



Max Planck Institute of
Immunobiology and Epigenetics

MAX PLANCK INSTITUTE OF
IMMUNOLOGY AND EPIGENETICS

INSTITUTE REPORT



2014
2016



MAX-PLANCK-GESELLSCHAFT

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Message from the Managing Director



“CREATIVE MINDS BROUGHT TOGETHER IN THE RIGHT RESEARCH ENVIRONMENT IS THE RECIPE FOR OUR SUCCESS.”

➤ Preparation of an Institute Report provides the opportunity for a critical reflection of productivity, innovation and also of scientific excellence of the institute. By reading the contributions of our group leaders, our team leaders of the scientific facilities and our non-scientific service units for this report I am both grateful and proud: grateful that the efforts of the entire faculty and staff members were able to establish a constant and successful development of the institute in the highly competitive field of modern biology; and proud that our aim of a close interaction between all these parts of the MPI-IE provides a strong fundament for state-of-the-art research and the needed freedom to develop and follow new ideas in the field.

MPI-IE is committed to providing an attractive and unique environment for two key areas of modern biology: Immunobiology and Epigenetics. We are interested in the basic mechanistic understanding of how cell-type identity versus cellular diversity is achieved. Moreover, how environment influences cellular response and behaviour are central questions at the heart of immunobiological and epigenetic research and thus join the overall concept of our institute. We have been very successful in maintain-

ing an outstanding research environment for senior and junior faculty. In the future, we aim to continue and maintain scientific quality and excellence at all levels. We also plan to expand on already established synergies amongst our research groups and to achieve long-term sustainability not only within MPI-IE but also within the wider Freiburg scientific community.

MPI-IE enjoys a vibrant and dynamic junior group leader faculty. Junior group leaders are recruited after an international and highly competitive call. This strategy not only ensures that the institute is engaged in mentoring and generating future leaders in the field but also brings in thematic diversity at the institute. This is also reflected in a regular turnover of junior faculty at the institute. Our success is exemplified by several faculty members moving on to prestigious positions following their stay at MPI-IE between 2014-2016: Hassan Jumaa accepted a professorship at the University of Ulm, Germany. Patrick Heun, who was awarded with an ERC starting grant during his time at the MPI-IE moved to a senior position at the Wellcome Trust Centre in Edinburgh, UK and Simona Sacconi, who was junior group leader in Freiburg till 2014, took the



next career step and went to the Institute for Research on Cancer and Aging in Nice, France.

The above-mentioned dynamics also means that the institute regularly welcomes the arrival of new members in the faculty. In 2014, Nicola Iovino and Tim Lämmermann and in 2015, Dominic Grün and Angelika Rambold joined the institute and successfully started their laboratory. Notably, in 2015 Erika Pearce was appointed new director as the successor of Rolf Kemler. She now heads the newly established department of Immunometabolism at the institute. At the same time Edward Pearce became member of our faculty as senior group leader in a joint University/Max Planck research group that will further strengthen our long-standing cooperation with the University of Freiburg. Erika and Ed joined us from the Washington University School of Medicine in St. Louis, USA and focus on studying the interplay between metabolism and immune response.

MPI-IE researchers are also very active in the local Freiburg scientific scene. A productive and fruitful collaboration with University of Freiburg has brought forward participation of several MPI-IE colleagues in various collaborative consortiums and at the same time several University groups participate in an international IMPRS-MCB PhD program at MPI-IE. Our very successful IMPRS-MCB PhD program was able to attract more than one hundred international young scientists from over twenty countries since its foundation in 2006. The constant recruitment of talented fresh minds, which joined the research groups of the institute and the university, shows the commitment of the MPI-IE to the idea that success in training of the next generation of scientist plays a key role in scientific excellence. By providing a comprehensive training program that ranges from scientific training to career development measures, we strive to create an environment that fosters the maturation of talented students into skilled young researchers. The success of our students is highlighted by the quality of first author publications. Two thirds of our IMPRS alumni continued their careers as postdoctoral fellows while one third moved into industry positions.

The scientific excellence of the MPI-IE has also been reflected in various other ways. Our scientists were able to attract highly competitive grants and prizes. In 2014, Michael Reth received the prestigious Paul Ehrlich and Ludwig Darmstaedter Prize and Thomas Boehm was awarded with the Ernst Jung Prize for Medicine, for their outstanding contributions in immunobiology. Our junior group lead-

ers were also very successful in attracting various grants and awards: For example, Eirini Trompouki secured a Marie Curie CIG Grant and recently Andrew J. Pospisilik obtained the highly-competitive ERC Consolidator Grant. He was also honoured with a Prize by the GlaxoSmithKline Foundation in 2015. Also in 2015 Dominic Grün was awarded with a grant by the Behrens-Weise-Foundation. At postdoctoral level, Claudia Keller received the prestigious Ingrid zu Solms Prize for Sciences in 2015, and very recently the IMPRS PhD student Ken Lam won the Otto Hahn Medal of the Max Planck Society in 2016. All these impressive achievements clearly highlight the scientific success and outstanding performance of the institute.

MPI-IE is live and kicking: Every member at the institute, be it a PhD student, postdoctoral fellow or a group leader, including the exceptional support of our administration and service units, is absolutely dedicated and committed to the success of the institute and to make it an unique place where fruitful ideas can flourish. To celebrate and to further encourage this common spirit we established the MaxDay in 2015. On this day all employees of the institute came together for joint activities and exchange between research groups, facilities and above all, between the scientific and non-scientific employees of the institute. Next event is planned for summer 2016, and is projected to be another highlight in the institutes' calendar.

The release of our new Institute Report coincides with the advent of the evaluation by the Scientific Advisory Board (SAB) in summer 2016. Firstly, I would like to thank all members of the SAB committee, who guided and supported us in the last decade; especially to those members who recently completed their term in the board: Markus Affolter, Adrian Hayday and Susan Gasser. Secondly, I want to welcome the new members of the board: Shelley L. Berger, Tasuku Honjo, Ellen V. Rothenberg and Ali Shilatifard. We looked forward to their input for shaping the institute in future.

Finally, I would like to thank everyone working at the MPI-IE for their constant commitment and efforts to make the institute an outstanding place to be. It was and still is a pleasure to work with such dedicated colleagues.

Enjoy reading!

Dr. Asifa Akhtar // Managing Director



01

About us



Max-Planck-Institut
für Immunbiologie und Epigenetik

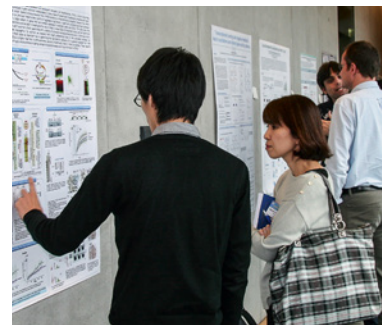
Institute Highlights



Grand Opening of the new building of the child care facility.



Ulrike Boenisch shows the deep sequencing facility to the public at a visitor event at the MPI-IE in August 2014.



Poster session during the 3rd Epigenetics Meeting at the MPI-IE

2014

January

Ernst Jung Prize for Thomas Boehm

Thomas Boehm is awarded with Ernst Jung Prize for Medicine. The prestigious award honours Boehm's groundbreaking contributions to the understanding of the development, differentiation and evolution of the immune system.

March

Paul Ehrlich & Ludwig Darmstaedter Prize to Michael Reth

Michael Reth receives the Paul Ehrlich and Ludwig Darmstaedter Prize for his outstanding achievements in the field of immunobiology.

New room for "Little Scientist"

The child care facility move into a large new building close to the MPI-IE. This extension allows the establishment of a new group for children aged 3 to 6 years. From now on, MPI-IE employees have the option of leaving their children in same child care facility from 3 months onwards until primary school.

May

Release of deepTools

Scientists of the bioinformatics facility of the MPI-IE developed a software solution to execute all relevant analytic steps that are necessary for genome analysis. This open source software provides an efficient, easy-to-handle and highly standardized way for advanced deep sequencing analyses. DeepTools as well as its comprehensive documentation are online available as freeware.

June

Nicola Iovino starts at the MPI-IE

Nicola Iovino starts his research group on epigenetic inheritance at the MPI-IE.

August

Visitor event at the MPI-IE

In cooperation with the local newspaper Badische Zeitung (BZ) the MPI-IE invited the lay public to visit the Institute. Talks and guided tours gave more than 130 visitors insights into life and work at the MPI-IE.

2014-2015

October

Tim Lämmermann starts at the MPI-IE

Tim Lämmermann, expert in the field of innate immune response, starts as a Max Planck research group leader.

December

3rd Epigenetics Meeting

The 3rd Epigenetics Meeting takes place. The high-profile conference represents a broad range of topics in the field of chromatin and epigenetics research and features more than 30 speakers. About 120 scientists participate in the meeting to share exciting data and novel ideas.

January

Asifa Akhtar new managing director of the MPI-IE

Asifa Akhtar is elected new managing director of the MPI-IE.

February

Greening the institute

Takeoff for the commitment of the MPI-IE in the ECOfit program 2015.



Employees and students of the MPI-IE at Science Fair 2015 in Freiburg



Performances during the poster session at the first MaxDay in July 2015.



Erika Pearce was recruited new Max Planck director in September 2015.

2015

The program initiated by the city of Freiburg and the state of Baden-Württemberg supports the institute to implement and optimize measures to save energy and protect the environment. The program accompanies the institute the entire year and includes a concrete action plan to become more sustainable.

July

Science Fair 2015

The MPI-IE participates in the Science Fair 2015 at the Freiburger Münstermarkt. The local event gave thousands of visitors the opportunity to get insights in the research of the Institute. On two days visitors could play different immunobiology and deep-sequencing games, observe fruit flies and look at tissue sections under the microscope.

MaxDay 2015

First MaxDay takes place. The central highlight of institut's life brings all employees of the MPI-IE together and promotes the exchange between research groups, facilities and above all between the scientific and non-scientific employees of the institute. A huge poster session with contribu-

tions from all labs and facilities as well as the administrative and service departments of the institute excited all employees. With the great feedback for the first official IMPRS graduation ceremony and the many other activities the MPI-IE is looking forward to the next round of the MaxDay in summer 2016.

Andrew Pospisilik receives Prize by the GlaxoSmithKline Foundation

Andrew Pospisilik, group leader at the MPI-IE, is awarded for his outstanding achievements in the field of metabolic diseases by the GlaxoSmithKline Foundation in the category "Basic Medical Research".

September

Dominic Grün starts at the MPI-IE

Dominic Grün working on quantitative single cell analysis starts as independent group leader at the Institute.

Erika Pearce becomes new Director

Erika Pearce is appointed new director at the MPI-IE. The American molecular and cell biologist joins the management board and become head of the newly established department of immunometabolism at the institute.

November

Edward Pearce joins the institute

Edward Pearce, expert in the field of immunometabolism, starts as an independent senior group leader at the MPI-IE.

Angelika Rambold starts at the MPI-IE

Funded by the Center for Chronic Immunodeficiency (CCI) Angelika Rambold starts her guest group 'Organelle Network Immunology' supported and located in the department of Thomas Boehm.

