochromatic RNAs and the localization of at least 16 core components (Fodor et al., 2010). Despite the identification of these key players that ensure heterochromatic integrity, it is still unknown how heterochromatin is initiated and by which mechanism(s) it remains discriminated from euchromatin.

We have recently identified the transcription factors Pax3 and Pax9 as redundant regulators of mouse heterochromatin, as they repress RNA output from major satellite repeats by associating with DNA within pericentric heterochromatin (Bulut-Karslioglu et al., 2012). Simultaneous depletion of Pax3 and Pax9 resulted in dramatic derepression of major satellite transcripts, persistent impairment of heterochromatic marks and defects in chromosome segregation. Genome-wide analyses of H3K9me3 showed enrichment at intergenic major satellite repeats only when these sequences retained intact bind-

ing sites for Pax and other transcription factors. Additionally, bioinformatic interrogation of all histone methyltransferase Suv39h-dependent heterochromatic repeat regions in the mouse genome revealed a high concordance with the presence of transcription factor binding sites. These data define a general model in which reiterated arrangement of transcription factor binding sites within repeat sequences is an intrinsic mechanism of the formation of heterochromatin (see Figure 2). In extension of this study, we are interested to identify additional transcription factors involved in heterochromatin formation. Furthermore, we would like to test our model that predicts a distinct arrangement of transcription factor binding sites as determinant for heterochromatin in human cells.

How to break heterochromatin

Although the Suv39h methyltransferases (KMTs) are known to ensure pericentric H3K9me3 methylation, the mechanisms that initiate and maintain mammalian heterochromatin organization remain elusive. While *Suv39h* dn cells display dispersed H3K9me3, H3K9me1 accumulates at pericentric regions, indicating the action of a specific H3K9me1 KMT. We developed a biochemical assay and used *in vivo*



Figure 2: A transcription factor-based model for mouse heterochromatin formation.

Model for the distinction between euchromatin and heterochromatin that is based on a synergistic vs. a more random organization of transcription factor binding sites. TF: transcription factor; KMT/Suv39h: histone methyltransferase; HP1: Heterochromatic Protein 1.



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analyses in murine embryonic fibroblast cells (MEFs) to identify Prdm3 and Prdm16 as redundant H3K9me1specific KMTs that direct cytoplasmic H3K9me1 (Pinheiro et al., 2012). The H3K9me1 is converted in the nucleus to H3K9me3 by the Suv39h enzymes to reinforce heterochromatin. Simultaneous depletion of Prdm3 and Prdm16 abrogates H3K9me1, prevents Suv39hdependent H3K9me3 and derepresses major satellite transcription. Most strikingly, DNA-FISH and electron microscopy reveal that combined impairment of Prdm3 and Prdm16 results in disintegration of heterochromatic foci and disruption of the nuclear lamina (see Figure 1). Our data identify Prdm3 and Prdm16 as H3K9me1 methyltransferases and expose a functional framework in which anchoring to the nuclear periphery helps maintain the integrity of mammalian heterochromatin.

We will now focus on the detailed molecular mechanism, by which these enzymes exert their function. In particular, we are interested in exploring a potential deposition related mechanism for H3K9me1 and signalling pathways that may regulate the enzymatic activity of Prdm3 and Prdm16.

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Genome-wide signatures for heterochromatin

Interstitial heterochromatin is required to restrict aberrant expression of retrotransposons, but it remains poorly defined due to the underlying repeatrich sequences. We dissected Suv39hdependent H3K9me3 by genome-wide ChIP-sequencing in mouse embryonic stem cells (ESCs) (Bulut-Karslioglu et al., submitted). Refined bioinformatic analyses of repeat subfamilies indicated selective accumulation of Suv39hdependent H3K9me3 at interspersed repetitive elements that cover ~ 5% of the ESC epigenome. The majority of the ~ 8,150 intact long interspersed nuclear elements (LINEs) and endogenous retroviruses (ERVs), but only a minor fraction of the > 1.8 million degenerate and truncated LINEs/ERVs, are enriched for Suv39h-dependent H3K9me3. Transcriptional repression of these intact LINEs and ERVs is dif-



Figure 3: Suv39h enzymes target retrotransposons in mouse ESCs. ChIP-seq analysis for H3K9me3 in wild-type and Suv39h dn ESCs and for Suv39h enzymes in Suv39h1- and Suv39h2-HA/FLAG knock-in ESCs. A representative region of 30 kb on chromosome 11. which contains several LINE and LTR elements, is shown.

ferentially regulated by Suv39h and other chromatin modifiers in ESCs but governed by DNA methylation in committed cells. These data provide a novel function for Suv39h-dependent H3K9me3 chromatin in the ESC epigenome and reveal that interstitial heterochromatin is restricted to the intact fraction of retrotransposon elements.

We plan to extend our genome-wide analyses to other core components of heterochromatin and to identify noncoding RNA moieties that associate with these factors.

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→ UNIVERSITY/MPI-IE GROUP HASSAN JUMAA

Regulation of proliferation and differentiation of B cells

The B cell antigen receptor and its downstream signaling in normal B cell development and B cell transformation +tam iCre TS/Cre iCre TS/Cre d7 d7 d7 d7 d3 d3 d3 d3 d1 d1 d1 d0 d0 d0

Figure 1: Inducible TEL-Syk (TS) expression leads to initial expansion of TS-expressing B cells in peripheral lymphoid organs. Photographs of spleens and lymph nodes from TS/iCre and iCre control mice at different time-points after tamoxifen (+tam) treatment.

The aim of our research group is to obtain a global understanding of the signalling pathways that regulate the proliferation and differentiation of B cells. B cells play a key role in adaptive immune response and enable the body to attack pathogens highly efficient and persistent. They bind pathogenic substances at the B cell antigen receptor (BCR). Signals emanating from the BCR or its precursor (pre-BCR) lead to changes in gene expression and determine the fate of developing B cells. Malfunctions in the signal transduction of B cells result in immunodeficiency, autoimmunity or leukaemia. Employing a multidisciplinary approach, we aim to provide a molecular and quantitative understanding of the signalling networks acting in B cells. Studying signal transduction in B cell development allows multiple interactions and interdisciplinary collaborations with research groups in both basic science and in the clinic.

Regulation of proliferation and differentiation of B cells and the development of lymphomas

The aim of our research is the characterization of molecular processes that regulate the normal development of B cells and the mechanisms that underlie B lymphocyte transformation. The BCR is a central regulator of B lymphocyte differentiation and proliferation. We are interested in how signalling from BCRs may promote lymphoma development and whether there are structural and/or functional features specific for BCRs expressed on lymphoma cells. Based on our previous studies on autonomous signalling of BCRs from polyreactive B cells, we have characterized BCRs from patients with Chronic Lymphocytic Leukemia (CLL). We could show that, in contrast to other B cell neoplasias, such as Multiple Myeloma, Mantle Cell Myeloma, Marginal Zone Myeloma and Follicular Lymphoma, CLL-derived BCRs induce antigenindependent, cell-autonomous signal-



Figure 2: CLL-derived BCRs possess autonomous signalling capacity

Fluorescence-activated cell sorting (FACS) analyses of Ca2+ flux after the addition of 4-hydroxytamoxifen (4-OHT) to TKO cells expressing the ERT2–SLP65 fusion protein and two representative CLL-derived BCRs or two representative BCRs from non-CLL lymphomas. ling which is dependent on the heavy chain complementarity determining region 3 (HCDR3) and an internal epitope in framework region 2 of the BCR heavy chain (HC) (Figure 2). Transferring the HCDR3 of CLL-derived BCRs confers autonomous signalling capacity to non-autonomously active BCRs, whereas mutations in the internal epi-



Figure 3: N46 glycosylation in μHC is essential for pre-BCR function.

Ca2+ mobilization in SLP-65, λ 5, RAG2 tripledeficient (TKO) cells expressing the ERT2-SLP65 fusion protein reconstituted with μ HC or the Nglycosylation mutant N46Q μ HC together with λ 5 stimulated with either 4-hydroxytamoxifen (4-OHT) only or with 4-OHT together with anti-HC antibody. Ca2+ mobilization upon stimulation with 4-OHT indicates autonomous pre-BCR signalling. The empty vector pair served as a control.



From left: Surova, Elena (PhD Student), Outaleb, Issam (PhD Student, until 4/2013), Wossning, Thomas (Postdoctoral Fellow, until 9/2013), Waheed, Oli (Master Student, until 4/2013), Krause, Arndt (Master Student, until 9/2013), Jumaa, Hassan (Group Leader), Dühren- von Minden, Marcus (Postdoctoral Fellow), Schneider, Dunja (PhD Student), Hug, Eva (Postdoctoral Fellow), Übelhart, Rudolf (Postdoctoral Fellow), Bach, Martina (Postdoctoral Fellow), Werner, Markus (Postdoctoral Fellow). Not present: Alkhatib, Alabbas (Postdoctoral Fellow), Iype, Joseena (PhD Student), Sainz Rueda, Concepcion (Technician)

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tope abolish this capacity. In this ongoing project, we further characterize structural and functional features of BCRs from lymphoma patients and are in the process of identifying bio-molecules suitable to specifically influence signalling from autonomously active receptors.

The role of N-glycosylation in BCRs of different classes and in precursor-BCRs

N-linked glycans are carbohydrates that are attached to multiple proteins that pass the secretory pathway and have an impact on e.g. protein stability and function. Similar to secreted antibodies, BCRs and their precursors are highly glycosylated. In this project, we aim at elucidating the impact of N-glycosylation on the function of BCRs at different developmental stages, of BCRs of different subtypes, and of BCRs from different lymphomas. We were able to show that the function of the pre-BCR is strictly dependent on the presence of an N-linked glycan in the µ-heavy chain constant region 1, where it promotes self-ligation between pre-BCRs on the very same cell. Self-ligation then leads to cell-autonomous signalling, proliferation and further development of the lymphocyte (Figure 3). During B cell development, the subtype of the HC of BCRs changes from a state where only the µ-type is expressed to a state where the µ-type is co-expressed with HCs of the δ -type. Since the variable region, important for antigen recognition remains the same, the reason for this is still poorly understood. We are interested in how signalling from BCRs with HCs of the μ - and δ -type differs depending on the particular subtype

and are studying the structural basis and functional consequences of these differences. Here, we focus on differential glycosylation, which we study e.g. by using a panel of glycan-binding proteins called lectins. Interestingly, the glycosylation pattern of BCRs derived from malignant B lymphocytes differs from that of healthy B cells. It has been shown that, for instance, in BCRs from Follicular Lymphomas, additional N-glycosylation sites are frequently introduced during the process of somatic hypermutation. In this part of the project, we seek to find out whether and how these changes in glycosylation influence BCR signalling and may contribute to the onset and/or progression of the disease.

Inducible activation of oncogenic Syk isoforms

The non-receptor protein tyrosine kinase Syk is a key mediator of signal transduction in B cells. By acting downstream of the BCR, Syk promotes signalling pathways involved in proliferation, differentiation and in the survival of transformed B cells. The goal of this project is to analyse the mechanisms and kinetics of Syk-induced proliferation and malignant transformation. To this end, we generated a mouse model for the inducible expression of the leukemia-derived TS fusion protein using a tamoxifen-inducible Cre mouse line for B cell-specific expression of TEL-Syk in adult mice. This study shows that TS expression leads to a marked transient expansion of the B cell pool in the periphery (Figure 1). However, our results suggest that inducible expression of TS in B cells is not sufficient for the transformation of B cells, as corresponding cells react with escape mechanisms leading to expression of tumor suppressors and initiation of terminal differentiation that limit the survival and expansion of the activated B cell. In a related project, we analyse the effects of expression of ITK-Syk, another leukemia-derived Syk fusion protein.