December

Andrew Pospisilik receives ERC Consolidator Grant 2015

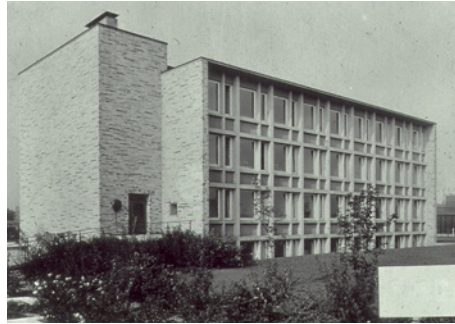
Andrew Pospisilik achieves one of the prestigious Consolidator Grants by the European Research Council in 2015 to continue his work on the genetic and epigenetic origins of complex diseases.

History of the Institute

➔ 1961



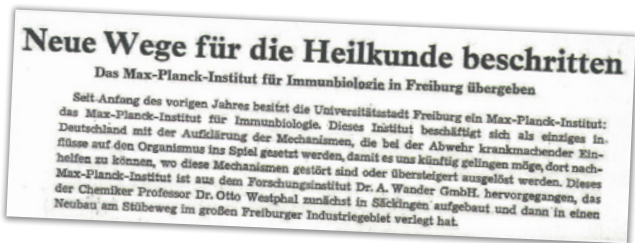
Otto Westphal, first director of the institute, with colleagues in the lab.



The institute in 1962.



Lab of Dr. S. Schlecht in the 60s.



press clipping from local news paper "Badische Zeitung" about the establishment of the institute in Freiburg, March 1963.

➔ 1984



Nobel Prize for director Georges Köhler



Nobel Prize celebration at the institute. From left: Westphal, Hess (vice president MPG), Köhler, Lüderitz and Eichmann.

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.

1970

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.

1981

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.

1984

In 1984, Niels Jerne, Georges Köhler and César 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 cell-cell 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.



Modern Lab in 2016 (Department of Thomas Boehm).



MPI-IB
1961-2010



MPI-IE
since 2010

In December 2010, the Max Planck Institute of Immunobiology was renamed to Max Planck Institute of Immunobiology and Epigenetics.

2010  2011

In December 2011, more than 200 guests celebrated the 50th anniversary of the MPI-IE.



In the late 1980s and also in 2007 two newly constructed buildings provided more space for new departments, research groups and facilities.



The institute in 2016.

2004

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 initiated 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 on epigenetics. To make an impact in the field of epigenetic research, the Kollegium decided to additionally appoint an epigenetics researcher as successor of Davor Solter.

2009

At the end of 2009, Asifa Akhtar was hired as the successor of Davor Solter (2006) focusing on chromatin regulation.

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 area of epigenetics and chromatin was founded.

2011

In December 2011 the institute celebrated its 50th anniversary. "Future needs ancestry" emphasized Peter Gruss, President of the Max Planck Society, and honoured the achievements of the MPI-IE.

2013

After a very successful career Rolf Kemler retired from the director position and established an emeritus group at the MPI-IE.

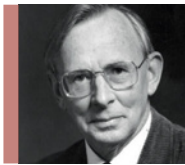
2015

In 2015, Erika Pearce was recruited as successor of Rolf Kemler (2013). She became head of the newly established department of immunometabolism at the institute.

Previous Directors of the Institute



Otto Westphal (Director from 1961-1982) 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 founding President of the German Society for Immunology.



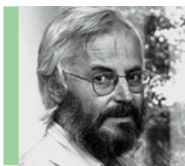
Herbert Fischer (Director from 1964-1981) had an ardent interest in macrophages at a time when the interest in the field of immunology was universally focused on lymphocytes. His group studied the role of phospholipid metabolism in the activation of macrophages and lymphocytes and its subsequent effects on the activation of the innate and adaptive immune systems.



Otto Lüderitz (Director from 1965-1988) 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 (Director from 1981-2004) 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.



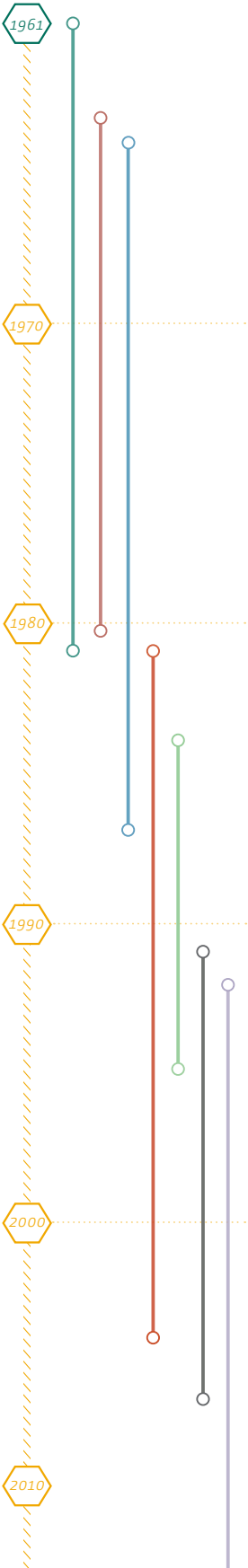
Georges Köhler (Director from 1984-1995) In the year 1981 Georges Köhler joined the Max Planck Institute of Immunobiology he was awarded the Nobel prize in Physiology or Medicine, together with Cesar Milstein and Niels Jerne for their pioneering work on the immune system and the generation of monoclonal antibodies using the hybridoma technique. His untimely death in 1995 was a great loss to the institute and the scientific community.



David Solter (Director from 1991-2006) studied in seminal experiments the developmental potential of maternal and paternal genomes by nuclear transplantation. He was one of the first to 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.



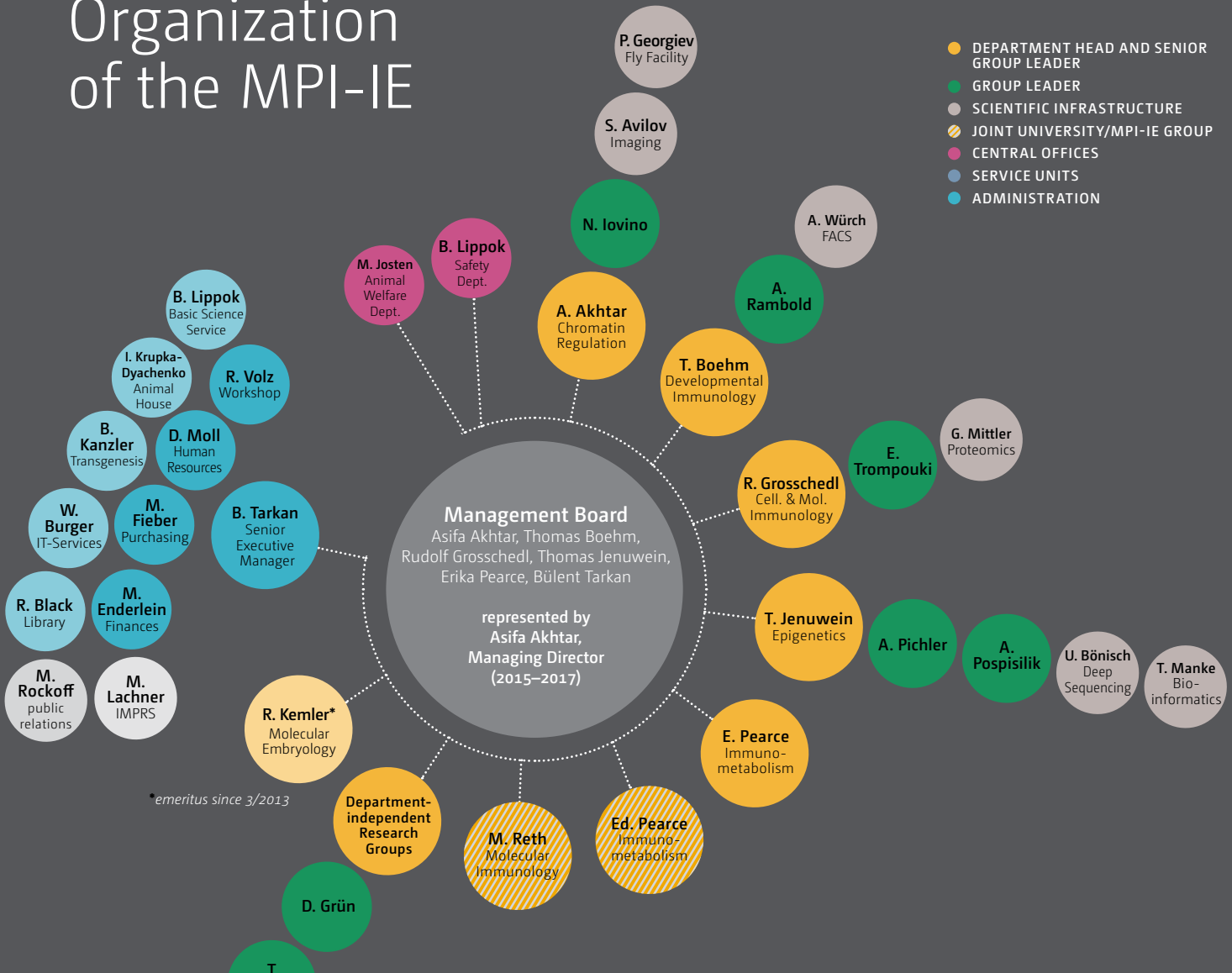
Rolf Kemler (Director from 1992-2013) identified the first cell-cell adhesion molecule in mouse development, E-cadherin. He discovered catenins as cytoplasmic anchorage proteins. Particularly β -catenin is well-known because of its dual function in cell adhesion and Wnt signaling. Kemler was the first 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.





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.

Organization of the MPI-IE



Board of Trustees

- | | | | |
|---|---|--|--|
| <p>Prof. Dr. Ali Shilatifard
Northwestern University
Feinberg School of
Medicine, Chicago, USA</p>  | <p>Prof. Dr. Amanda Fisher
(Chair person)
Imperial College London
London, UK</p>  | <p>Dr. Axel Glatz
Managing Director
Pfizer Deutschland GmbH
Freiburg, Germany</p>  | <p>Prof. Dr. Kerstin Krieglstein
Dean of the Medical Faculty,
University Medical Center
Freiburg, Germany</p>  |
| <p>Prof. Dr. Tasuku Honjo
Kyoto University,
Kyoto, Japan</p>  | <p>Prof. Dr. Ellen V. Rothenberg
California Institute of Tech-
nology, Pasadena, USA</p>  | <p>Prof. Dr. Martin Haag
Mayor of the City
of Freiburg
Freiburg, Germany</p>  | <p>Dr. Nicola von Lutterotti
(Chair person)
Free Journalist
Zurich, Switzerland</p>  |
| <p>Prof. Dr. Philip Avner
EMBL Monterotondo,
Monterotondo, Italy</p>  | <p>Prof. Dr. Shelley L. Berger
University of Pennsylvania
Philadelphia, USA</p>  | <p>Dr. Christian Hodeige
Managing Director
Badische Zeitung Group
Freiburg, Germany</p>  | <p>Prof. Dr. Gunther Neuhaus
Vice Rector for Research
University of Freiburg
Freiburg, Germany</p>  |
| <p>Prof. Dr. Max D. Cooper
Emory University School of
Medicine, Atlanta, USA</p>  | <p>Prof. Dr. Dan Littman
Langone Medical Center,
Skirball Inst. New York, USA</p>  | <p>Michael Kleiner
Ministerialdirigent
Ministry of Science, Re-
search and Art
Stuttgart, Germany</p>  | <p>Dr. Dieter Salomon
Lord Mayor of the City of Freiburg
Freiburg, Germany</p>  |

Scientific Advisory Board



➤ MANAGEMENT BOARD

Scientific Members of the Institute

- Asifa Akhtar (since 2009)
Managing Director
- Thomas Boehm (since 1998)
- Rudolf Grosschedl (since 2004)
- Thomas Jenuwein (since 2008)
- Erika Pearce (since 2015)

Senior Executive Manager

- Bülent Tarkan (2011-2016)



➤ EXTERNAL SCIENTIFIC MEMBERS

- Michael Sela (since 1967) Department of Immunology, Weizmann Institute of Science, Rehovot, Israel
- Barbara B. Knowles (since 2002) Emeritus Professor, The Jackson Laboratory, Bar Harbor, USA
- Michael Reth (since 2002) Department of Molecular Immunology, University of Freiburg, Germany / Associate Professor MPI-IE, Freiburg, Germany
- Paolo Sassone-Corsi (since 2011) Center for Epigenetics and Metabolism, University of California, Irvine, USA
- Alain Fischer (since 2015) Unité Hématologie Immunologie Infectiologie à Hopital Necker-Enfants Malades, Paris, France

➤ The Max Planck Institute of Immunobiology and Epigenetics (MPI-IE) is organized in five departments, plus two joint appointments 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'.