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BCL6 enables Ph+ acute lymphoblastic leukemia cells to survive BCR-ABL1 kinase inhibition.

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Übelhart R, Bach MP, Eschbach C, Wossning T, Reth M, Jumaa H. (2010) N-linked glycosylation selectively regulates autonomous pre-BCR function. Nat Immunol. 11(8): 759–65. → GROUP ANDREA PICHLER

SUMO and Ubiquitin control at the level of E2 enzymes



Upstream and downstream signals of E2 regulation

Figure 1: Ubiquitin and SUMO, small proteins regulating protein functions.

▶ Posttranslational modifications are powerful tools to reversibly modulate protein function. They allow dynamic control of cellular processes like transcription, DNA repair, cell cycle progression or meiosis without the need of de novo protein synthesis. Besides phosphorylation, methylation or acetylation, the attachment of ubiquitin and its relatives are amongst the most frequently used reversible modifications.

Ubiquitin and SUMO (small ubiquitin related modifier) are small proteins involved in the dynamic regulation of protein function including stability, activity, intracellular localization etc.. Both modifications are essential for viability in most organisms. We investigate the regulation of covalent attachment of these modifiers to their substrates. In both cases, modification is performed by a hierarchical enzyme cascade involving E1, E2 and E3 enzymes. We are interested in the consequences of regulation at the level of E2 enzymes in mammalian and yeast cells. As deregulation of the SUMO and ubiquitin system has been implicated in various diseases ranging from diverse types of cancer to several neuropathological diseases it is important to understand all levels of regulation. In our studies, we put strong emphasis on biochemical approaches in combination with general cell biology to gain

novel mechanistic insights in the powerful complexity of such regulatory enzymes.

Regulation of sumoylation by its sole E2 enzyme Ubc9

SUMO is an essential posttranslational modification with roles in most cellular pathways. It is generally conjugated to its substrates as a single moiety although SUMO chain formation is involved in some functions. The consequences of sumoylation for a respective substrate are highly diverse including changes in interactions, localisation, stability and activity. SUMO conjugation occurs via an ATP-dependent enzymatic cascade involving one E1 activating, one E2 conjugating and a few E3 ligating enzymes, whereas deconjugation is performed by SUMO specific proteases.

In understanding how regulation is performed the focus in the field concentrates on regulation via E3 ligases and proteases because these enzymes mainly ensure substrate specificity. Whether and how E2 enzymes contribute to regulation is poorly understood but is expected to have widespread consequences on all downstream events (E3 ligases and substrates). In line, different studies correlate E2 upregulation with various types of cancer emphasizing the importance to understand the



Figure 2: SUMO conjugation via E1, E2 and E3 enzymes and deconjugation via SUMO specific proteases (Senps).



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molecular basis of E2 enzyme regulation. We aim to understand mechanisms of E2 regulation in E3 dependent and independent manner and its consequences on the respective substrates. We investigate the consequences of SUMO E2 deregulation in mammalian and yeast cells.

Regulation of ubiquitination by the E2-enzyme E2-25K

Ubiquitination regulates thousands of cellular proteins involved in multiple cellular functions requiring a tightly regulated system. Ubiquitin is attached to its substrate either as a single moiety or as a polymeric chain. How ubiquitin is linked to the substrate (monoubiquitin or differently linked ubiquitin chains) determines the fate of this protein and results in either proteasome dependent degradation or other non-proteolytic regulatory consequences. Like sumoylation, ubiquitination is performed by an energy dependent tri-enzyme cascade involving E1, E2 and E3 enzymes and deconjugation depends on deubiquitinating enzymes. Deregulation of only one E3 enzyme can have severe consequences for the cell. It is not surprising then that different studies have connected deregulation of ubiquitin E2 conjugating enzymes to distinct diseases as these enzymes cooperate with a set of E3 ligases. We aim to understand mechanisms of ubiquitin E2 regulation and its consequences on cooperating E3 ligases and substrates. Currently, we focus on E2-25K, a mammalian ubiquitin E2 enzyme which upon deregulation is involved in neuropathological diseases such as Alzheimer's disease.



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Figure 3: Ubiquitin conjugation via E1, E2 and E3 enzymes and deconjugation via deubiquitinating enzymes (Dubs).

→ GROUP J. ANDREW POSPISILIK

Epigenetic control of complex disease



Chromatin plasticity in metabolic disease

Figure 1: Recent studies have shown beta-cell de-differentiation to be a hallmark of diabetes progression. We find that PcG disruption can induce normal islets (left) to de-differentiate (right), without altering key cellular processes such as proliferation or apoptosis.

■ Current estimates place the prevalence of diabetes and obesity in the range of 300 million to beyond 1 billion by the year 2030. As critical risk factors for heart disease, cancer and stroke, obesity and diabetes currently represent one of the world's chief economic and health care challenges. While studies have established elegant genetic frameworks for our current understanding of these complex disorders, the contribution of a number of critical regulatory layers, in particular developmental and epigenetic regulation, remains poorly understood.

Our lab is interested in understanding epigenetic regulatory systems that contribute to the susceptibility and development of complex disease. In particular, we are interested in signaling to and signaling from chromatin, and how canonical signaling modules define for instance phenotypic variation. These paradigms are broad and include, among others, signaling mediated changes to post-translational modifications of histones, non-coding RNAs, and modifiers of chromatin stability such as the Polycomb-Trithorax Groups. What is clear at present is that these epigenetic effectors play a critical role in defining set-points for entire functional gene sets; the fundamental outstanding question we are interested in is how these epigenetic cues influence the susceptibility and development of human disease. In addition to examining novel genetic determinants of metabolic disease control our chromatin efforts are split into two avenues:



Figure 2 left: Hedgehog mutant mice (KO) are born normal and live healthy lives despite having virtually no fat tissue. These mice are the first completely healthy lipoatrophic mice generated to date.



Epigenetic mapping of mammalian disease

The first comprehensive attempts to provide an epigenetic framework on a genome wide scale have now been realized (Bernstein et al. 2007). These efforts, primarily in stem cells, have provided the seminal cataloguing system from which to organize and compare information of epigenetic marks under varying experimental conditions, including disease states. A significant body of evidence supports the existence of a robust layer of epigenetic control in metabolic tuning and in complex disease. Our first long-term goal is to characterize using ChIP-seq and chromatin interaction mapping the plasticity of chromatin regulatory circuits in the contexts of metabolic disease (obesity, diabetes, and cancer). Our primary approach is to combine the rapidly evolving potential of the next generation sequencing approaches with gene-targeting in mice and will be complemented with characterization of highly characterized human patient samples (together with H. Esterbauer, Vienna) as well as in the context of three larger-scale consortia: Epigenesys - a EU network of excellence on Systems Biology and Epigenetics; Medep - a DFG funded collaborative research center focusing on Medical Epigenetics; and DEEP - the BMBF funded German Epigenome Project. The long-term goal will be to help build an international re-



From left: Villegas, Florian (MSc Student), Tiko, Theo (MSc Student, until 2012), Gossens, Klaus (Postdoctoral Fellow), Selvaraj, Madhan (PhD Student), Teperino, Raffaele (Postdoctoral Fellow), Öst, Anita (Postdoctoral Fellow), Pospisilik, John Andrew (Group Leader), Lempradl, Heidi (Postdoctoral Fellow), Ruf, Marius (Technician), Esterbauer, Harald (Visiting Scientist 2012), Einwallner, Elisa (Visiting Scientist 2012), Dalgaard, Kevin (PhD Student), Jayaramaiah Raja, Sunil (Postdoctoral Fellow), Welz, Steffi (Technician Trainee), Lu, Tsai-Hsiu (PhD Student), Not present: Heyne, Steffen (Postdoc toral Fellow), Longinotto, John (PhD Student)

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ously unknown obesity factors. Using a novel high throughput method we were able to interrogate over 10,000 genes (~75% of Drosoph*ila* protein coding genes) for their ability to influence fat levels in living adult Drosophila

source correlating chromatin plasticity with DNA accessibility, gene expression and disease state.

Functional translation of epigenetic cues

A compliment to the first strategy, our second long-term goal is to functionally characterize disease-specific epigenetic modifications in vivo. These studies, which will capitalize on the integration of targeted mouse genetics and systems biology approaches will address causality and mechanism of action. This approach is being complimented by Drosophila genetic and RNAi-based in vivo screening approaches (Figure 3) as well as collaborative ventures examining epigenetic-targeted mouse mutagenesis systems. Using this approach we have already identified several novel epigenetic modulators of metabolism. Our ongoing projects currently span the characterization of a unique epigenetically-sensitive stochastic obesity phenotype, an inducible cell fate "lock" in adipocyte stem-cells (Figure 2), understanding beta-cell dedifferentiation (Figure 1) as well as a novel Warburglike metabolic shift in non-transformed cells. These studies will help us define novel disease regulatory elements and broaden our understanding and therapeutic arsenal for human disease.

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→ UNIVERSITY/MPI-IE SENIOR GROUP MICHAEL RETH

Signal processes in B cells: from analysis to synthesis



Synthetic and nanoscale biology to decipher structure and function of the B cell antigen receptor

Figure 1: Confocal microscopic image shows proximity of BCRs detected by Fab-PLA, nuclei are stained with DAPI and depicted in blue while the PLA signals are in red.

→ Research in the Department of Molecular Immunology seeks a better understanding of the organisation and regulation of receptors and intracellular signalling pathways in normal and diseased lymphocytes. Our research is largely focused on the development and function of B lymphocytes. We have proposed a new model for the structure and activation of the B cell antigen receptor (BCR) and have discovered new signalling components in activated B cells. Several of our findings have contributed to a better understanding of human diseases such as leukemias and autoimmunity. Furthermore, we are among the first to adapt synthetic biology approaches for studying signalling in mammalian cells. In a collaboration between the University of Freiburg and the MPI-IE, the department also organizes an advanced study program for molecular immunology. This popular program started in 1997 and attracts students both from Freiburg and from other Universities.

Over the last two decades, more than 100 components have been discovered to be part of the diverse signalling pathways connected to the BCR. How can we ever reach a comprehensive understanding of such a complex signalling system that is essential for the normal life of B cells and whose malfunction is associated with many human diseases? The loss-of-function approaches (knock-out) prominently conducted in recent years gave important clues about the biological role of each signalling element but failed to reveal, in detail, the signalling mechanisms of these elements. For this, the rebuilding of minimal functional signalling systems is a new powerful tool stemming from the field of synthetic biology. We have devel-

oped a method

allowing the tran-

sient and induc-

ible co-expression

of up to 12 genes

in the S2 Dro-

With this syn-

thetic biology ap-

proach, we have

rebuilt the BCR

and its proximal

ele-

signalling

sophila cell line.