In addition, currently eight 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 fa-

cilities, and ensures a collaborative atmosphere at the institute. Monthly meetings of the faculty facilitate internal communication, identification of solutions, and dissemination of information.

Further, to ensure the high quality and productivity of research, the 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.

The Board of Trustees ("Kuratorium") provides the institute with valuable advice in social and science-political issues and supports further developments of the institute. The board meets once a year to interact with the management board of the MPI-IE and with other members of the Max Planck Society.

Administration & Service

Mission Statement: Excellent science flourishes in the right research environment. The main goal of the institute's administrative and service personnel is to provide optimal service so that the scientists can stay focused on their research.



Dr. Bülent Tarkan
Senior Executive
Manager



Finance Department



Human Resources



Our ECOfit-Team

In 2015 the MPI-IE took part in the ECOfit program by the city of Freiburg. To improve the economic efficiency of our infrastructure but most important to become a more sustainable institute several administrative departments teamed up. Since the beginning of 2016 the MPI-IE is officially "ECOfit-certified".



Safety Department

"Little Scientists" – child care facility

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. Since spring 2014 a new and larger

building in immediate vicinity of the institute enabled us to add a 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 child care facility until primary school right next to the institute. The entire service is optimally adjusted to the international environment at the MPI-IE: German and English native speaking educators care for the children, opening hours are very flexible and very limited holiday closing days support the parents.





Workshop



Reception



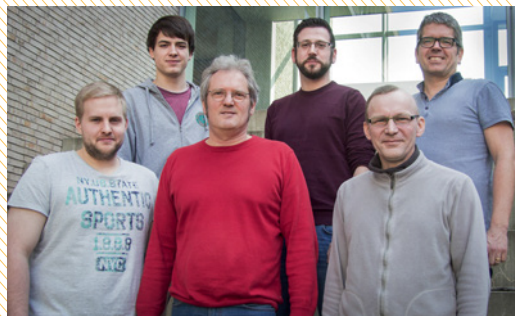
Basic Science Service



Library



Purchase Department



IT-Service

Senior Executive Manager: Dr. Bülent Tarkan (2011-2016)

Assistant to Senior Executive Manager: Nathalie Schulz

Finance Department

Head: Martina Enderlein
 Jasmin Haas
 Manuela Mattmüller
 Saskia Moos
 Florian Stiegler

Human Resources

Head: Daniel Moll
 Manfred Groß
 Veronika Klank
 Dominique Schädler
 Patricia Schätzler-Ott
 Sabine Stallone

Purchase Department

Head: Michele Fieber
 Regina Burger
 Melanie Maurer
 Sven Mußmann

IMPRS-MCB

Head: Monika Lachner
 Lisa Breitner

Public Relations

Marcus Rockoff

Reception

Head: Daniela Moll
 Sabrina Fögele
 Gabriele Prosch

Safety Department

Head: Bernadette Lippok
 Sabine Fietzeck
 Ute Lenz
 Marianne Piteau
 Andreas Rolke

Library

Rose Black
 Susanne Demme

Cleaning

Andrea Aukthun
 Irmgard Bregenhorn
 Michael Breithaupt
 Olga Lai
 Heike Nsusu
 Annerose Streit

Workshop

Head: René Volz
 Uwe Bachstein
 Günther Barth
 Adalbert Bleichner
 Thomas Fischer
 Michael Herm
 Roland Kaiser
 Steffen Pappenfuß

Basic Science Service

Head: Bernadette Lippok
 Nadine Beck
 Doris Hoppe
 Ute Lenz
 Yan-Qing Li
 Petra Lüderitz
 Patricia Mayer
 Madalena Moreira
 Roswitha Meller
 Andrea Reinecke

IT-Service

Head: Wolfgang Burger
 Daniel Andris
 Wolfgang Arndt
 Ralf Gritzner
 Christian Pagel
 Wilhelm Rüsing

Trainees

Luca Schuler-Köblle
 Theresa Cimentepe
 Sophia Schulze
 Till Wörpel

Staff Restaurant & Lounge

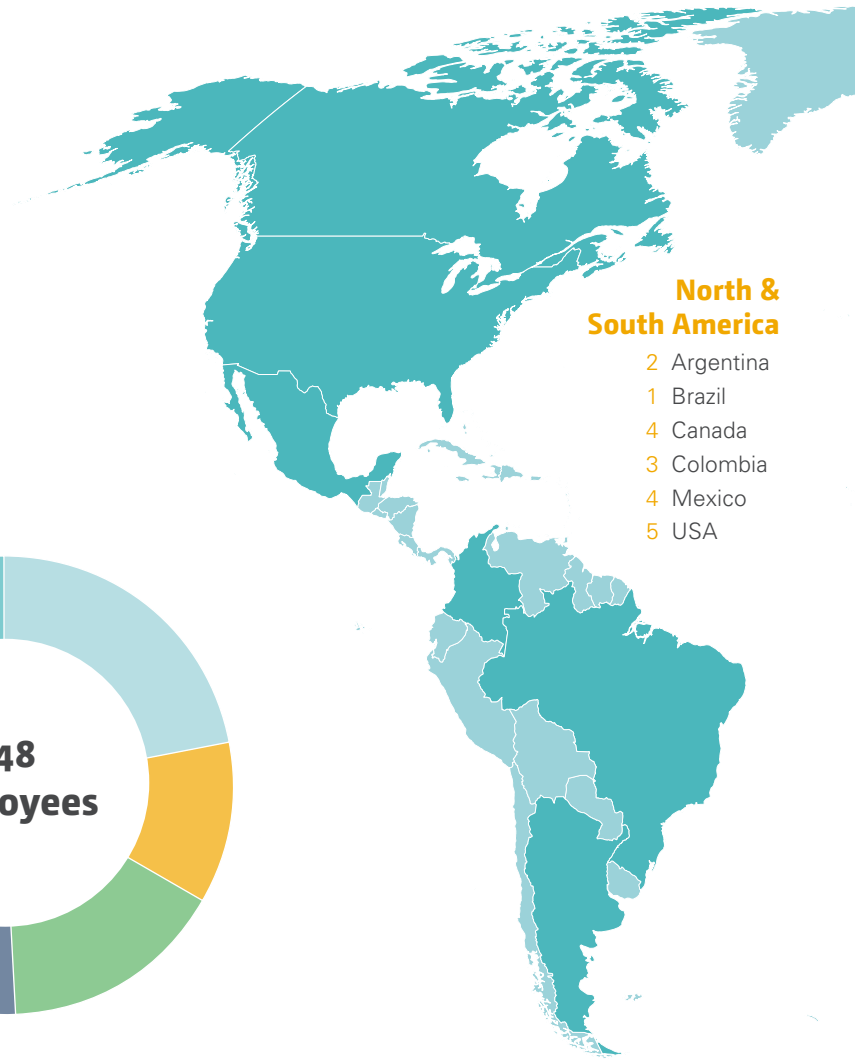
Martin Haberstroh
 (Tenant)

Facts

The 348 employees of the MPI-IE stem from all 5 continents and represent more than 45 countries of the world.

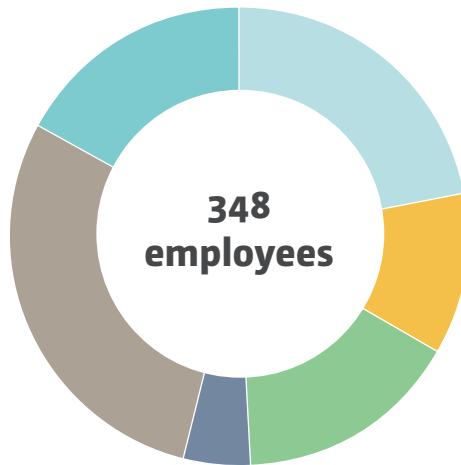
data as of Dec 2015

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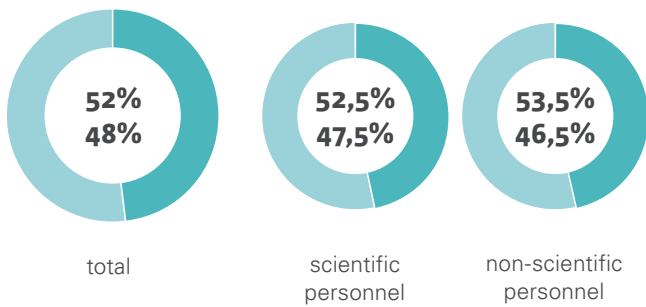


Personnel

- 77** Postdocs
- 7** Senior Group Leaders
- 9** Group Leaders
- 39** Ph.D. Students
- 56** Scientific Assistants
- 16** Trainees, student assistants
- 101** Administration & non-scientific personnel
- 59** Guest Scientists