Figure 2: Schematic drawing of the dissociation activation model (DAM). According to this model the BCR has an auto-inhibitory oligomeric structure on resting B cells. In the presence of antigen, the equilibrium between closed BCR oligomers and monomers is shifted towards the open clustered monomers. The dissociation of BCR oligomers leads to B cell activation.

ments and obtained insight into the working principles of these molecules. In this way, we discovered that a direct positive-(BCR/Syk)-feedback amplifies the signal of the BCR once it meets its cognate antigen. We then made the surprising discovery that Syk is a dualspecific kinase that can switch from tyrosine to serine phosphorylation, thus reversing its role from activation to suppression of BCR signalling (our paper describing these results was chosen as a signalling breakthrough of the year 2011). We are currently using the same methods to study the diverse ways that the cytoskeleton is associated with the resting and activated BCR.

Nano-scale organization of the BCR on resting and activated B cells

The exact organization of proteins in the membrane of living cells is still poorly understood, but a better knowledge of this topic is of great importance for biological research and medical applications.

We have recently showed with a quantitative bimolecular fluorescence complementation assay (BiFC) that the B cell antigen receptor (BCR) forms auto-inhibitory dimers on the surface of resting B cells. This discovery lead us to develop the dissociation activation model (DAM) whereby the dissociation and reorganization of BCR oligomers are proposed to be key events during B cell activation. To obtain direct evi-



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Since 2007 Scientific director of the Center for Biological Signalling Studies (BIOSS) funded by the excellence program of the German government

dence for the proposed BCR dissociation process, we have improved the *in situ* proximity ligation assay (PLA). *In situ* PLA detects the close proximity of two target proteins by amplifying a proximity signal using oligo-coupled secondary (2-PLA) or primary (1-PLA) antibodies. By conjugating oligos directly to Fab fragments (Fab-PLA), we improved the detection limit of PLA down to 10-20 nm and could directly monitor the dissociation of BCR oligomers on the surface of both murine and human naive B cells.

Currently, we are combining Fab-PLA studies with state-of-art super-resolution microscopy techniques, to investigate the structure, organization and dynamics of the BCR and its interaction with co-receptors. In contrast to the Singer-Nicolson fluid mosaic model, our studies suggest that many membrane proteins are not freely diffusing monomers but rather multicomponent protein complexes pre-organized in nano-size protein islands.

Studying B cell survival and function *in vivo* with the Cre/loxP technique

Our department was one of the first to

develop a tamoxifen-regulated Cre recombinase system for regulating gene activity. To date, we have sent our vectors and B cell specific mice to more than 200 labs all over the world. Using our B-cell specific, tamoxifen-inducible Cre mice, we are able to activate or delete any floxed gene in the B cell linage.

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Currently, we are using this technique to study the role of BCR components as well as the kinase Syk during B cell development and survival. The deletion of either the gene encoding the heavy chain (HC) or the BCR signalling subunit Ig- α results in a loss of BCR expression on the surface of mature B cells. However, while HC deficient B cells die rapidly, Ig-α deficient B cells are long-lived (up to 200 days) in tamoxifen-treated mice, even though they lack surface BCR. In the absence of the kinase Syk, B cell development is arrested at the pre-B cell stage. However, the deletion of the Syk gene in mature B cells, results in only a partial B cell deficiency and more than 30 %of the mature B cells survive for longer times without Syk. Our current studies suggest that in the absence of Syk, the





BCR coreceptor CD19 and the BAFFreceptor can provide an essential prosurvival signal for Syk-negative mature B cells.

Phosphatases clearly play an important role in suppressing hyperactive B cells associated with autoimmune diseases. We have generated mice deficient for the phosphatase PTP1B specifically in B cells and found that these mice develop an autoimmune disease. Furthermore, we discovered that B cells from patients suffering from an important human autoimmune disease show a down-regulation of PTP1B.

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Molecular mechanisms of gene regulation

NF-κB family of transcription factors as paradigm for the molecular basis of transcriptional specificity

Figure 1: Heatmap: image of genome-wide H3K9me3 around enhancers.

The Nuclear Factor kappa B (NF- κ B) is a family of transcription factors that play a crucial role in regulating a number of genes controlling the immune system, apoptosis, cell growth and tissue differentiation. NF- κ B exists in most vertebrate cell types as a combination of five structurally related Rel/NF- κ B proteins which, in order to function, must associate in homo- and hetero-dimeric forms. NF- κ Bs dimers are retained in the cytoplasm by interaction with I κ Bs. Upon activation, the I κ B proteins are phosphorylated and

targeted for proteasomal degradation; the NF- κ B dimers are then free to translocate into the nucleus, bind to target sequences (termed κ B sites) in the promoters of several genes, and activate transcription. *In vitro* studies have shown that the different NF- κ B dimers have almost the same ability to bind any given κ B site; however *in vivo* there are cases in which the different κ B sites are bound preferentially by specific NF- κ B dimers and with higher affinity than others. While the basic mechanisms of NF- κ B-mediated gene activation are



relatively well studied, very little is understood at the molecular level about how promoter and cellular specificity is achieved. Depending on the cell type and the stimulus, each NF-κB dimer has been shown to regulate specific targets, even though some genes respond to several NF-KB species in a redundant fashion. Our lab studies the molecular mechanisms which regulate gene expression, using the NF-kB family of transcription factors as a model system. A major focus is to understand how the activities of transcription factors can be controlled in promoter-specific and cell type specific fashion. The NF-кВ family of transcription factors is crucial for the expression of multiple genes involved in cell survival, proliferation, differentiation and inflammation. The molecular basis by which NF-KB activates endogenous promoters is largely unknown, but it seems likely that it should include the means to tailor transcriptional output to match the wide functional range of its target genes. To dissect NF-kB-driven transcription at native promoters, we disrupted the interaction between NF-kB p65 and the Mediator complex. We found that expression of many endogenous NF-kB target genes depends on direct contact between p65 and Mediator, and that this occurs through the Trap-80 subunit and the TA1 and TA2 regions of p65. Unexpectedly, however, a subset of p65-dependent genes are transcribed normally even when the interaction of p65 with Mediator is abolished. Moreover, a mutant form of p65 lacking all transcription activation domains previously identified in vitro can still activate such promoters in vivo. We found that without p65, native NF-kB target promoters cannot be bound by secondary transcription



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factors. Artificial recruitment of a secondary transcription factor was able to restore transcription of an otherwise NF- κ B-dependent target gene in the absence of p65, showing that the control of promoter occupancy constitutes a second, independent mode of transcriptional activation by p65. This enables a subset of promoters to utilize a wide choice of transcription factors, with the potential to regulate their expression accordingly, whilst remaining dependent for their activation on NF- κ B.

Determinants of specificity and redundancy are still poorly defined. We are interested in elucidating the molecular mechanisms which control how particular promoters are able to recruit specific NF- κ B dimers, and not others; and to understand how this specificity is further modulated by signals emanating from different cellular stimuli. We have also shown that *in vivo* some genes can recruit specifically only a single dimer, whereas others can recruit more than one, either sequentially (showing a complete replacement of the different NF-κB subunits bound to the promoter over time) or simultaneously. This promoter specificity can in turn be regulated in different cell types by differences in the chromatin structure of the promoter itself, in particular by covalent modifications to histones. We recently identified a subset of tightly regulated inflammatory genes whose promoters, when inactive, are associated with high levels of dimethylated H3K9 (van Essen et al., Mol Cell 2010). Upon stimulation of dendritic cells (DCs) with LPS, this methylation is erased, and transcription of these genes is induced. By initially analysing cells treated with amine-oxidase inhibitors, and subsequently by shRNAmediated knock-down of candidate enzymes, we cloned and characterized a novel H3K9 demethylase responsible for this effect. We found that without H3K9 demethylation, stimulus-induced NF-kB



Figure 3: Promoter H3K9 dimethylation regulates the recruitment of activating transcription factors. Stimulus-induced H3K9me2 demethylation by Aof1 is required for activation of in ammatory genes in DCs. The c-Rel subunit of NF- κ B functions as a targeting signal for Aof1.

recruitment to these promoters is prevented, and transcription is blocked, indicating that H3K9me2 represses the activity of these promoters by limiting transcription factor access. Interestingly, we noticed that low-level, prestimulus binding of c-Rel to promoters was unaffected, and in further experiments we established that this binding acts as an essential targeting signal for stimulus-induced promoter derepression, by directly guiding demethylase recruitment (van Essen et al., Mol Cell 2010). Current experiments are aimed at elucidating the signal-dependent upstream steps which control this.

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van Essen D, Engist B, Natoli G, Saccani S. (2009) Two modes of transcriptional activation at native promoters by NF-kappaB p65. PLoS Biol. 7(3): e73. → GUEST SCIENTIST RITWICK SAWARKAR

Chaperoning gene expression

The interplay of DNA sequence, chromatin and chaperones in transcriptional control

→ A typical metazoan cell expresses

only a fraction of all the genes it in-

herits from the mother cell. How does

a cell decide which genes to express?

Organisms have evolved sophisticated

logical networks, which select genes

for expression. An important element

in these networks is chromatin that

influences the activity of RNA poly-

merase enzyme complex. Over the past

few years, it has been shown that RNA

polymerase II (pol II) gets recruited to

promoters of several genes, but pauses

shortly thereafter. This appears to be a

critical rate-limiting step for gene regulation. The precise reasons and conse-

quences of this event, especially in the

context of chromatin, remain largely

unknown. Furthermore, how misregulation of pol II pausing is instrumen-

tal in diseases such as cancer is unex-

plored. By employing systems biology,

genetics and single cell approaches in

Drosophila and mammalian systems, we will elucidate the basic principles of

the crosstalk between chromatin and

Crosstalk between chromatin factors

RNA pol II pausing is a dynamic pro-

cess regulated during development,

which suggests that both epigenetic

and genetic factors influence pausing.

Conversely, paused RNA pol II complex

RNA pol II pausing.

and RNA pol II pausing

itself can be instructive in shaping the chromatin around promoters. We aim to carefully dissect the crosstalk between core transcriptional machinery and chromatin modifying systems. In particular we will take a systems biology approach to decipher how DNA sequences and chromatin factors function in concert to regulate pol II pausing.

DNA sequences at promoters are thought to influence pol II pausing, and also to recruit chromatin modifying machinery. The quantitative principles of this are yet to be learnt and we will approach this issue by integrating chromatin profiles with sequence- and pausing information. By systematically changing DNA sequences *in vivo*, we will identify motifs that regulate chromatin modification and RNA pol II pausing.

Most studies rely on knock-down of chromatin modifying proteins to study how they influence pol II activity in cell populations. Given the dynamic nature of the pausing process, the slow depletion of factors by the knock-down approach is unlikely to lend itself for precise analyses. We will develop new genetic tools that rapidly degrade proteins *in vivo* such that pausing analyses can be performed in single or groups of cells. In combination with global transcriptome and chromatin profiling, such a method will answer fundamen-

CDC37



Figure 2: A cartoon depicting the process of RNA polymerase II pausing and elongation, analogous to traffic lights. Pol II pausing may influence promoter chromatin and vice versa [(ii)] in the figure], and the rules of this exciting communication are being unraveled. NELF: Negative elongation factor, DSIF: DRB sensitivity inducing factor, P-TEFb: positive transcription elongation factor b. Picture adapted from Sawarkar and Paro (2013) Trends in Cell Biology.

complex is not known.