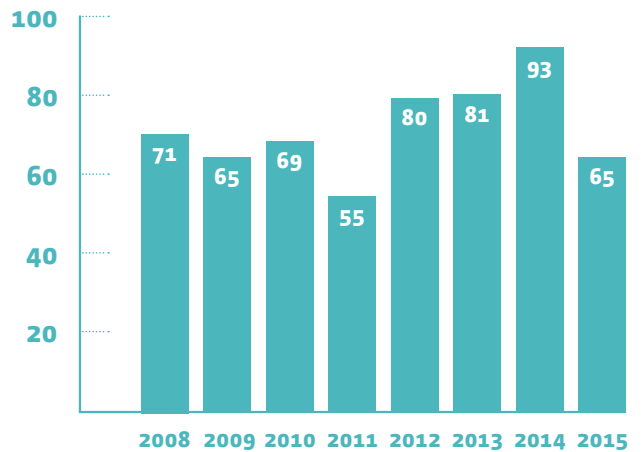


Gender



Publications

Over 560 publications since 2008

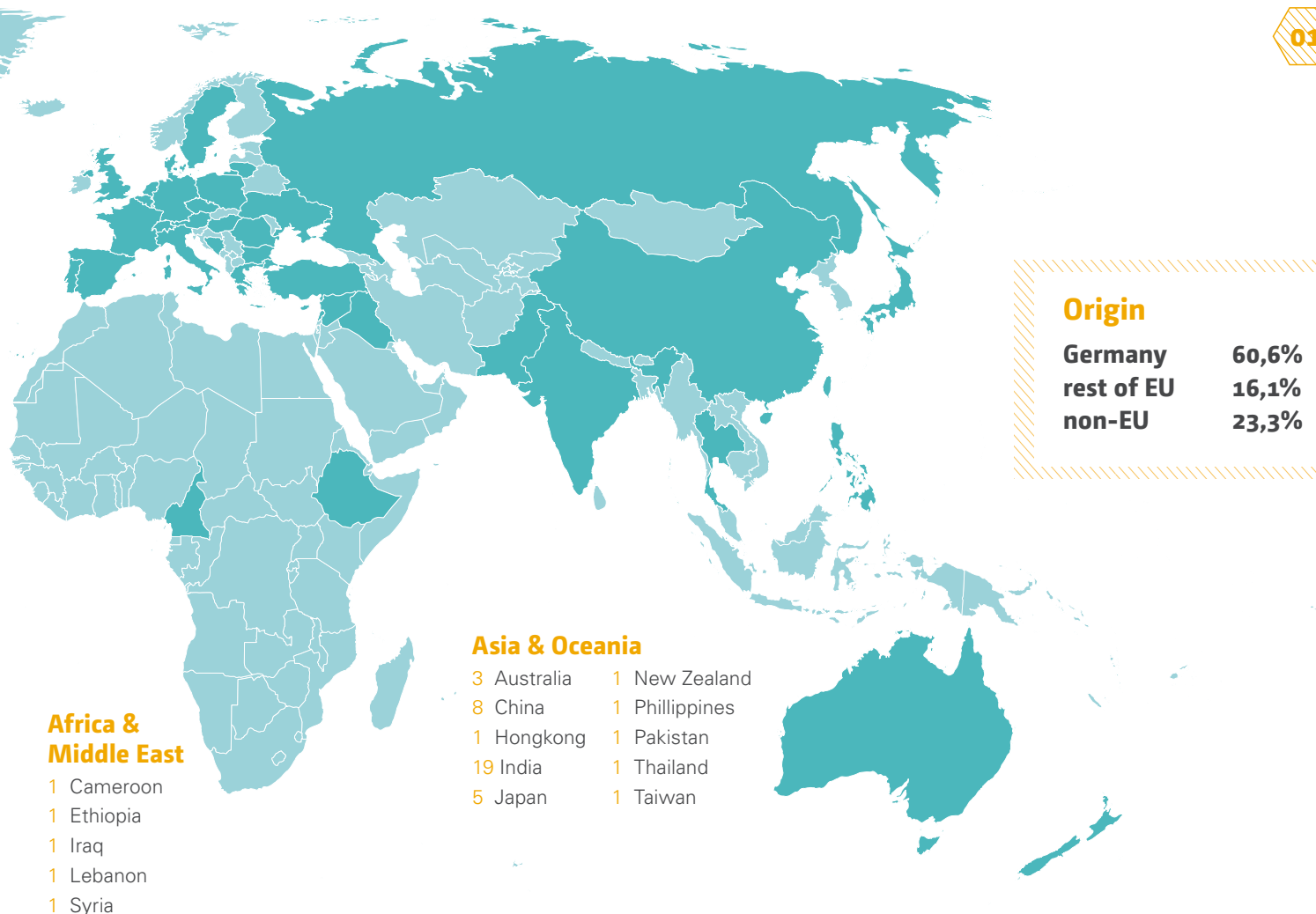


(Source: Scopus 2015)

Europe

3 Austria	3 Bulgaria	2 Cyprus	10 France	1 Hungary	2 Netherlands	1 Romania	1 Sweden
1 Bosnia	1 Switzerland	1 Czech Rep.	211 Germany	5 Italy	7 Poland	3 Russia	3 Turkey
1 Belgium	7 Great Britain	1 Denmark	4 Greece	1 Lithuania	2 Portugese	5 Spain	3 Ukraine

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Origin

Germany	60,6%
rest of EU	16,1%
non-EU	23,3%

Africa & Middle East

1 Cameroon
1 Ethiopia
1 Iraq
1 Lebanon
1 Syria

Asia & Oceania

3 Australia	1 New Zealand
8 China	1 Phillipines
1 Hongkong	1 Pakistan
19 India	1 Thailand
5 Japan	1 Taiwan

Collaborations



➔ 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: SFB746 "Functional specificity by coupling and modifications of proteins", SFB850 "Control of Cell Motility in

Morphogenesis, Cancer Invasion and Metastasis", SFB992 "Medical Epigenetics – From basic mechanisms to clinical applications" SFB1140 "Kidney disease – From genes to mechanisms" and SFB1160 "Immune-mediated pathology as a consequence of impaired immune reactions".

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.



The International Max Planck Research School



Lab rotations
initial rotation period in different labs to find the most suitable environment and research topic

Scientific training

- Advanced Methods
- Critical Analysis of Literature
- Laboratory Skills

Transferable skills

- Scientific Writing & Presentation
- Project & Self Management

Career Development

- Application Training
- Funding Opportunities

Conferences
support for international scientific conferences & regional symposia

TACs
regularly thesis advisory committees to guarantee scientific excellence & success of each student

IMPRS-MCB at a glance



IMPRS-MCB
From Molecules to Biological Systems

IMPRS COORDINATOR



Dr. Monika Lachner

IMPRS Office
Lisa Breitner

More Information
www.imprs-mcb.mpg.de



Mission of IMPRS-MCB

➔ The International Max Planck Research School for Molecular and Cellular Biology (IMPRS-MCB) was established in 2006 as a joint initiative of scientists from the University of Freiburg and the MPI-IE. It is our goal to provide talented students with excellent training opportunities in the fields of Molecular and Cellular Biology, Epigenetics and Immunobiology, thereby supporting their development into promising young researchers.

Organization and Structure of IMPRS-MCB

The IMPRS-MCB program is managed by the IMPRS office in collaboration with the two IMPRS Spokespersons and the members of the Graduate Committee. We currently have 49 students from 22 countries, and 18 faculty members (15 from MPI-IE and 3 from the University of Freiburg). We are also very proud of our growing Alumni group, which comprises 50 graduates by now. Most of our alumni continued their careers as postdoctoral fellows (67%) or moved into industry positions (22%).

Overview: Ph.D. at IMPRS-MCB

One of the distinctive aspects of our program is a rotation period. Students spend three months in three different laboratories before starting their Ph.D. project. This is an important step towards identifying a suitable laboratory for their Ph.D. thesis, as it allows them to experience three distinct lab environments and provides further insight into particular research topics. The rotations are also a chance for group leaders to ascertain which student will be the best fit for their groups.

Finally, this rotation phase enhances communication and networking within the Institute.

After the rotation period, our fellows join the lab, in which they will carry out their Ph.D. work. The Ph.D. project constitutes the core of the scientific training. Our students benefit from the supervision of the respective group leader, the interactions within the laboratory and collaborations within and outside the MPI-IE. Furthermore, the individual progress of all IMPRS-MCB students is regularly monitored by their thesis advisory committees (TACs).

IMPRS-MCB students also participate in a broad curriculum that complements their laboratory training. This additional training program covers three major aspects – advanced scientific training, transferable/soft skills and networking. In particular, we offer courses that relate to

- Advanced scientific methods
e.g. bioinformatics, statistics, imaging
- Critical analysis of scientific literature
- Scientific Writing
- Scientific Presentation
- Good Scientific Practice
- Career Development
- Funding Opportunities

We strongly encourage the participation of our students in the annual Ph.D. retreat organized by the Ph.D. representative. Furthermore, we support their attendance at regional symposia and international scientific conferences. Students also have the possibility to learn German and to obtain advice in planning their future career.

Focus Immunobiology

➤ 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.

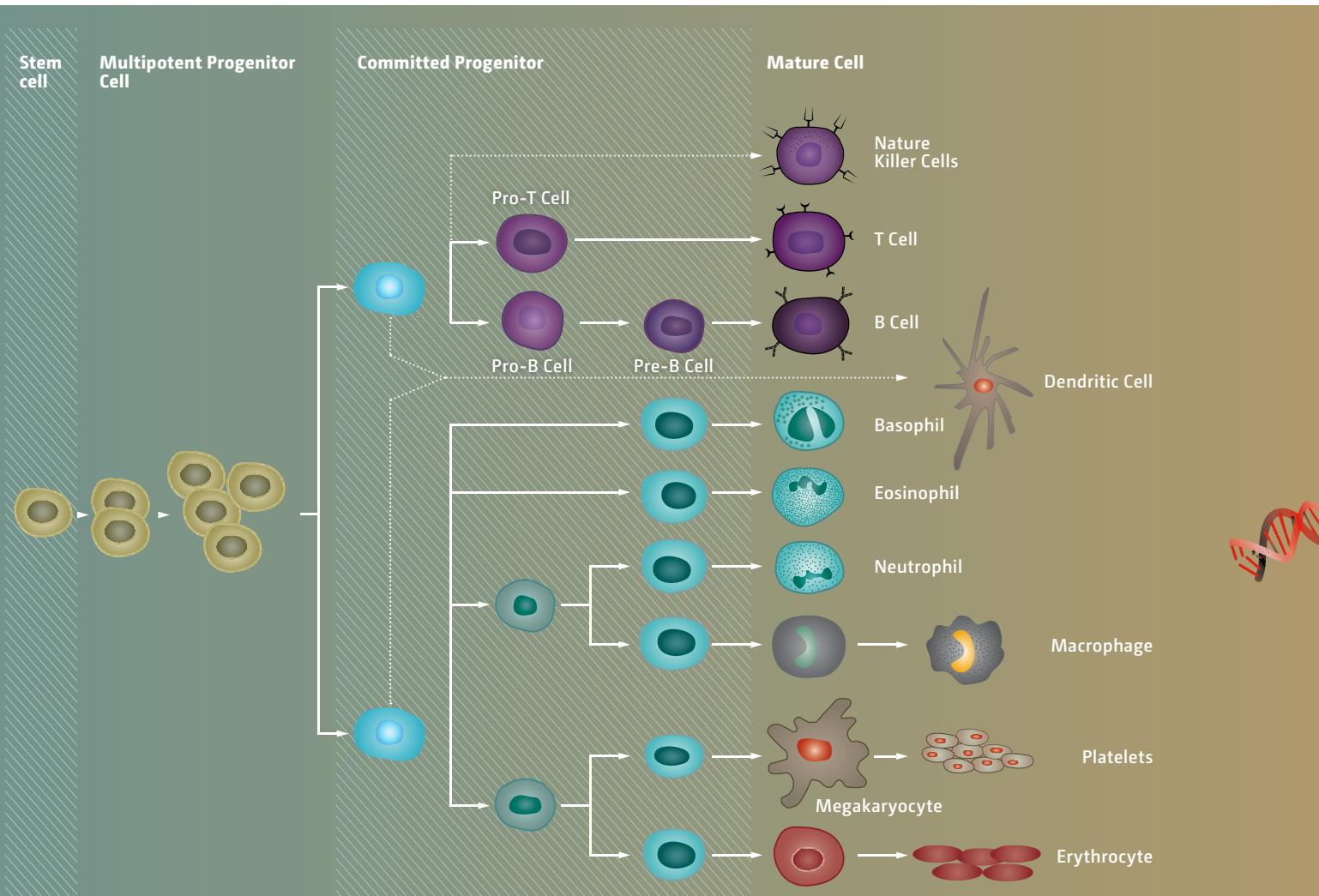
Also for humans, a properly functioning immune system is of central importance. Indeed, of all branches of medicine, the translation of results from immunological research to medical treatments over the last two centuries has probably had the most significant impact on human life expectancy. Many devastating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



The evolutionary and life-time development of hematopoietic 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.

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

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Focus Epigenetics

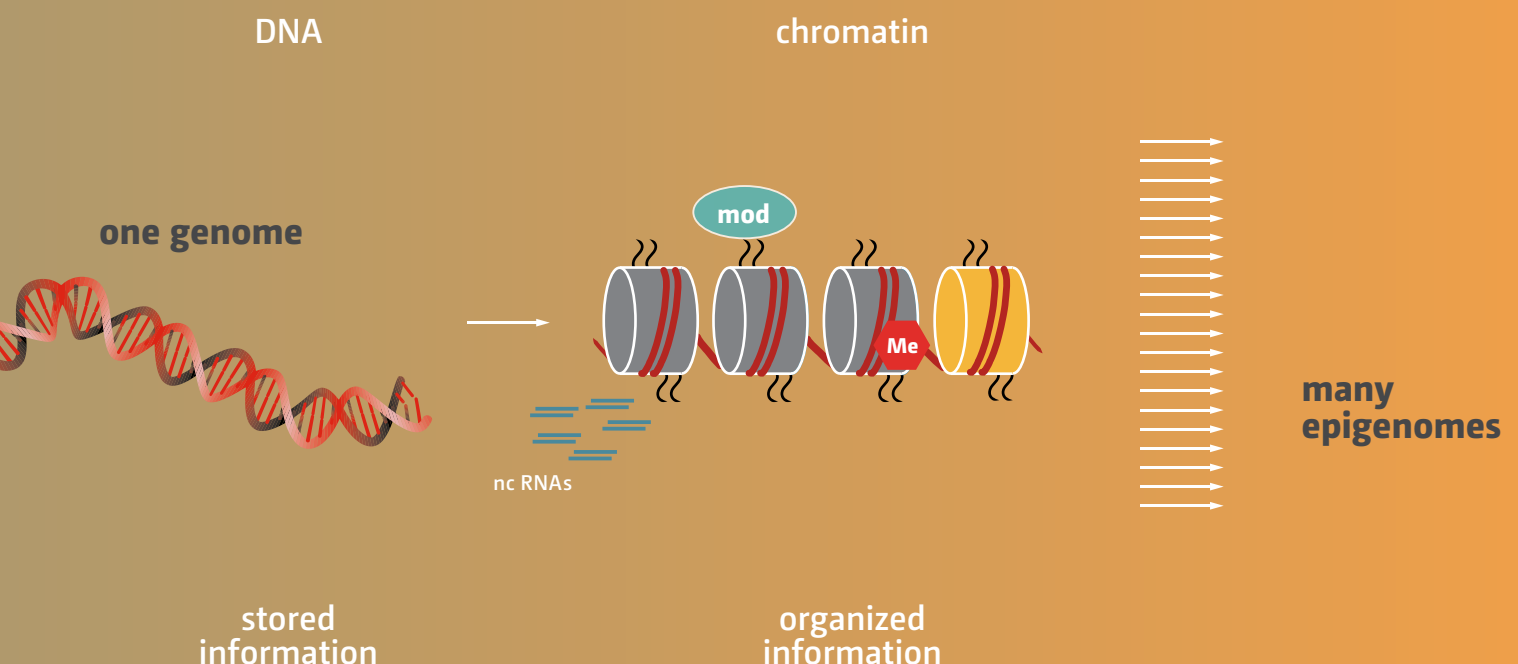
➔ “Are we more than the sum of our genes and how can environmental cues alter gene expression?” While almost all cell types within a human body share an identical DNA sequence, its utilization will differ significantly according to the designated function of a cell. The DNA template within the cell nucleus 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 dysregulation 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.

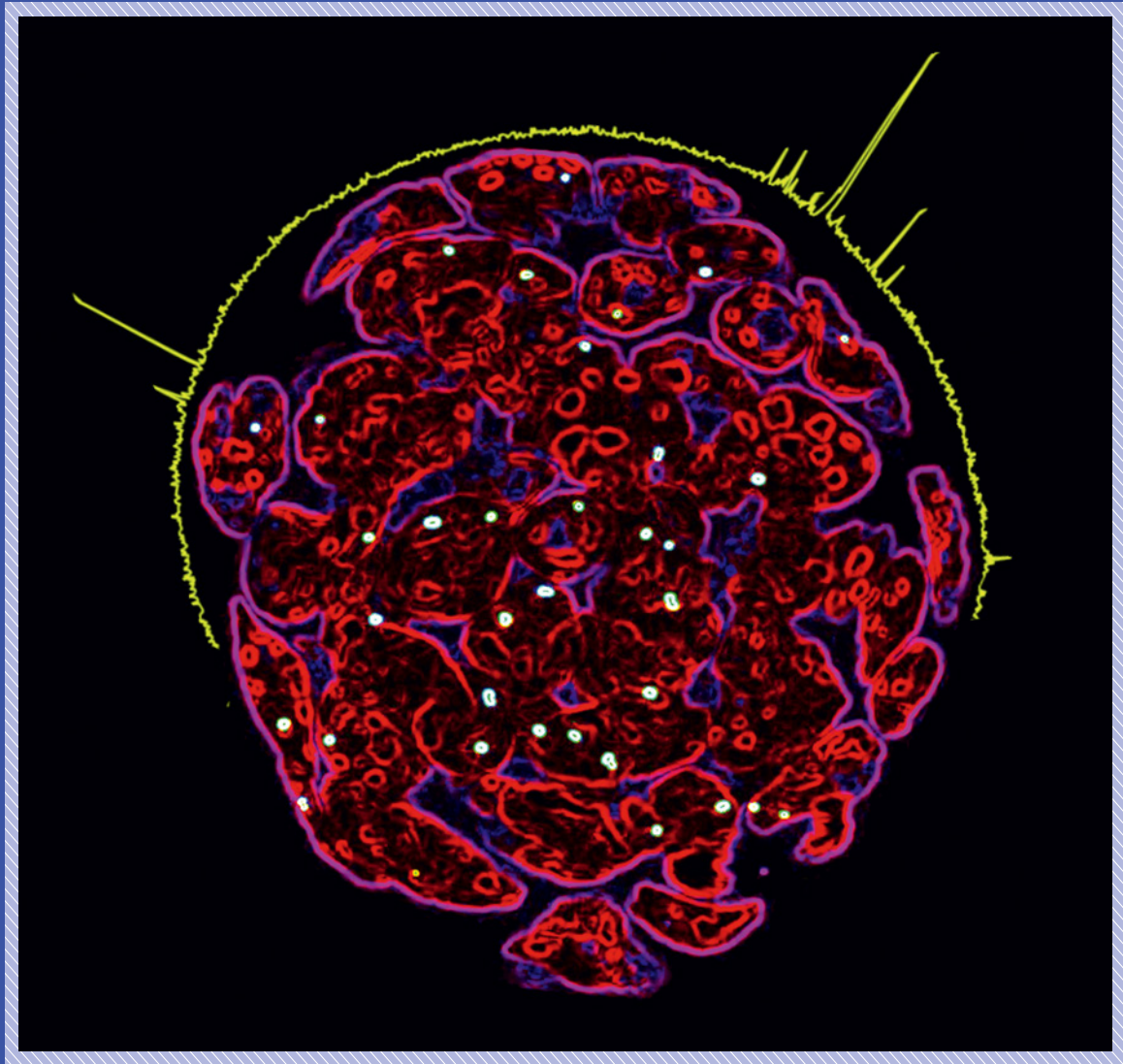


The DNA template within the cell nucleus is not naked, but wrapped and protected by specific proteins (histones). This DNA-histone polymer is called chromatin. Histone modifications and other chromatin alterations are important elements of epigenetic gene control. This is critical for embryonic development,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



Research Activities





🔍 LAB ASIFA AKHTAR

Immunofluorescence image of a multicellular colony of female mouse embryonic stem cells: in culture, the cells were probed with RNA-FISH probe specific for Tsix-DXPas34 (green/yellow dots). The yellow signal surrounding the upper hemisphere of the cell colony is the ChIP-Sequencing readout for MSL2 chromatin binding in the region of the X inactivation center. The most pronounced peak showcases the binding of MSL2 to Tsix enhancer – DXPas34.



➤ LAB MEMBERS

Administration

Linda Schmidl

Technicians

Herbert Holz, Thiebault Lequeu, Janine Seyffert, Thomas Stehle

Postdoctoral Fellows

Tugce Aktas Ilik, Felicia Basilicata, Benjamin Herquel, Ibrahim Avsar Ilik, Claudia Keller, Ken Lam, Amol Panhale, Maria Shvedunova, Sarah Toscano, Abdullah Sahyoun, Bilal Sheikh

Ph.D. Students

Aindrila Chatterjee, Aline Gaub, Raed Hmadi, Shantanu Iyer, Adam Karoutas, Cecila Pessoa, Giuseppe Semplicio

➤ GROUP LEADER ASIFA AKHTAR

1971 Born in Karachi, Pakistan, Undergraduate studies in Biology at University College London, United Kingdom

1994-1997 Ph.D. studies at Imperial Cancer Research Fund, London, United Kingdom

1998-2001 Postdoctoral fellow at EMBL, Heidelberg and the Adolf-Butenandt-Institut, Munich, Germany

2001-2009 Group Leader at EMBL, Heidelberg, Germany

2009-2013 Max Planck Investigator, Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

Since 2013 Director at the MPI-IE, Department of Chromatin Regulation, Freiburg, Germany

Since 2015 Managing Director at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



➤ SELECTED PUBLICATIONS

Chlamydas S, Holz H, Samata M, Chelmicki T, Goergiev P, Pelechano V, Dündar F, Dasmeh P, Mittler G, Tavares Cadete F, Ramirez F, Conrad T, Wie W, Raja S, Manke T, Luscombe NM, Steinmetz LM and Akhtar A (2016). Functional interplay between MSL1 and CDK7 controls RNA polymerase II serine 5 phosphorylation. **Nature Structural and Molecular Biology (in press)**.

Ramirez F, Lingg T, Toscano S, Lam KC, Georgiev P, Chung HR, Lajoie B, de Wit E, Zhan Y, de Laat W, Dekker J, Manke T, Akhtar A (2015). High-affinity sites form an interaction network to facilitate spreading of the MSL complex across the X chromosome in *Drosophila*. **Molecular Cell** **60**, 146-62.

Meunier S, Shvedunova M, Van Nhuong N, Avila L, Vernos I, Akhtar A (2015). An epigenetic regulator emerges as microtubule minus-end binding and stabilizing factor in mitosis. **Nature Communications** **6**, 7889.

Chelmicki T, Dündar F, Turley M, Khanam T, Aktas T, Ramirez F, Gendrel AV, Heard E, Manke T and Akhtar A (2014). MOF complexes use short and long-range interactions to ensure stem cell identity and Xist repression. **eLife****10**, 7554.

Dias J, Van Nguyen N, Georgiev P, Gaub A, Brettschneider J, Cusack S, Kadlec J and Akhtar A (2014). Structural analysis of the KANSL1/WDR5/KANSL2 complex reveals that WDR5 is required for efficient assembly and chromatin targeting of the NSL complex. **Genes & Development** **28**, 929-942.