Figure 1: Activity of individual genes can be visualized by staining the giant chromosomes of salivary glands of *Drosophila*. In this picture, Cdc37 co-chaperone of heat-shock protein 90 (Hsp90) is shown to bind to specific loci on polytene chromosome. This co-chaperone helps Hsp90 in stabilizing protein kinases. Which kinases are substrates of Cdc37-Hsp90



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tal questions relating chromatin and transcription.

Nuclear proteostasis in transcriptional dynamics

Proteins are folded and stabilized by chaperones and degraded by proteasomal machinery. Both these important arms of protein homeostasis or proteostasis function not only in cytosol but also at chromatin, as recent exciting studies have shown. Our longterm goal is to systematically quantitate proteostasis at chromatin in the context of transcription. How these processes are regulated when the cellular environment changes during development, stress, ageing and disease will be investigated. In particular the following two aspects of proteostasis will be studied in detail.

Chromatin-based chaperone network:

The chromatin proteins controlling RNA pol II activity are stabilized by chaperones such as heat-shock protein 90 (Hsp90). Inhibitors of Hsp90 are in advanced clinical trials for cancer treatment implicating the chaperone in cancer pathology. Using a combination of proteomics, fly genetics and systems biology approaches, we are mapping the chromatin-based chaperone network and its influence on DNA transactions such as transcription and replication. We will elucidate how this network is integrated with the extranuclear signaling and how this affects cancer initiation and progression.

Chromatin-based degradation control:

Cells respond to external environment by typically mounting a rapid transcriptional response. These processes require rapid removal of chromatin factors from specific loci, and hence are degraded at chromatin. How this process achieves the specificity and is regulated by external cues remains a mystery. We will utilize systematic approaches to decipher the mechanisms and controls operating. By providing synthetic and specific degradation machinery at chromatin, we will test the design principles of nuclear proteostasis.



Figure 3: A silver stained gel depicting the outcome of a tandem affinity purification of the molecular chaperone Hsp90 from chro-matin. Nuclear-localisation signal containing red fluorescent protein (NLS-RFP) acts as a negative control.

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→ GROUP EIRINI TROMPOUKI

Signals and hematopoietic stem cells

Zebrafish hematopoietic progenitor cells



Figure 1: Whole mount *in situ* hybridization of zebrafish hematopoietic stem and progenitor cell.

The role of inflammatory pathways in hematopoietic stem cell biology

Hematopoiesis is the process of generating new cells of all the blood lineages. Exceedingly rare hematopoietic stem cells (HSCs) are responsible for maintaining the balance between different blood lineages and replenishing the system in case of stress such as infection, trauma or irradiation. Signal transduction pathways continuously deliver information to the cells by mediating the actions of signaling transcription factors. We are particularly interested in inflammatory pathways that have recently been implicated in HSC quiescence and regeneration. HSCs were thought to respond to inflammatory signals indirectly, just to replenish immune hematopoietic cells. Only lately, it has been proven that HSCs can directly sense and respond to inflammatory signals, not only in cases of infection but also under steady state conditions. Acute inflammatory signaling affects HSC quiescence and chronic exposure can lead to HSC exhaustion and may cause hematopoietic malignancies. Using zebrafish and mice as model organisms we want to understand how these pathways control HSC quiescence, and affect hematopoietic differentiation and regeneration. We are mainly interested in studying how inflammatory signals formulate the transcriptional landscape of HSCs. The ultimate goal is to understand how we can manipulate HSCs and improve hematopoietic outcomes during stress and disease.

Inflammatory signaling during hematopoietic development

Like all cells, HSCs are influenced by a complex cascade of signaling pathways that are either synergistic or antagonistic and ultimately determine all cell decisions. Inflammatory signaling pathways like TLR, TNF, IFN pathways were mostly associated with immune hematopoietic cells, but recently, it was shown that they have the capacity to directly stimulate HSCs both under steady-state and stress conditions. We reason that since organisms do not live in sterile environments inflammatory signals can possibly affect the development of HSCs. We will focus on understanding how inflammatory signaling affects HSC formation during hematopoietic development.

We will undertake a reverse genetic and chemical screens to elucidate the role of inflammatory pathways in HSC formation during development. Zebrafish is an ideal system to study hematopoietic development since the hematopoietic system is conserved and the HSC formation adequately described. *In vivo* microscopy will be used to understand how inflammatory signals affect HSCs in the context of the whole organism and serial and competitive transplantation will determine their effect on adult HSCs. The identified pathways will be studied for their effects on transcriptional regulation. Genome-wide techniques like ChIP-seq, RNA-seq, Hi-C will be used to establish how inflammation affects hematopoietic development. Our ultimate goal is to understand how these pathways are used during development and thus be able to manipulate them during disease.

Crosstalk between inflammatory and developmental signaling pathways

Developmental signaling pathways, like the Wnt and the BMP pathways, and inflammatory pathways like the TNF, IFN, TLR, affect HSC quiescence and subsequently their ability to reconstitute irradiated recipients. Inhibition of the Wnt signaling in the hematopoietic niche or activation of the IFN pathway both affect HSC proliferation. Interactions between these pathways have been observed during gene transcription, for example it was shown that signaling responsive transcription factors like TCF7L2 (formerly TCF4), SMAD1 and STAT1 bind to the same genomic regions. In addition ChIPseq for the Wnt mediator TCF7L2 on progenitor hematopoietic cells shows binding on inflammatory genes and specifically on STAT1 regulatory elements. We hypothesize that these pathways may interact and control normal and stress hematopoiesis. We will use



From left: Yin, Na (Lab Manager), Trompouki, Eirini (Group Leader)

in vivo imaging and transplantation assays in zebrafish and mice to dissect possible interactions between these pathways. Genome-wide studies will reveal how these pathways interact on the transcriptional level while epistasis experiments in zebrafish will identify interactions between the signaling cascades. Since deregulation of these pathways leads to a variety of cancers we expect that fine tuning of these pathways is necessary to combat disease.



Figure 2: Identification of inflammatory signaling pathways that affect hematopoietic stem cell formation and migration.

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Cell adhesion and Wnt/β-catenin signaling in mouse development

Key molecules in signal transduction and cell proliferation



Figure 1: Wnt/β-catenin signaling regulates Telomerase in stem cells and cancer cells. Telomere length determined by quantitative fluorescence in situ hybridization analysis.

LAB MEMBERS



From left: Özgün Korkusuz, Nasiye (Technician, until 10/2013), Kemler, Rolf (Group Leader), Hoffmeyer, Katrin (Postdoc), Raggioli, Angelo (Postdoc), Not present: Schneider, Rosemary (Administrative Assistant)

Embryogenesis relies on the precise interplay of signaling cascades to activate tissue-specific differentiation programs. An important player in these morphogenetic processes is β -catenin, which is a central component of both the cadherin-mediated cell adhesion and the canonical Wnt signaling pathway.

The dual role of β-catenin in cadherinmediated adhesion and as the downstream effector of the canonical Wnt signaling pathway is addressed by combining in vitro and in vivo analysis. We conditionally deleted beta-catenin from the entire posterior region of the embryo after gastrulation. In addition, we are dissecting the adhesive and signaling functions of β-catenin prior to and throughout gastrulation by conditionally replacing the wild type protein with mutant isoforms impaired in specific molecular interactions and functions. We have isolated several ES cell lines with pre-determined genotypes for E-cadherin and β -catenin.

We found that β -catenin is required to maintain genomic stability in mouse ES cells. Additionally, we report a molecular link between Wnt/β-catenin signaling and the expression of the telomerase subunit Tert. β-Catenindeficient mouse embryonic stem (ES) cells have short telomeres; conversely, ES cells expressing an activated form of β -catenin (β -cat-delEx3/+) have long

telomeres. We show that β-catenin regulates Tert expression through the interaction with Klf4, a core component of the pluripotency transcriptional network. β-Catenin binds to the Tert promoter in a mouse intestinal tumor model and in human carcinoma cells. We uncover a previously unknown link between the stem cell and oncogenic potential whereby β -catenin regulates Tert expression, and thereby telomere length, which could be critical in human regenerative therapy and cancer.

Techniques used include homologous recombination in embryonic stem (ES) cells, heterotypic expression, biochemical and immunochemical investigation of protein interactions of the cadherin-catenin-cell adhesion complex with cytoskeletal actin microfilaments, and the activation of target (or reporter) gene expression by nuclear β-catenin. The Cre/loxP system is used to conditionally inactivate E-cadherin and β -catenin in specific cell lineages and tissues.

→ ROLF KEMLER

1945 born in Burghaun, Germany; Studies in veterinary medicine, University Giessen, Germany

1971–1973 PhD at the MPI of Immunobiology, Freiburg, Germany

1974–1981 Postdoctoral studies at the Institute Pasteur, Paris, France



1981–1987 Group Leader, Friedrich-Miescher-Laboratorium of the Max Planck Society, Tübingen, Germany 1987–1992 Group Leader, MPI of Immunobiology, Freiburg, Germany

1992–2013 Director at the MPI-IE, Freiburg, Germany Since 2013 Emeritus Group Leader, MPI-IE, Freiburg, Germany

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Wnt/β-catenin signaling regulates telomerase in stem cells and cancer cells.



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Past Research Groups (2011–2013)

During 2011 and 2013 several colleagues have ended their work as group leaders at the Max Planck Institute of Immunobiology and Epigenetics (MPI-IE) and moved to other positions or reached retirement.

Prof. Dr. Wolfgang Schamel (2002–2012) Present affiliation: Biologie III, Faculty of Biology, University of Freiburg

Our interest are the molecular mechanisms of the activation of T lymphocytes by pathogens. We develop new biochemical techniques for the

identification and analysis of multiprotein complexes on a large scale (quantitative proteomics, systems biol-

em. Prof. Dr. Marina Freudenberg (1981–2012) retired



The major interests of the laboratory are the elucidation of the mechanisms underlying the biological activity of the bacterial endotoxin lipopolysaccharide (LPS) and other

bacterial components, the role of these

the protein complexes that change in time and space within the signaling cascades of the antigen TCR. We have determined the TCR stoichiometry, by showing that complexes of different sizes co-exist on the cell membrane. Interestingly, they play different roles in T cell activation. We have detected a conformational change at the TCR induced by ligand-binding that is – together with TCR clustering – required for T cell activation. Our new "permissive geometry model" of TCR triggering unifies so far irreconcilable findings as crystallographic data of the TCR sub-

ogy). The methods are used to define

components in the microbial recognition by the innate immune system and the conditions under which the activity of such components can be altered. Of particular interest is the infection induced LPS hypersensitivity and its role in the innate immune response and defense against intruding pathogens. Sensitivity to LPS is determined by a locus on mouse chromosome 4, designated the lps gene. Mutations of this gene result in unresponsiveness to the lethal and other biological effects of LPS.