Senior Group Asifa Akhtar

EPIGENETIC MECHANISMS MEDIATED BY HISTONE ACETYLATION

X chromosome regulation as a paradigm to analyze
transcriptional complexity

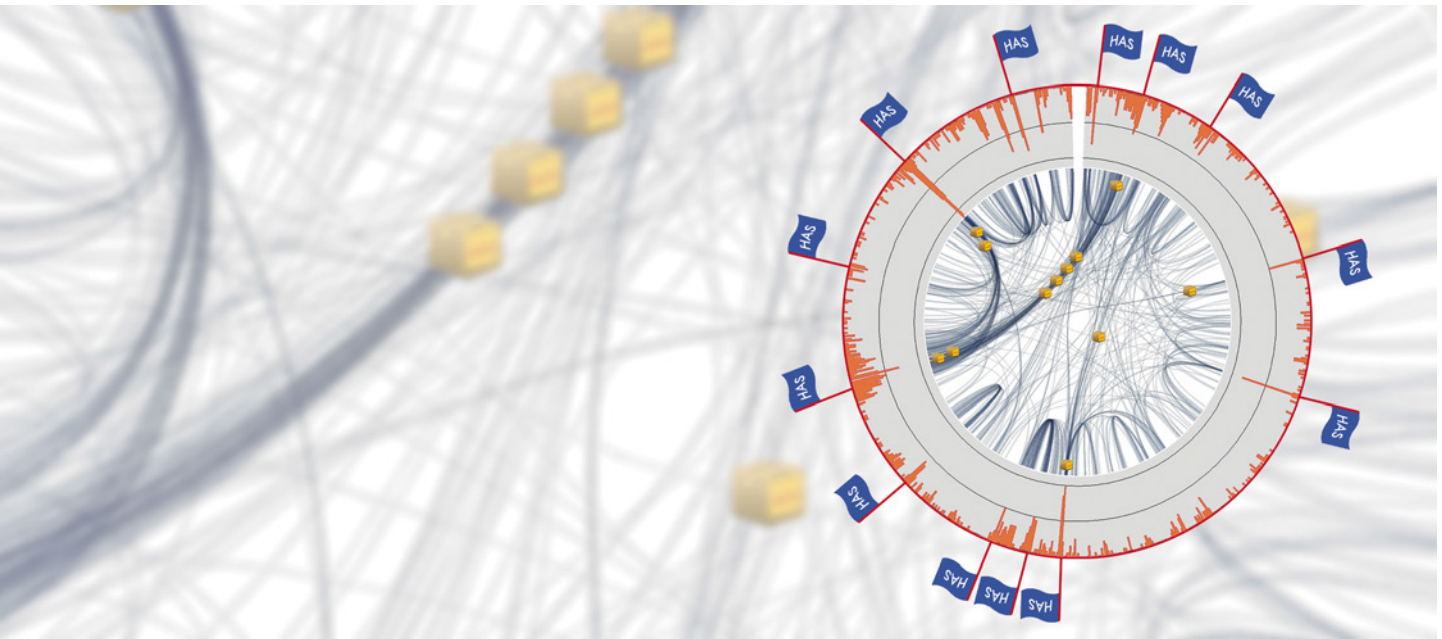


Figure 1 Logistics on fly X chromosome for the MSL complex: The high-affinity sites dispersed on the X chromosome and are well-connected to each other and operate as central hubs to distribute the MSL complex over the X chromosome to achieve dosage compensation.

➤ 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 plays an essential role in the regulation of gene expression. Structural changes in chromatin 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 two-fold hyper-transcription of the genes on the male

X chromosome. Genetic studies have identified a number 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]. The hyperactive X is also specifically hyper-acetylated at histone H4, acetylation which is achieved by the MOF histone acetyl transferase.

One of our major goals 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],

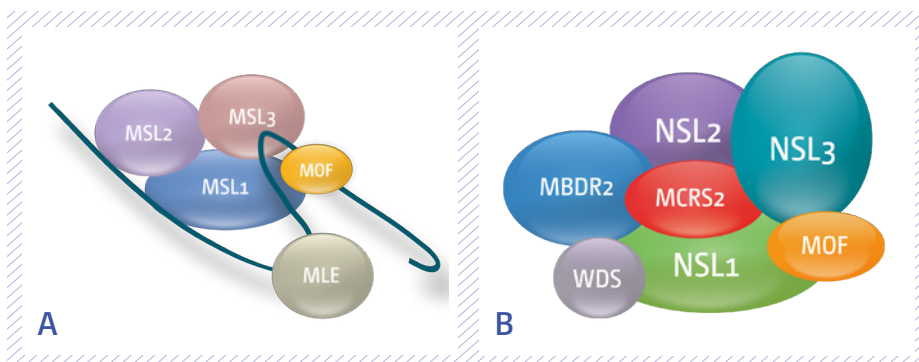


Figure 2 MOF histone acetyl transferase is part of two distinct multiprotein complexes

A The Male-Specific-Lethal (MSL) complex in *Drosophila*, consisting of two non-coding RNAs and five proteins, is a key factor in regulation of the X chromosome by the process of dosage compensation.

B The Non-Specific-Lethal (NSL) Complex binds to all chromosomes. It is enriched on promoter regions and is involved in the regulation of many housekeeping genes in *Drosophila*.



gets targeted to the X chromosome. In addition, we are studying the mechanism by which the MSL complex modulates X chromosomal transcriptional output at a single cell resolution all the way to chromosomal and organismal level.

The role of non-coding RNA in epigenetic regulation

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 regulation. 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 RNA-protein interactions regulate transcriptional and post-transcriptional events and thereby influencing cellular homeostasis.

The chromosome dynamics and gene expression

It is becoming increasingly clear that chromosomal organization as well as gene positioning has the potential to influence gene expression. 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. Our recent work employing chromosome conformation techniques (HiC and 4C) and high resolution imaging indicates that high affinity sites located on the X chromosome form an interaction network to facilitate dosage compensation.

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. We have identified an interesting evolutionary link between the fly and mammalian MSL complexes in X chromosomal regulation. By targeting an enhancer which regulates *Tsix*, an antisense transcript controlling the expression of *Xist* long-non-coding RNA, the MSL complex members ensure that the X chromosome remains active in embryonic stem cells. In future, we plan to explore further the mechanism by which MSL proteins play a role during X chromosome inactivation in mammals.

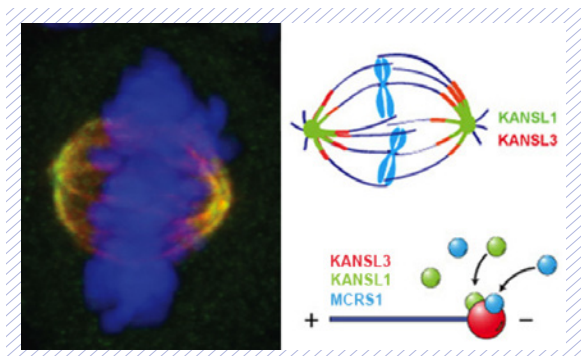
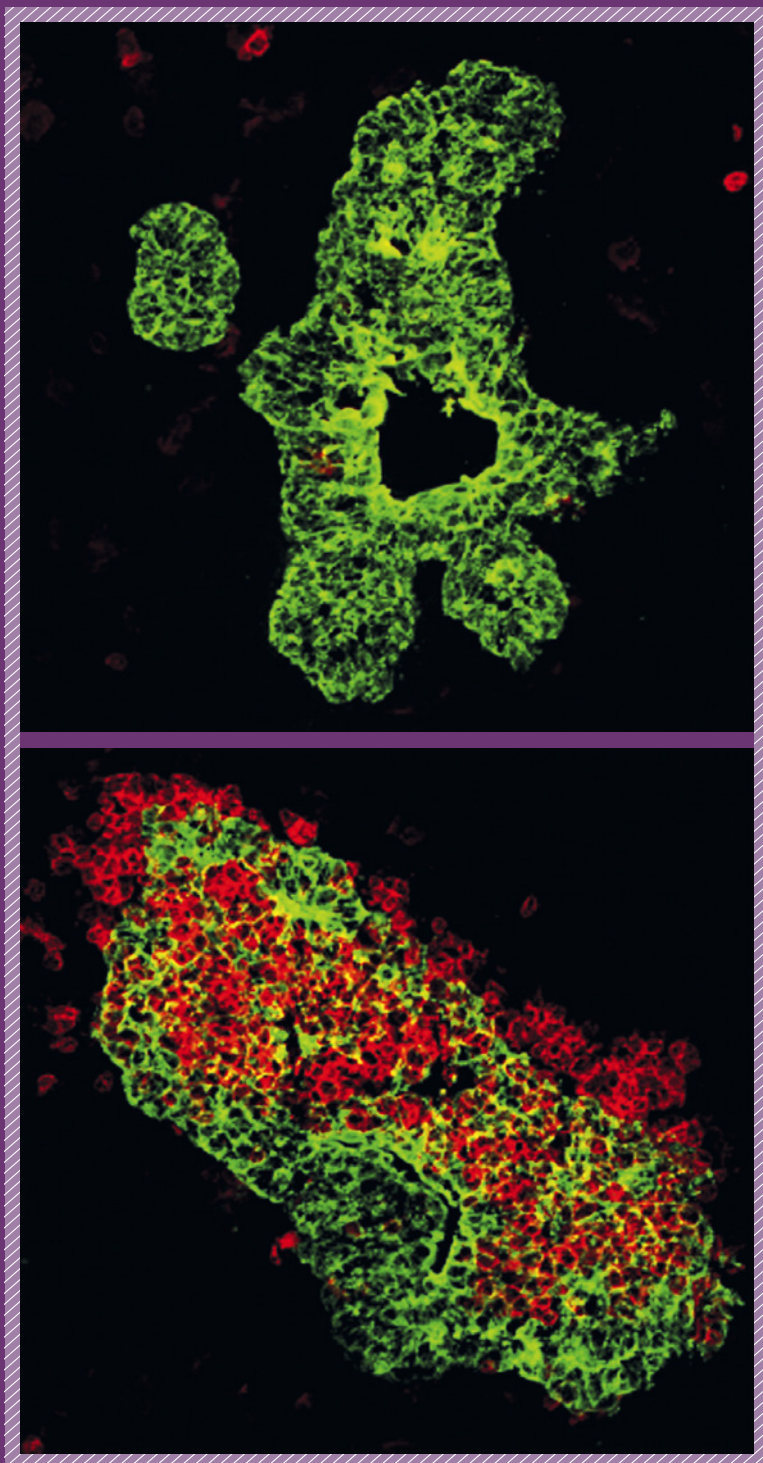


Figure 3 KANSL proteins, how NSL proteins are called in mammals, localize to the poles of the mitotic spindle. Left panel: Immunofluorescence of the mitotic spindle. KANSL3 is shown in green, tubulin in red and DNA in blue. Overlaps between red and green appears as yellow. Right Panel: Schematic representation of KANSL proteins on the mitotic spindle (upper half) and microtubules.

Our recent work has also revealed that the mammalian MSL and NSL complexes are not only major regulators of housekeeping gene function but are also involved in tissue specific regulation by targeting a variety of enhancers in mammals. Moreover, cellular function of NSL proteins extends beyond their epigenetic role during interphase. We have shown that mammalian KANSL1 and KANSL3 proteins decorate the mitotic spindle and have identified KANSL3 as a novel microtubule minus end binding protein. Thus, our work revealed novel functions of NSL complex members during different stages of cell cycle. In future we plan to explore how interphase versus mitotic functions are coordinated by NSL complex members in mammals. ◀

➤ LAB THOMAS BOEHM

In vivo engineering of the thymic epithelial niche (green). The dysfunctional thymic epithelial niche (top) supports T cell development (T cells in red) only after engineered expression of the Cxcl12 chemokine and the Notch ligand Dll4 (bottom).



Senior Group Thomas Boehm

Design principles of
adaptive immunity
in vertebrates



➤ LAB MEMBERS

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Connor Patrick O'Meara, Katarzyna, Sikora, Inês Trancoso

Ph.D. Students
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Visiting Scientists
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Hiroaki Suetake (04/15-04/16)

➤ GROUP LEADER THOMAS BOEHM

1956 Born in Gelnhausen, Germany, study of Medicine at the Johann Wolfgang Goethe University Frankfurt, Germany

1982-1986 Fellow in Biological Chemistry and Pediatrics, University of Frankfurt Medical School, Germany

1987-1991 Staff Scientist at the Laboratory of Molecular Biology, Cambridge, England

1992-1994 Professor at the University of Freiburg Medical School, Germany

1995-1997 Professor at the German Cancer Research Center, Heidelberg, Germany

Since 1998 Director at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



➤ SELECTED PUBLICATIONS

Swann, JB, Weyn A, Nagakubo D, Bleul CC, Toyoda A, Happe C, Netuschil N, Hess I, Haas-Assenbaum A, Taniguchi Y, Schorpp M and Boehm T (2014). Conversion of the thymus into a bipotent lymphoid organ by replacement of Foxn1 by its paralog, Foxn4. *Cell Reports* **8**, 1184-1197.

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Boehm T and Swann JB (2014). Origin and evolution of adaptive immunity. *Annual Review of Animal Biosciences* **2**, 259-283.

Senior Group Thomas Boehm

THYMOPOIESIS: FROM EVOLUTIONARY ORIGINS TO FUTURE THERAPIES

Design Principles of adaptive immunity in vertebrates

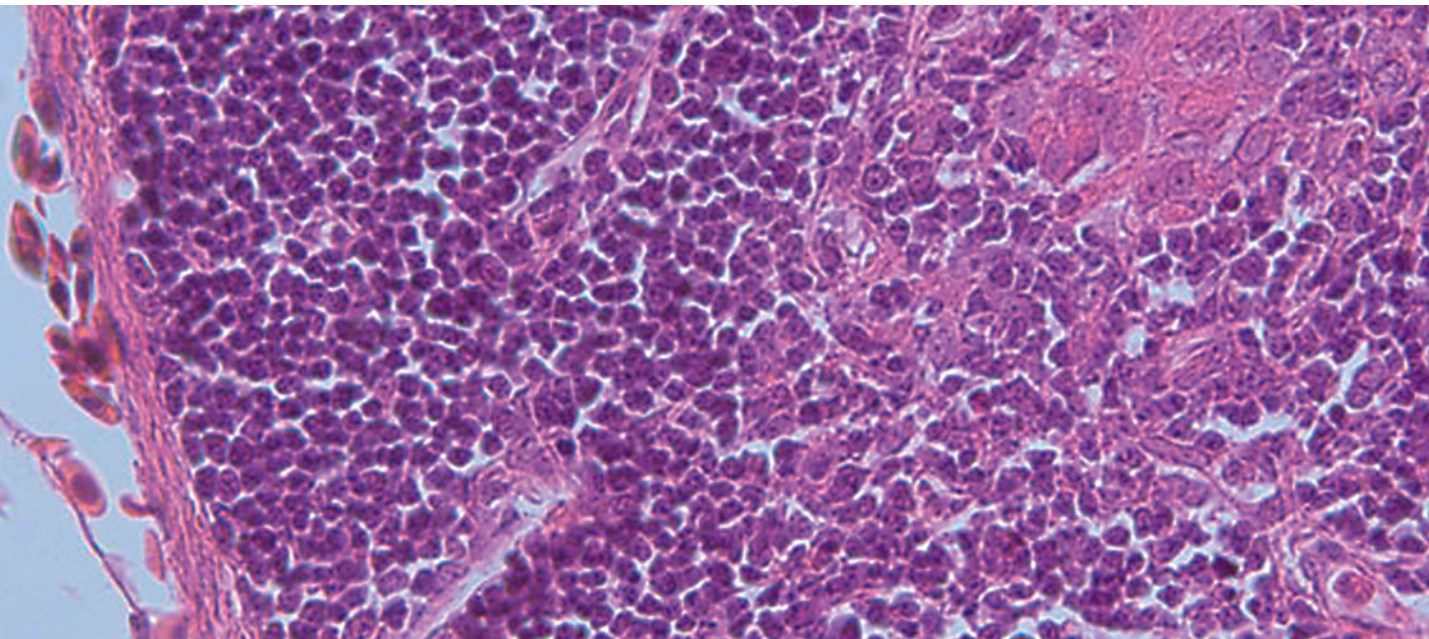


Figure 1 A histological section of thymus tissue of a young shark shows that the organ structure has remained essentially unchanged since about 500 million years.

➤ 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 these organs. In an iterative process, we combine forward genetic screens and methods of precise genetic interference in model systems to examine the role of single genes or combinations thereof in the formation of the epithelial 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, and are studying the molecular mechanisms 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 transgenic mice nullizygous for *Foxn1* by re-expression of individual target genes of the *Foxn1* transcription factor, singly or in combinations. To date, we have achieved the reconstitution of T cell development until the CD4⁺CD8⁺-double-positive stage of developing thymocytes 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. We are also interested in examining the molecular basis of thymus involution, a physiological process that leads to reduced output of naïve T cells in ageing individuals.

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 haematopoiesis. 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. Using genetic interaction analysis, we aim at generating a draft version of the genetic networks underlying vertebrate T cell development. We also use long-term live imaging analysis with our mutants and novel transgenic fish lines to further 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.

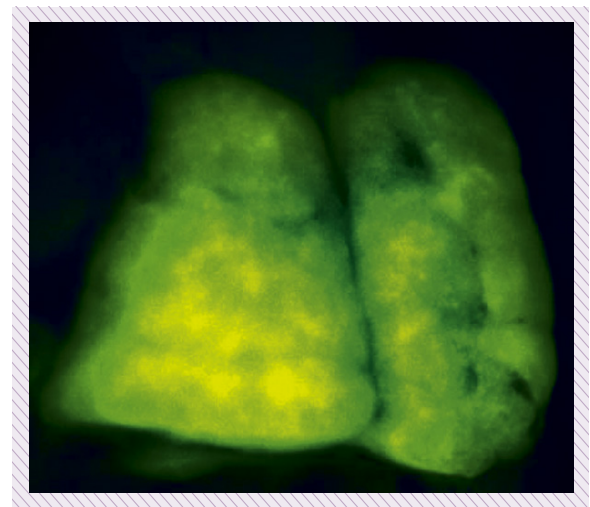


Figure 3 Macroscopic view of an embryonic mouse thymus. The fluorescence originates from the epithelial compartment.

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 defense. 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 aims at elucidating the design principles of adaptive immune systems. At present, we are focussing on studies of the lamprey immune system, with particular emphasis on T cell development. ↩

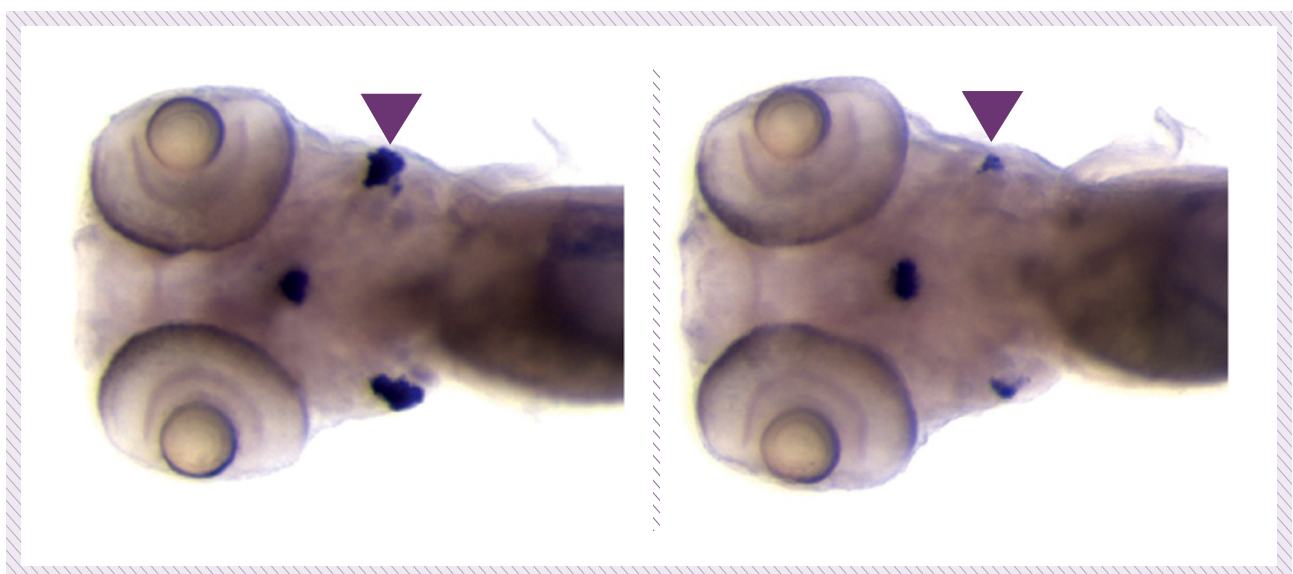


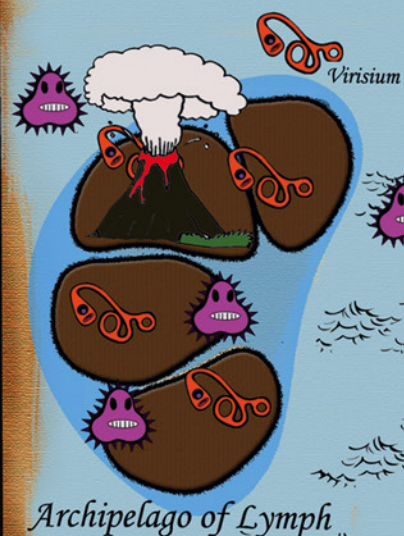
Figure 2 Thymocyte development in wild-type (left panel) and mutant (right panel) fish, visualized by RNA *in situ* hybridization. The thymus is marked with red arrowheads.

CHROMATIN TALES: GUARDIANS OF THE B CELLS

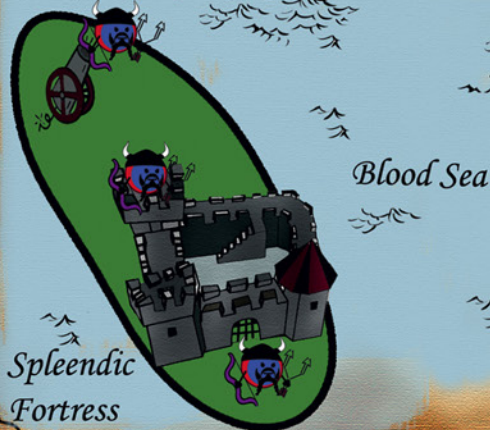
Once upon a time, the realm of Immunity came under siege from a combined attack from the evil Bacterium and Virisium. The invaders had tunneled through the volcano on Mount Node of the Archipelago of Lymph and entered the Blood Sea.