Prof. Dr. Tilmann Borggrefe (2005–2012)

Present affiliation: Justus Liebig Universtity Giessen, Germany



Chromatin-based mechanisms significantly contribute to embryonic and postnatal development and cell type identity by transducing extracellular signals into changes in gene expression. Taking the Notch signal transduction cascade in T-cell development as our model system, my laboratory investigates dynamic changes in chromatin upon presence or absence of a Notch signal. Understanding these mechanisms of gene expression has important implications in the understanding of fundamental processes such as development and cellular transformation. In particular, we want units, studies on the geometry of the ligand (MHC peptide), the presence of multimeric TCR on the cell surface and the role of self-peptide MHC in T cell activation. Blocking the effectors of the conformational change, allowed us to inhibit T cell activation. Thus, auto-immune diseases could be treated by this mechanism. Lastly, we work on the human CD3gamma-deficiency. We have developed a humanized CD3gammadeficient mouse strain, that shows the same defects as the human patients, and is now used to study this disease in detail.

The identification of the lps gene and its product, with participation of our group, end of 1998, as toll-like receptor 4 (TLR4) brought the break-through into a new era for the field of endotoxin research and of microbial products generally. Since the discovery of TLR4, ten different TLRs acting as signaling receptors for microbial components have been identified. This enabled further the study of signaling pathways involved in the activation of cells of the innate immune system.

to elucidate the molecular mechanism how chromatin modifiers set up responsiveness of genes regulated by the Notch signaling pathway. We want to understand the molecular mechanism of the transcriptional regulation at Notch genes, the regulation of histone marks by Notch and the bridging function of the mediator complex between transcriptional regulators as Notch and the general transcription machinery around RNA polymerase II.

Past Research Groups (2011–2013)

Dr. Robert Schneider, (2002–2012) Present affiliation: Institute of Genetics and Molecular and Cellular Biology, Strasbourg, France



One of the major goals of post-genomic biology is to understand the molecular basis and physiological role of covalent protein modifications. We are usbe "histone code"

ing histones and the "histone code" as models to study multi-site protein

Dr. Marinus Lamers (1984–2013) retired



Classically the main function of the immune system was seen in the defence to pathogenic microorganisms. Now, it is presumed that the immune sys-

Dr. Peter Nielsen (1985–2013)

Present affiliation: Senior Scientist, Department of Molecular Immunology, MPI-IE, Freiburg, Germany



B cells are derived from hematopoietic stem cells, are relatively shortlived and circulate throughout the body. They mature further to plasma cells and a small

number of activated B cells will give rise to long-lived memory B cells. For

modifications. Our aim is to identify new modifications, to decipher how these modifications are epigenetically inherited and how they can regulate gene expression and chromatin structure. The best studied examples for multi-site protein modifications are currently histone proteins. The complexity and diversity of histone (and other chromatin-associated) modifications add largely to the capacity of the genome to store and process information. We are currently only beginning to understand the many implications

tem is in a constant dialogue with the environment, i.e. with the microbial world and its products at the boundary layer between – defending – organism and outside world. This dialogue is necessary to maintain an ecosystem that allows survival of beneficial microorganisms, but the disposal of deleterious ones. The difference between beneficial and deleterious is most likely not absolute, but rather sliding, and thereology and disease. Whilst it is still under discussion if histone modifications form a true "code", it has now been established that changes of histone modifications and of protein complexes binding to specific modifications are involved in the regulation of most – if not all – genes in eukaryotic cells. Therefore the significance of studying chromatin modifications extends far beyond the field of chromatin research, because changes in the modification pattern are likely to affect all biological processes.

of this epigenetic information for bi-

fore, the recognition strategies of the host is not tailored on pathogens only. Although the immune system most likely evolved at the demarcation line between in and out, immune processes at this line are still a poorly studied part of the immune system. For understanding the immune system, it may actually be the most important one.

the generation of a large repertoire of antigen-specific membrane-bound Bcell receptors (BCR), gene segments are successively and variably recombined in a process that includes minimization of self-reactivity. The later stages of B cell development are triggered by signaling cascades, emanating both from the antigen-bound BCR and from several other surface co-receptors. Maturation includes proliferation and BCR modifications. The resulting plasma cell is highly specialized to synthesize, assemble and secrete large amounts of immunoglobulin. Crucial to all of these processes is the ability to temporally and quantitatively regulate chromatin structure and gene transcription. We are interested in understanding how the regulation of gene activity defines B-cell development. In particular, we are studying how the transcriptional activity of the immunoglobulin kappa locus is regulated during B-cell development and the role that the transcriptional activator protein Bob1 (also called OBF1 or OCA-B) plays during Bcell development.

Research Facilities



Laboratory Animal Facility

→ CAROLINE JOHNER

1997–2001 PhD studies, GSF-Zentrum für Umwelt und Gesundheit (now Helmholtz-Zentrum) & veterinarian in GSF animal facility, Munich, Germany 2001–2003 Head of the barrier and experimental rodent facilities at the Zentrale Klinische Forschung, University Hospital, Freiburg, Germany Since 2004 Head of Animal Facility at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



→ LAB MEMBERS

▶ The Animal Facility at the Max Planck Institute of Immunobiology and Epigenetics has supported research for more than 45 years. It provides scientists at the institute with an ideal environment in the field of laboratory animal science to perform studies on their chosen animal models. The most common animal models are the mouse (*Mus musculus*), the zebrafish (*Danio rerio*) and the fruitfly (*Drosophila melanogaster*).

With a constant and decisive move towards highly educated and motivated staff, the animal facility maintains a basic breeding and husbandry service for more than 450 genetically modified and 20 wild type mouse strains.



The animals are kept either in high barrier (SPF – specified pathogen free) areas with restricted access for animal care staff only or in low barrier (conventional) areas with additional access for licensed scientists. In addition, intensively trained and skilled animal care staff handle immunodefficient and germ-free mice in isolator units and thus ensure optimal animal care and research conditions. By using various layers of physical barriers and standard operating protocols, we are strongly committed to the well-being and health of our animal colonies. In both barrier systems, we offer IVC (individually ventilated caging) and open caging depending on the need of the scientist and the room quality.

The Mouse House offers high standard services that include:

- Animal colony maintenance
- Training for scientists, caretakers and trainees
- Assistance in experimental design and techniques
- Embryo- and sperm-cryopreservation and rederivation
- IVF (in vitro fertilization)
- Sterile embryo transfer
- Germfree hysterectomy
- Tissue biopsies, blood and organ collection
- Import/ export of animals

For mouse strain management and coordination of the services offered, a software program (TierBase) developed by Peter Nielsen (MPI-IE), has become indispensable. TierBase provides users with 24-hour access to their data, facilitates communication between animal care staff and researchers, and provides an ideal tool for the mandatory documentation. In the past years, the system has been developed further and with its animal welfare tool allows users and the animal welfare officer to monitor procedures and animals that have been licensed by the local authority.

➡ SELECTED PUBLICATIONS

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Transgenic Mouse Facility

→ BENOIT KANZLER

 1996 PhD thesis in Cellular and Molecular Biology with Prof. Danielle Dhouailly, Albert Bonniot Institute, University Joseph Fourier of Grenoble, France
 1996–1999 Postdoctoral Fellow, Max Planck Institute, Freiburg, Germany, Laboratory of Dr. Moisés Mallo (Department of Developmental Biology)
 Since 1999 Head of the Transgenic Mouse Facility at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



LAB MEMBERS

From left: Hiltl, Clarissa (Research Assistant), Lopez, Elsa (Technical Assistant), Kemmer, Svenja (Research Assistant), Kanzler, Benoit (Head of Facility)

→ The Transgenic Mouse Facility of the Max Planck Institute of Immunobiology and Epigenetics has been established in 1995 to enable the successful and efficient generation of genetically modified mice, thus allowing the scientific investigators to focus their research efforts on biological rather than technological aspects. We strive to provide cutting-edge transgenic and genetargeting technology, in both a time effective and cost efficient manner. New mouse lines are generated either by transgenesis (via pronuclear microinjection of specific DNA constructs into fertilized oocytes) or targeted mutagenesis (via microinjection of embryonic stem (ES) cells into blastocysts or 8-cell stage embryos, as well as via diploid or tetraploid ES/morulas aggregations), e.g. "knockouts" or conditional mutants.

Our barrier facility maintains a specific pathogen free (SPF) health status, housing gene modified mice in ventilated cage systems (IVCs) that are serviced by intensively trained and skilled animal care staff. Microinjected embryos are reimplanted under strict sterile conditions into pseudopregnant foster mothers inside this barrier. Serology testing of selected animals is systematically completed to confirm their health status, thus allowing the transfer of the transgenic founders or positive gene targeted offspring to other areas of the animal house for further breeding and analysis. After germline transmitters have been identified the investigators become responsible for breeding and

analysis of mouse lines in compliance with the German law.

In the last three years, the Transgenic Mouse Facility has generated:

- Through ES cell microinjection: 238 injection days: 900 ES-cell derived mouse lines or chimeric embryos, corresponding to 48 distinct targeting constructs
- Through DNA pronuclear injection: 159 injection days: 516 new transgenic mouse lines or embryos, corresponding to 58 distinct transgenes

In parallel, we strive to implement and develop new technologies, e.g. establishment of new methods for ES cell derivation or targeted mutagenesis using Zinc-finger nucleases (ZFNs) or Transcription activator-like effector nucleases (TALENs).

In order to keep track of all experimental parameters, conduct data analysis and edit statistics and reports, a database has been developed. For each new mouse line generated, researchers are responsible for keeping accurate and regularly updated records (e.g. mating details, breeding performances, genotyping and phenotyping results) in our internal mouse colony management database, TierBase. This software enables efficient communication with the scientists and the German authorities (Regierungspräsidium Freiburg), complying with the current legislation on animal welfare.

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Fish Facility

→ MICHAEL SCHORPP

1993–1996 PhD at University of Freiburg and German Cancer Research Center, Heidelberg, Germany

1996–2000 Postdoctoral Research Fellow at the German Cancer Research Center, Heidelberg and at the Max Planck Institute of Immunobiology, Freiburg, Germany

Since 2000 Project Leader and Head of Fish Facility (since 2009) at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



► LAB MEMBERS

→ In general, higher vertebrates such as mouse and rat are used as model organisms in immunological research. Nevertheless, the zebrafish and Medaka model systems offer some advantages over higher vertebrates which can also be exploited to answer questions in immunological science. Zebrafish and Medaka are extraordinarily fecund. This opens up the possibility to carry out genetic studies such as mutagenesis screens. Such screens are also performed to identify genes and pathways which are important for the development and function of the immune system. Sequencing of fish genomes and analysis of gene functions have shown that there are less differences between fish and humans than expected. This implies that results from mutagenesis screens in fish can be transferred to and used for studies on hereditary diseases affecting the human immune system.

The rapid extracorporal development and the transparency of zebrafish and Medaka embryos is another advantage of these model organisms, making them superior to higher vertebrates for certain experimental approaches. For example, transgenic lines make it possible to monitor the development of the embryo and its organs *in vivo* on a cellular level. Furthermore, the fish embryo is easily accessible allowing manipulation of and interference with developmental processes.

Service

The fish facility at the Max Planck Institute of Immunobiology and

Epigenetics was opened in 2007. It houses zebrafish (Danio rerio) and Medaka (Oryzias latipes) fish. Each room is equipped with an independent water treatment unit. Access to the facility is restricted and automatically recorded; technical parameters of the entire facility are remotely controlled. A separate quarantine room provides opportunity for short-term experiments without compromising the high hygienic standards of the main facility. Currently, about 50 different lines carrying ENUinduced mutations affecting different developmental processes and several transgenic lines are kept in the system. Wild-type strains are maintained for breeding experiments as well as for general egg and embryo supply. The facility is run by a group of four staff members.

The following procedures are routinely used:

- Automated whole mount *in situ* hybridisation
- BAC transgenesis
- Cell transplantations in embryos and adult fish
- Homozygosity mapping/positional cloning
- Pressure-driven microinjection of mRNA, DNA, or antisense morpholino oligonucleotides into fertilized eggs
- Sperm cryoconservation and *in vitro* fertilisation

➡ SELECTED PUBLICATIONS

Hess I, Boehm T. (2012) Intra-vital imaging of thymopoiesis reveals dynamic lympho-epithelial interactions. Immunity. 36(2): 298–309.

Iwanami N, Mateos F, Hess I, Riff el N, Soza-Ried C, Schorpp M and Boehm T. (2011) Genetic evidence for an evolutionary conserved role of IL-7 signaling in T cell development of zebrafish. J Immunol. 186(12): 7060–7066.

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Proc Natl Acad Sci USA, 107(40): 17304-17308.

Fly Facility

→ PLAMEN GEORGIEV

2004–2007 PhD studies in Molecular biology/Genetics at Cambridge University, Cambridge, UK

2007–2010 Postdoctoral fellow at the Babraham Institute, Cambridge, UK Since 2010 Head of Fly Facility at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



LAB MEMBERS
From left: Georgiev, Plamen (Head of Facility), Lenz, Ute (Technician). .
Not present: Gutierrez, Noel (Technician)

The fruit fly *Drosophila* is one of the most extensively characterized metazoan organisms. Over more than 100 years since it was introduced as an experimental animal model for biological research, it has allowed for key biological concepts and phenomena to be elucidated, leading to some of biology's fundamental findings, including the chromosomal theory of heredity and basic genetic mechanisms underlying animal development and evolution.

Importantly, many aspects of development and behaviour in *Drosophila* parallel those in humans. The completion of both the human and *Drosophila* genome sequencing projects revealed that more than 75% of human genetic disease genes have clear homologues in the fruit fly. Taking advantage of the significantly shorter life cycle, large number of offspring and powerful array of genetic and molecular tools available in *Drosophila*, it is now feasible to perform large-scale genetic screens in *Drosophila* to identify novel drugs and therapeutic targets.

The fly facility was set up in 2010 upon recruitment of Dr. Asifa Akhtar. The facility contains a fly room with 7 dissection microscopes and CO_2 supply



for day-to-day fly pushing and a stereo microscope for fluorescent imaging. Attached to the fly room are 25°C and 18°C light-, temperature- and humidity-controlled rooms for fly stocks. In addition, there are separate rooms allocated for large population cages allowing embryo collections for chromatin isolation and an 18°C room for a collection of mutant and transgenic lines maintained to facilitate rapid amplification upon request. In addition, a fly food preparation kitchen with a large production capacity has been established for the maintenance of these flies as well as for the daily needs of the fly groups. A state-of-the-art microinjection unit equipped with an inverted microscope, Eppendorf Femtojet, micromanipulator and embryo aligning stage, allows Drosophila embryo transformation and generation of transgenic animals using transposase-mediated random insertion as well integrase-mediated site-specific integration of transgenic constructs.

At present there are four research groups at the MPI-IE using *Drosophila* as a model organism and the fly facility aims to offer an organized infrastruc-



ture, consult and support in applying advanced genetic techniques (such as gene targeting by homologous recombination or TALENS, ZFNs, CRISPR/ Cas-based methods for genome engineering), designing large-scale forward genetic screens and developing new genetic techniques for the specific needs of the fly-related research of these groups.

SELECTED PUBLICATIONS

Ilik IA, Quinn JJ, Georgiev P, Tavares-Cadete F, Maticzka D, Toscano S, Wan Y, Spitale RC, Luscombe N, Backofen R, Chang HY, Akhtar A. (2013) Tandem stem-loops in roX RNAs act together to mediate X chromosome dosage compensation in Drosophila.

Mol Cell. 51(2): 156–73.

Hallacli E, Lipp M, Georgiev P, Spielman C, Cusack S, Akhtar A*, Kadlec J*. (2012) Msl1-mediated dimerization of the dosage compensation complex is essential for male X-Chromosome regulation in Drosophila. **Mol Cell. 48(4): 587–600.** *co-corresponding authors

Georgiev P, Chlamydas S, Akhtar A. (2011) Drosophila dosage compensation: males are from Mars, females are from Venus. Fly (Austin). 5(2): 147–54.

Flow Cytometry and DNA Sequencing Facility

→ ANDREAS WÜRCH

1986 Undergraduate studies in Biology, University of Freiburg , Freiburg, Germany

1993–1999 Research Assistant, Department of Cellular and Molecular Immunolgy, Prof. Dr. Klaus Eichmann, Max Planck Institute of Immunobiology, Freiburg, Germany

Since 2000 Head of FACS Facility at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



From left: Knoblauch, Maike (Technician), Würch, Andreas (Head of Facility), Wild, Doris (Technician). Not present: Hobitz, Sebastian (Technician), Burger, Claudia (Technician)

▶ The Flow Cytometry Unit, utilizing high quality flow cytometry instrumentation, provides comprehensive and technically sophisticated cell analysis and sorting services as well as flow cytometry training and education for new users.

Flow cytometry is a method that allows measurements of various characteristics of individual cells by using fluorescent probes. Our laboratory has the capability to run a broad diversity of advanced flow cytometry applications.

The DNA Sequencing Unit provides access to automated DNA sequencing. The sequencing service processes samples on a 48-capillary ABI 3730 DNA Analyser. In 2012, we had a total of 50.000 sequences.

Currently available applications include:

- cloning and cell sorting
- multi-color immunofluorescence
- DNA cell cycle analysis
- measurements of apoptosis
- calcium flux
- fluorescence resonance energy transfer (FRET)

The facility is equipped with the most advanced, state-of-the-art flow cytometers. For sorting, we have a Beckman Coulter MoFlo XDP sorter, two Becton Dickinson (BD) FACSAria sorters, a (BD) Influx sorter and a Miltenyi autoMACS magnetic sorter. For analysis of

cell samples our facility provides three LSR II, one LSRFortessa and two FACS-Caliburs (all from BD). The MoFlo, the Influx and the FACSArias are versatile high speed sorters equipped with up to five lasers, enabling the measurement of up to nine fluorescent parameters (MoFlo and FACSAria II) and up to twelve fluorescent parameters (Influx and Aria III), respectively. Additionally the Influx is the worldwide unique steam-in-air sorter equipped with the octagon and trigon optical arrays. The light from the five spatially separated laser beam spots is delivered by fiber optics to the octagon and trigon detector arrays increasing the sensitivity and flexibility of the BD Influx, yielding more information from each sample.

All flow cytometers can simultaneously sort four populations at up to 30.000 cells per second with greater than 99,8% purity and high recovery. Selected populations can be sorted into test tubes or deposited directly into a 96well plate. Sterile cell sorting is available upon request allowing subsequent culturing of sorted cells. The magnetic cell separation system (autoMACS) can isolate large numbers of cells for a single surface marker with high purity in a short period of time. The cell sorters are available on weekdays upon special arrangement with the operators. These instruments are often booked weeks in advance. In 2011 we sorted more than 6.000 samples.



The Influx sorter is a worldwide unique steam-in-air sorter equipped with the octagon and trigon optical arrays.

Proteomics Facility

→ GERHARD MITTLER

1998–2002 PhD studies (Protein Biochemistry and Molecular Cell Biology), Gene Center LMU and Helmholtz Center Munich

2002–2005 Postdoctoral Fellow, laboratory Prof. Dr. Matthias Mann, Center of Experimental Bioinformatics, University of Southern Denmark, Odense, Denmark

Since 2005 Group Leader and Head of Proteomics Facility (since 2010), Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



► LAB MEMBERS
From left: Cohen, Meital (Staff Scientist), Nerz, Gabriele (Technician), Mittler, Gerhard (Head of Facility), Richter, Florian (Postdoc)

The proteomics facility is offering a state-of-the-art Mass Spectrometry (MS) analysis service and is collaborating with research groups at the institute in order to develop custom-tailored strategies to address important biological questions. We are specialized in functional proteomics, which is concerned with correlating physiological changes at the cellular level with alterations in protein composition and protein modifications as a major discovery and hypothesis generating tool.

To pursue this we employ metabolic labeling using stable isotopes (SILAC) in order to not only achieve a comprehensive *qualitative* but also a *quantitative* description of proteomes using nanoLC-MS technology. This enables us to study both complex proteomes (organelles) and interactomes like protein-protein or protein-DNA complexes.

The facility is running three hybrid electrospray ionization (ESI) FT-MS instruments that are coupled online to nanobore liquid-chromatography systems. One system consists of a LTQ-FT Ultra mass spectrometer that possesses unrivaled mass resolution (greater one million) and mass accuracy (sub-ppm). The second system is a LTQ Orbitrap XL+ETD mass spectrometer, which offers excellent resolution (>100.000), mass accuracy (sub-ppm) and high sensitivity. This instrument is equipped with ETD (electron transfer dissociation) and HCD (high collision energy dissociation) capabilities that

can give additional structural information useful for de-novo sequencing and post-translational modification (PTM) analysis. A Q Exactive high-performance benchtop quadrupole Orbitrap MS represents the most recent addition to our facility, offering very high sensitivity, resolution (140.000) mass accuracy (low ppm) and HCD MS/MS acquisition speed (>10 Hz) making it the instrument of choice for shotgun proteomics as well as targeted ID and quantification experiments ("quanfirmation").

For offline protein and peptide chromatography, SMART and ETTAN-LC microbore HPLC systems are used. Isoelectric focusing (IEF) of peptides is performed on an Agilent 3000 off-gel fractionator. Sample preparation commonly involves in gel, in solution, FASP or on bead trypsin digestion followed by clean up using C18 and SAX STAGE tip solid phase extraction protocols.

For peptide and protein identification we use an in-house Mascot database server along with tools from the MSQuant open-source environment. Quantitative proteomics experiments (SILAC and label free) are analysed by MaxQuant and Perseus software that are both developed in the department of Prof. Matthias Mann at the MPI for Biochemistry.

Standard service includes protein identification and peptide mapping (using multiple proteases) from both colloidal coomassie and silver-stained gel bands and low-complexity proteomes (e.g. protein complexes, affinity pulldowns etc.). PTM characterization and quantitative proteomic analysis by either SILAC or label free approaches are much more time consuming and are therefore considered as special analysis. Global proteomic projects are evaluated and ranked by an in-house committee whose current members are A. Pichler, R. Grosschedl and G. Mittler.

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Mzb1 (pERp1) regulates calcium homeostasis, antibody secretion and integrin activation in innate-like B cells.

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Mittler G, Butter F and Mann M. (2009) A SILAC-based DNA-protein interaction screen that identifies candidate binding proteins to functional DNA elements. Genome Res. 19(2): 284–293.

Imaging Facility

→ PETRA KINDLE

1983–1985 Training to medical technical assistant, MTA-Schule Freiburg
 1985–2005 Employee at the University Hospital of Freiburg
 Since 2005 Research Assistant, Imaging Facility, Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



LAB MEMBERS
 N.N. (Head of Facility), Kindle, Petra (Interim Head of Facility)

→ Understanding the roles gene products play in biological systems is a key challenge in the post-genomic era. To accomplish this task, visualization techniques based on fluorescence microscopy have become indispensable for gathering information on localization, morphology and dynamics of biological structures. Today, a broad range of reagents, including organelleselective dyes and antibody conjugates, permit specific fluorescence labeling of virtually every cellular component. Furthermore, genetically encoded probes, such as fluorescent proteins greatly facilitate labeling and monitoring of dynamic events in vivo. Due to improvements in fluorescence labeling, electronic light sensors and the introduction of digital imaging techniques, fluorescence microscopy is a key technology in modern bio-medical research. The imaging facility at the Max Planck Institute of Immunobiology and Epigenetics provides a collection of state-of-the-art light microscopy instruments and image processing tools, including assistance through the microscopic imaging process.

Overview of the Equipment

Confocal microscopes and Live cell imaging

- Zeiss LSM 510 Meta NLO with 4 lasers, Argon (458, 477, 488, 514 nm), DPSS 561 nm, HeNe 633 nm, Chameleon (tunable 705-980 nm), heating table for temperature control, HBO 100W for Vis mode, filter sets for GFP, RFP, DAPI, CFP, YFP and NDD (non descanned detection for 2P)
- LSM 780 Zeiss (new GaAsP detectors), with integrated Fluorescence Correlation Spectroscopy (FCS), Flu-



Postdoc Carmen Galán at the spinning disc microscope.

troscopy (FCCS), and Raster Image Correlation Spectroscopy (RICS), 4 lasers (Argon (458, 488, 514nm)), DPSS 561nm, HeNe 633nm, Diode 405nm, HXP 120 W for Vis mode, Filtersets for GFP, RFP and DAPI Spinning Disc Zeiss: special type of

orescence Cross-Correlation Spec-

- spinning Disc Zeiss, special type of confocal microscope for very fast live cell imaging with 2 cameras Mrm (CCD) and Evolve (EMCCD), 3 lasers, OPSL 488 nm, Diode 561 nm, Diode 405 nm, small incubation chamber for CO₂/Temperature control, HXP for Vis mode, filter sets for GFP, RFP, DAPI
- Axiovert 200M Zeiss with Eppendorf Femto Jet Colibri with LED 385, LED 470, LED 555 and LED 625, DICII and DIC III, filter sets for GFP, RFP, DAPI/ GFP/ Red/ FarRed and Mrm camera
- Tokai Hit Incubation unit for CO₂ and temperature control, versatile for all inverted Zeiss microscopes

Structured Illumination

- Zeiss Imager Z1 (upright) with Apo-Tome slider with XBO 75 W, 6 filter cubes for GFP, RFP, CFP, YFP, FarRed and DAPI, DICII and DIC III, Mrm and Mrc camera, Axiovision 4.82 software
- Zeiss Imager Z1 (upright) with ApoTome slider with HXP 120 W, 6 filtercubes for GFP, RFP, CFP, YFP, FarRed and DAPI, DICII and DIC III, Mrm camera, Zen 2012 software.

Deep Sequencing Facility

→ ULRIKE BÖNISCH

2002–2007 Undergraduate studies in Nutritional Sciences, University of Hohenheim, Stuttgart, Germany

2007–2012 PhD Student, Department of Microsystems Engineering, University of Freiburg, Freiburg, Germany

Since 2012 Scientist, Deep Sequencing Facility, Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



 LAB MEMBERS
 From left: Bönisch, Ulrike (Scientist), Betancourt, Emily (Technician), Arrigoni, Laura (Postdoc)

▶ The Deep Sequencing Facility at the Max Planck Institute of Immunobiology and Epigenetics (MPI-IE) offers high-throughput sequencing services mainly for in-house research groups. The facility is equipped with modern state-of-the-art technology, highly qualified staff and interacts closely with the bioinformatics facility at the MPI-IE. Next to standard sample processing huge effort is put into the optimization, automation and standardization of existing workflows according to internationally emerging standards.

Apart from serving all groups at the institute, the unit is also part of a local (MEDEP) and a large German-wide research consortium (DEEP) for which it produces ChIP-seq data and reference epigenomes from various tissue types. As one of the six epigenome production centers in Germany, the facility has set up a complete semi-automated ChIP-Seq pipeline that enables chromatin extraction, chromatin immunoprecipitation, library preparation and deep sequencing according to highest international standards and quality controls.

Technology

The unit employs the Illumina technology that uses massively parallel sequencing to generate several billion bases of high quality nucleotide sequences per run. To this end DNA molecules are attached to a surface, amplified and the complementary DNA strand re-synthesized by consecutive incorporation of fluorescent reversible terminator deoxyribonucleotides (a technology referred to as sequencing-by-synthesis). Surface-images are analysed to generate high-quality sequences. Using the Illumina sequencing technology maxi-

> mum read length of 100 bp (HiSeq2500[®], high-output mode), 150 bp (HiSeq2500[®], rapid-run mode) as well as 300 bp (MiSeq[®]) is feasible. Furthermore pairedend sequencing can be applied as well as barcoding to in-



Ulrike Bönisch loads the MiSeq system. In the foreground: HiSeq2500® that conducts up to 3 billion single reads.

crease the number of parallel analysed samples.

A robust instrumentation infrastructure is in place for standardized and quality controlled sample preparation. This includes a Covaris S220, an Agilent Bioanalyser, a Qubit Fluorometer as well as liquid handling stations for automated chromatin immunoprecipitation and library preparation. To manage the huge data flow generated by the facility, we utilize a large data center and computer cluster for storage, analysis and visualization.

Our service covers

- Preparation of multiplexed libraries for cluster generation and sequencing on the HiSeq2500 and MiSeq instrument
- Tutorials and training of researchers in sample and library preparation techniques

Main applications supported by the Deep sequencing facility are

- ChIP-Seq
- RNA-Seq (Poly-A, Ribo-Minus,
- directional/non-directional),
- gDNA-Seq
- Amplicon-Seq

Bioinformatics Facility

→ THOMAS MANKE

1994–1998 PhD studies (Theoretical Physics) at Cambridge University, UK
 1998–2010 Postdoc at Tsukuba University, Japan, at Columbia University, New York, USA, and at Max Planck Institute for Molecular Genetics, Berlin, Germany
 Since 2010 Group Leader and Head of Bioinformatics/NGS Unit, Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



LAB MEMBERS

From left: Ramirez, Fidel (Postdoc), Richter, Andreas (Postdoc), Diehl, Sarah (Research Assistant), Manke, Thomas (Head of Facility), Dündar, Friederike (PhD Student)

→ The regulation of gene expression is controlled by multiple mechanisms, such as the sequence-specific binding of transcription factors to DNA, epigenetic signals and a dynamic chromatin state. An unbiased understanding of these processes requires access to largescale experiments and the capacity to analyse genome-wide data. Apart from supporting many collaborative projects at the MPI-IE, the group interacts very closely with the Deep Sequencing Facility that generates data at an unprecedented scope, resolution and rate. The bioinformatics facility is operating a powerful Data Center to process, analyse and visualize this information. We also provide regular training and help our colleagues to interpret genomewide data.

Data Center

We operate a state-of-the-art data center with a dedicated cooling system, more than 500 high-speed cores, 100TB storage and backup. This infrastructure is constantly upgraded and provides a powerful backbone for primary analysis of sequencing data, various web services, and for the extensive secondary data analysis by the bioinformatics facility and other internal users. We also implemented hundreds of standardized tools, protocols and Linux software for data management and analysis.

Epicenter Web Service

Apart from offering direct access to our Linux servers, we also host a number of different web-services (Galaxy, Rstudio, GenomeBrowser) that help to access primary deep-sequencing data, perform standardized analyses and visualizations. We provide an interactive web interface for data storage, management and sharing. At its core the Galaxy web-service includes many customizable tools and extensive workflows for deep-sequencing analysis, visualization and data integration.

Training

We offer regular bioinformatics training courses and interactive tutorials on genome-wide data analysis, visualization, and statistical interpretation. These courses take place twice per year and are open to all members of the MPI-IE and PhD students.

> Raw sequences are obtained as large images with different colour codes for different nucelotides. Based on the measured intensities, it is possible to accurately assign a nucleotide to each spot of the image. The enormous degree of parallelization allows for the simultaneous determination of billions of basepairs.

→ SELECTED PUBLICATIONS

Bulut-Karslioglu A, Perrera V, Scaranaro M, de la Rosa-Velazquez IA, van de Nobelen S, Shukeir N, Popow J, Gerle B, Opravil S, Pagani M, Meidhof S, Brabletz T, Manke T, Lachner M, Jenuwein T. (2012) A transcription factor-based mechanism for mouse heterochromatin formation. Nat Struct Mol Biol. 19(10): 1023–30.

Lam KC, Mühlpfordt F, Vaquerizas JM, Raja SJ, Holz H, Luscombe NM, Manke T, Akhtar A. (2012) The NSL Complex Regulates Housekeeping Genes in Drosophila.

PLoS Genet. 8(6): e1002736.

Thomas-Chollier M, Hufton A, Heinig M, O'Keeffe S, Masri NE, Roider HG, Manke T, Vingron M. (2011) Transcription factor binding predictions using TRAP for the analysis of ChIP-seq data and regulatory SNPs.

Nat Protoc. 6(12): 1860–9.



04 Around the Institute



Life at the Institute



The lounge with its relaxing atmosphere is the central meeting point for members of the institute.



Max Planck Health Day

How to avoid back pain? How to recognize burnout? And how to eat healthy even when in a hurry? During June 2013, all employees of the MPI of Immunobiology and Epigenetics (MPI-IE) and the MPI of Foreign and international Law (MPICC) were invited to the 1. Max Planck Health Day. With this initiative, the two Max Planck Institutes set a sign for a healthier working environment. The employees received advice for ergonomic work places, participated in back coachings and learned about strategies of stress prevention.



Annual Institute Retreat

Each year in October, all scientific staff at the institute, including students and research assistants, take part in a 2-day retreat that aims at an intensified scientific exchange between members of the MPI-IE. In 2011 and 2012, the event took place in Saint Hippolyte in Alsace, France. In 2013, Phd students, postdocs and group leaders attended individual retreats that were tailored to focus on specific questions in these peer groups.



Marathon Men and Women

It is a great festival every year in April: after winter the runner's scene meets in Southern Baden to start the new season with the Freiburg Marathon. For this event, members of the MPI-IE build a runners team, consisting of both Marathon newcomers and experts. Everybody is welcome to take part in the MPI-IE running team.

Little Scientists

The institute offers child care premises located right next to the institute. The facility is run by Concept Maternel, a well-respected non-profit organization which runs several child care facilities and a primary school in Freiburg. Its pedagogical concept is based on the principles of Montessori, Freinet and Pikler and promotes the individual development of each child. Since June 2012, Concept Maternel provides education for children aged between 6 months to 3 years. In autumn 2012, building operations started for the erection of a large new building which will also be in immediate vicinity of the MPI-IE. The extension will enable us from December 2013 to add a "Kindergarten" group for children aged 3 to 6 years to the facility. This way, MPI-IE employees will have the option of leaving their child in the same facility until primary school.





The district Stühlinger: close to the city center and popular with students.

➢ Freiburg is most commonly known as the capital of The Black Forest and is located in one of the most scenic corners of Southern Germany, situated between the borders to Switzerland and France. Freiburg is a very young and lively city due to a high percentage of students. The town with its approximately 200.000 inhabitants is



Daily event: Market in Freiburg.

characterized by 25,000 students at the University of Freiburg. 15% among them are from abroad, half European and non-European, which adds to the international flair of the town.

The calm climate and the local cuisine of Baden, crowned with numerous first class restaurants in and around Freiburg, make Freiburg a town highly attractive to visitors from all over the world.

The cultural life of Freiburg is also very diverse. A big concert hall, several theaters, and many clubs offer events on a regular basis. During the summer time, various open air concerts and festivals take place, the Zeltmusikfestival ZMF being one of the biggest.

Life around Freiburg

Within the last 50 years, the upper Rhine valley has undergone a tremendous change from a nearly rural area, narrowed by borders, to the heart of Europe, with Milan and Paris being closer than the German capital Berlin. Since August 2013, a TGV train directly connects Freiburg and Paris, in 3.5 hours. The Black Forest, flanking Freiburg in the east, has lots to offer, besides the

characteristic cuckoo clocks and traditional costumes. It is one of Germany's highest mountain ranges, with the "Feldberg" only 45 minutes from Freiburg. It is an ideal terrain not only for nature lovers for hillwalking skiing, mountain biking, and paragliding. The nearby vineyards of Kaiserstuhl and Markgräfler Land provide Freiburg and beyond with excellent wines such as "Weissburgunder" and "Spätburgunder". In the French region of Alsace, Colmar and the village Riquewihr are attractive locations, not only due to their historical city center. From Freiburg, the door to Switzerland is Basel. Many museums reside in or next to the Swiss city. Basel is also called the "pharmaceutical city" since the headquaters of various pharmaceutical companies are located here. From there, some of the most prominent Swiss mountains like the Eiger can be reached easily.



View from the Feldberg to the Swiss Alps.



Mountainbiking and Paragliding in the Black Forest.

Special Guest Seminar Series

The Special Guest Seminar Series has long been established as a regular event featuring talks by highly recognized scientists from across the globe. The topics range from science directly relevant to the research of the MPI-IE to novel and innovative research from other areas of biomedical science.

2011

13. January 2011

Daniela Rhodes

MRC Laboratory of Molecular Biology, Cambridge, UK Chromatin structure and regulation of its compaction

03. February 2011

Günter Hämmerling German Cancer Research Center, Heidelberg, Germany *Consequences of weak and strong interactions between T cells and antigen-presenting cells*

10. February 2011

Tom Owen-Hughes University of Dundee Dundee, UK *Chromatin remodelling and genome organization*

10. March 2011

Howard Cedar Hebrew University Medical School, Jerusalem, Israel Programming DNA methylation during development

28. March 2011

Mark Shlomchik Yale University School of Medicine, New Haven, USA Germinal center selection and the development of memory B and plasma cells

07. April 2011

Alfred Singer

National Institute of Cancer, NIH, Bethesda, USA *How MHC specificity is imposed in the thymus*

Kevin Struhl Harvard Medical School Boston, USA *An epigenetic switch linking inflammation to cancer and the role of polycomb complexes in cancer stem cells*

28. April 2011

Shiv Pillai Harvard Medical School Boston, USA Clonal ignorance and genetic susceptibility to human autoimmune disorders

12. May 2011

Daniela Finke University of Basel Basel, Switzerland Intrinsic and extrinsic signals regulating the development and function of lymphoid tissues

19. May 2011 Ichiro Taniuchi RIKEN Research Center for Allergy and Immunology Yokohama, Japan *Transcriptional regulation of thymocyte development*

26. May 2011

Evan Rosen Harvard Medical School Boston, USA *Epigenomic approaches to pathway discovery in adipose biology*

07. July 2011

Sidonia Fagarasan

RIKEN Research Center for Allergy and Immunology Yokohama, Japan Dynamic interactions between bacteria and immune cells in the gut

29. September 2011

Gerhard Christofori University of Basel Basel, Switzerland

Molecular dissection of epithelialmesenchymal transition (EMT)

30. September 2011

Ben Black University of Pennsylvania Philadelphia, USA *The molecular underpinnings of centromere identity and maintenance*

11. October 2011

Bing Ren UCSD School of Medicine San Diego, USA Decoding the epigenome

18. October 2011

Nikolaus Rajewsky

Max-Delbrück Center for Molecular Medicine Berlin-Buch Berlin, Germany *Gene regulation by small RNAs and RNA binding proteins*

17. Novemeber 2011

Matthias Schäfer German Cancer Research Center, Heidelberg, Germany Dnmt2-mediated RNA methylation and endogenous RNA stress

28. November 2011

Mitchell Lazar

University of Pennsylvania Philadelphia, USA Epigenomic regulation of metabolism



Discussion in the lounge after the seminar.

2012

02. February 2012

Lars Jansen

Instituto Gulbenkian de Ciência Oeiras, Portugal *The centromere: A showcase for epigenetic inheritance*

09. February 2012

Ronald Hay University of Dundee Dundee, UK How SUMO talks to ubiquitin

23. February 2012

Eric Vivier Centre of Immunology Marseille-Luminy, France *Natural Killer cells*

1. March 2012

Neil Brockdorff University of Oxford Oxford, UK Polycomb – a complex problem

15. March 2012

Nicholas Proudfoot

University of Oxford Oxford, UK Ending the message: Roles of Rloops, gene loops and non coding RNA in terminating transcription in eukaryotes 3. May 2012

Peter Lane

University of Birmingham Birmingham, UK Evolution and involution of CD4 memory and effector function

10. May 2012

Peter Vogt The Scripps Research Institute La Jolla, USA *PI3K signaling in cancer*

11. May 2012

Tasuku Honjo Kyoto University Graduate School of Medicine Kyoto, Japan *An evolutionary view of the mechanism for immune and genome diversity*

14. May 2012

Bruce Beutler University of Texas, Dallas, USA *Genetic analysis of immune sensing and signaling in mammals*

5. June 2012

Howard Chang Stanford University School of Medicine, Stanford, USA Genome regulation by long noncoding RNAs 5. July 2012

John Schwabe University of Leicester Leicester, UK Insights into the assembly, regulation and specificity of HDAC: corepressor complexes

23. August 2012

Matthias Mann

Max Planck Institute of Biochemistry, Martinsried, Germany The modern, mass spectrometric toolbox for proteomics and its application in biology and biomedicine

11. September 2012

Ellen Rothenberg California Institute of Technology, Pasadena, USA *Forging T-lymphocyte identity:*

transcriptional and epigenetic control

12. September 2012

Sadaf Farooqi Cambridge Institute for Medical Research, Cambridge, UK *Genetic and molecular mechanisms involved in human obesity*

27. September 2012

Gioacchino Natoli European Institute of Oncology Milan, Italy *The macrophage epigenome and the control of inflammatory gene expression*

24. April 2013

Steffen Jung Weizmann Institute of Science Rehovot, Israel Intestinal macrophages in gut homeostasis and inflammation

7. May 2013

Oliver Rando University of Massachusetts Massachusetts, USA *Genomic approaches to epigenetic inheritance in yeast and mouse*

23. May 2013

Robin Allshire University of Edinburgh Edinburgh, UK *Establishing and propagating specialised chromatin states*

13. June 2013

Jesper Svejstrup Cancer Research UK London Research Institute London, UK The interface between transcription and genome instability 12. October 2012

Patrick Tam

University of Sydney Sydney, Australia Intersection of transcriptional and signalling activity for head morphogenesis in mouse embryos

18. October 2012

Richard Treisman

Cancer Research UK London Research Institute London, UK *G-actin as a regulator of protein function*

14. November 2012

Steven Shoelson

Harvard University Medical School, Boston, USA Targeting inflammation in diabetes and cardiovascular disease

20. November 2012

Karen Adelman

National Institute of Environmental Health Science, NIH North Carolina, USA *Promoter-proximal pausing of RNA polymerase II: a novel means to regulate immune responses*

22. November 2012

Kai Simons

Max Planck Institute of Molecular Cell Biology and Genetics Dresden, Germany *Lipids organizing cell membranes*

2013

10. January 2013

Johan Auwerx

Ecole Polytechnique Fédérale Lausanne, Switzerland *Mitochondrial proteostasis and the regulation of metabolism on longevity*

24. January 2013

Bruce Edgar

Ruprecht-Karls-University of Heidelberg, Heidelberg, Germany Stem cell-mediated homeostatic and dysplastic growth in the Drosophila intestine

7. February 2013

Edvard Moser

Norwegian University of Science and Technology Trondheim, Norway *Brain maps for space*

7. March 2013

Catherine Dargemont Institute Jacques Monod Paris, France *Ubiquitin conjugation: a timing mechanism for nuclear functions*

14. March 2013

Giacomo Cavalli Institute of Human Genetics Monpellier, France *Genome organization and epigenetic control of development*

21. March 2013

Angelika Amon Massachusetts Institute of Technology, Cambridge, USA *Consequences of aneuploidy*

4. April 2013

Eran Segal Weizmann Institute of Science Rehovot, Israel Unraveling the effect of DNA sequence on chromatin and transcriptional regulation using thousands of designed promoters

.....

27. June 2013

Sten Eirik Jacobsen University of Oxford

Oxford, UK Cellular pathways and molecular determinants of early lymphomyeloid fate decisions

7. October 2013

Job Dekker University of Massachusetts Worchester, USA 3D folding of genomes

14. October 2013

Thomas Eickbush

University of Rochester New York, USA Dynamic features of the rDNA locus allow the long term survival of a mobile element

7. November 2013

Patrick Matthias Friedrich Miescher Institute Basel, Switzerland Analysis of HDAC function in lymphoid cells and beyond



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By public transport (from main train station)

Tramway Station at the south end of the main station. Take either tram no. 1 (dir. Littenweiler), 3 (dir. Vauban) or 5 (dir. Rieselfeld) until "Bertoldsbrunnen" (2 stops). Then change to tram no. 2 (dir. Zähringen) until "Tullastraße" (6 stops from "Bertoldsbrunnen"). Walk along the "Tullastraße" until "Zinkmattenstraße" (roundabout) and follow this street until "Stübeweg" on your left (a 15–20 min walk).

Alternatively, you can use busses 7200/7206 from the ZOB (central bus station), next to the main train station. Take the bus until "Stübeweg" (approx. 14 min ride). After you leave the bus, Stübeweg is on the right. The MPI-IE is on the right hand side.

🚔 By car

From the A5 Autobahn, Karlsruhe to Basel, take the Exit "Freiburg Nord"; keep in the left lane and take Highway B294 in the direction of Freiburg. After B 294 runs into Highway B 3 towards Freiburg, take the Exit "Industriegebiet Nord". At the traffic light, go through the large intersection and make a halfleft turn into "Hans-Bunte-Straße". Turn left at Stübeweg. The MPI-IE is on your right.

🔶 By airplane

Via Frankfurt International Airport After arrival at the Frankfurt International Airport go to the long-distance train station (Fernbahnhof). From there, it is a 2h train ride to Freiburg main train station.

Via Basel/Mulhouse/Freiburg Airport After arrival take the Airport bus to Freiburg (app. 1h bus ride). It will stop at Freiburg main train station.



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