To combat this attack the brave mecenary clans of Macrophages and NK Cells were sent out. Unable to contain the enemy, the realm had no choice but to train the fledglings to become specialised warriors. The wise guardian and mentor of the B cell warriors, the two headed dragon EBF the First was awoken to guide the young ones.

Will the mighty EBF with the help of the centaurs PAX5 and the friendly blacksmith Troll MZB1 successfully lead the young ones out of the Forest of Fate onto the Path of Identity to become B cell warriors...????



Archipelago of Lymph



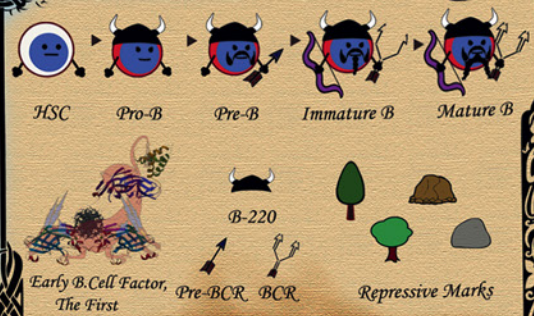
Splendid
Fortress



Nicheburg

Forest of Fate

Path of Identity



HSC Pro-B Pre-B Immature B Mature B

Early B. Cell Factor, The First B-220 Pre-BCR, BCR Repressive Marks

Senior Group Rudolf Grosschedl

Regulatory Circuitries
underlying
B Lymphopoiesis



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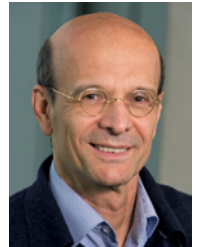
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1999-2004 Professor and Director of Gene Center, University of Munich, Germany

Since 2004 Director at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



➤ SELECTED PUBLICATIONS

Boller S, Ramamoorthy S, Akbas D, Nechanitzky R, Burger L, Murr R, Schübeler D, Grosschedl R (2016). Pioneering Activity of the C-Terminal Domain of EBF1 Shapes the Chromatin Landscape for B Cell Programming. **Immunity** **44**, 527-541.

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Nechanitzky R, Akbas D, Scherer S, Györy I, Hoyler T, Ramamoorthy S, Diefenbach A and Grosschedl R (2013). Ebf1 is essential to maintain B cell identity and to prevent alternative cell fates in committed cells. **Nature Immunology** **14**, 867-875.

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Kieslinger M, Hiechinger S, Dobreva G, Consalez GG, Grosschedl R (2010). Early B cell factor 2 regulates hematopoietic stem cell homeostasis in a cell-nonautonomous manner. **Cell Stem Cell** **7**, 496-507.

Senior Group Rudolf Grosschedl

REGULATORY CIRCUITRIES UNDERLYING B LYMPHOPOIESIS

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

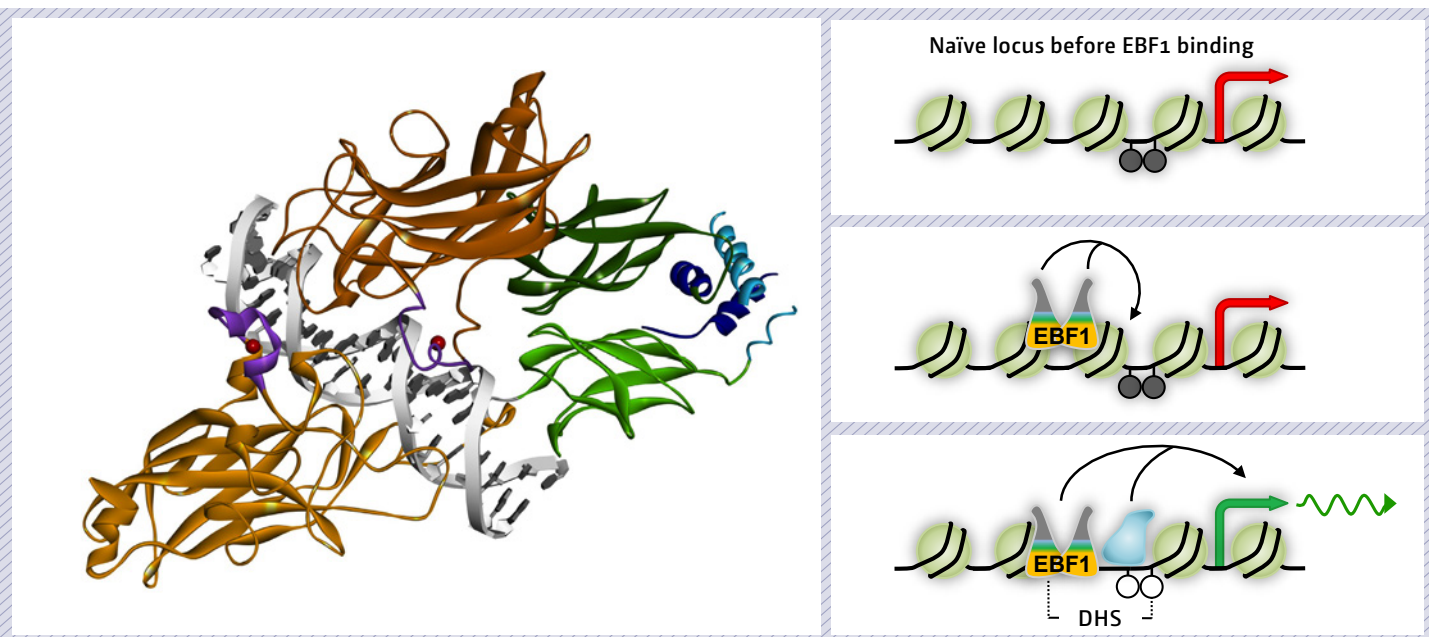


Figure 1 Structure of the EBF1: DNA complex and scheme of the role of EBF1 in conferring lineage-specific changes in progenitor chromatin.

➤ The developmental potential and trajectory of a cell are influenced by external signals and internal conditions, including the epigenetic state and the activity of transcription factors. Hematopoiesis is one of the best characterized developmental systems for studying cell fate decisions, differentiation, lineage-specific gene expression and plasticity of transcriptional and chromatin states. In particular, B lymphopoiesis is an excellent paradigm for the stepwise differentiation of multipotent progenitors into terminally differentiated effector cells. Differentiation of multipotent progenitors into effector lineages requires multiple changes in transcriptional and chromatin states. These include (i) multilineage priming of enhancers implicated in setting a chromatin state permissive for gene activation, (ii) expression of lineage-specific transcription factors that establish de novo accessibility of cis-regulatory elements, (iii) combinatorial action of transcription factors that form complex regulatory networks and activate lineage-specific gene programs and (iv) repression of transcriptional programs associated with alternative cell fates to stabilize lineage decisions and commit cells to a specific cell fate. We address questions of how lineage-specific transcription factors establish accessibility in naïve chromatin of hematopoietic progenitors, how specific combinations of transcription factors activate a

lineage-specific program of gene expression and how transcription factors and cis-acting sequences form regulatory networks that establish and maintain B cell identity. Other questions include the role of higher-order chromatin structure and the function of transcription factors in the regulation of stem cell pluripotency. Finally, we seek to understand which signals from stromal cells influence transcriptional determinants of B lymphopoiesis and which genes regulate functional differences between conventional and innate-like B cells.

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 cell-type-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 a subset of stromal cells in the bone marrow. Loss- and gain-of-function experiments indicated that EBF1 functions in a complex regulatory network with

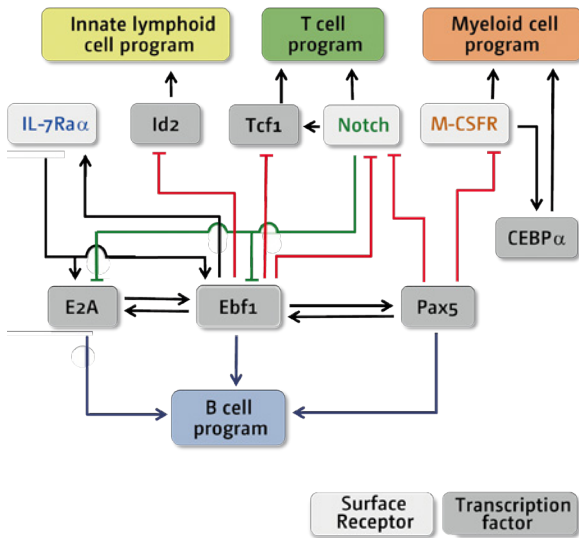


Figure 2 Schematic representation of the regulatory network in which the transcription factor EBF1 acts.

other transcription factors, in which positive feedback loops and cross-antagonism stabilize the establishment and maintenance of the B cell program. Genome-wide analysis of EBF1-bound regions and EBF1-regulated gene expression indicated that EBF1 activates genes that define the B lineage and represses genes that promote alternative hematopoietic lineages. In addition, EBF1 “poises” genes for expression at later stages by inducing histone H3K4 dimethylation. Recently, we identified a domain in EBF1 that enables binding to inaccessible chromatin (naïve state) in progenitor cells. EBF1 allows for cell fate determination by facilitating the binding of other transcription factors and the loss of DNA methylation. Moreover, we found that the maintenance of the B cell fate involves a “double lock mechanism” in which EBF1 and Pax5 repress distinct genes that mediate the permissiveness and responsiveness of cells to alternative cell fate signals.

Stem cell pluripotency & higher-order 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 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 and/or the SUMOylation of Satb2 contributes to the heterogeneity of ES cells in the expression of pluripotency genes. Satb2 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 IgH locus in Satb2-deficient pro-B cells, we anticipate to unravel the molecular basis of Satb2 function in the regulation of higher-order 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’, poly-reactive antibodies. Therefore, these cells are considered to bridge the innate and adaptive immune responses. Previously, we identified and cloned a gene, termed *Mzb1*, which is abundantly expressed in marginal zone B cells and B1 cells. *Mzb1* is an endoplasmic reticulum-localized protein that regulates antibody secretion, calcium homeostasis and integrin-mediated cell adhesion. In particular, *Mzb1* function is required under conditions of ER stress that occurs naturally during plasma cell differentiation and under conditions of DNA damage. *Mzb1* interacts with the chaperone Grp94 and seems to act as a substrate-specific co-chaperone. Current efforts focus on the mechanism by which *Mzb1* regulates functions specific to innate-like B cells.

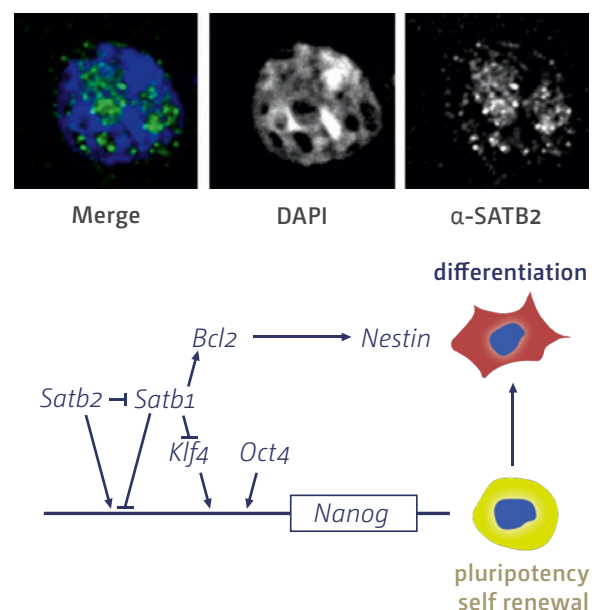
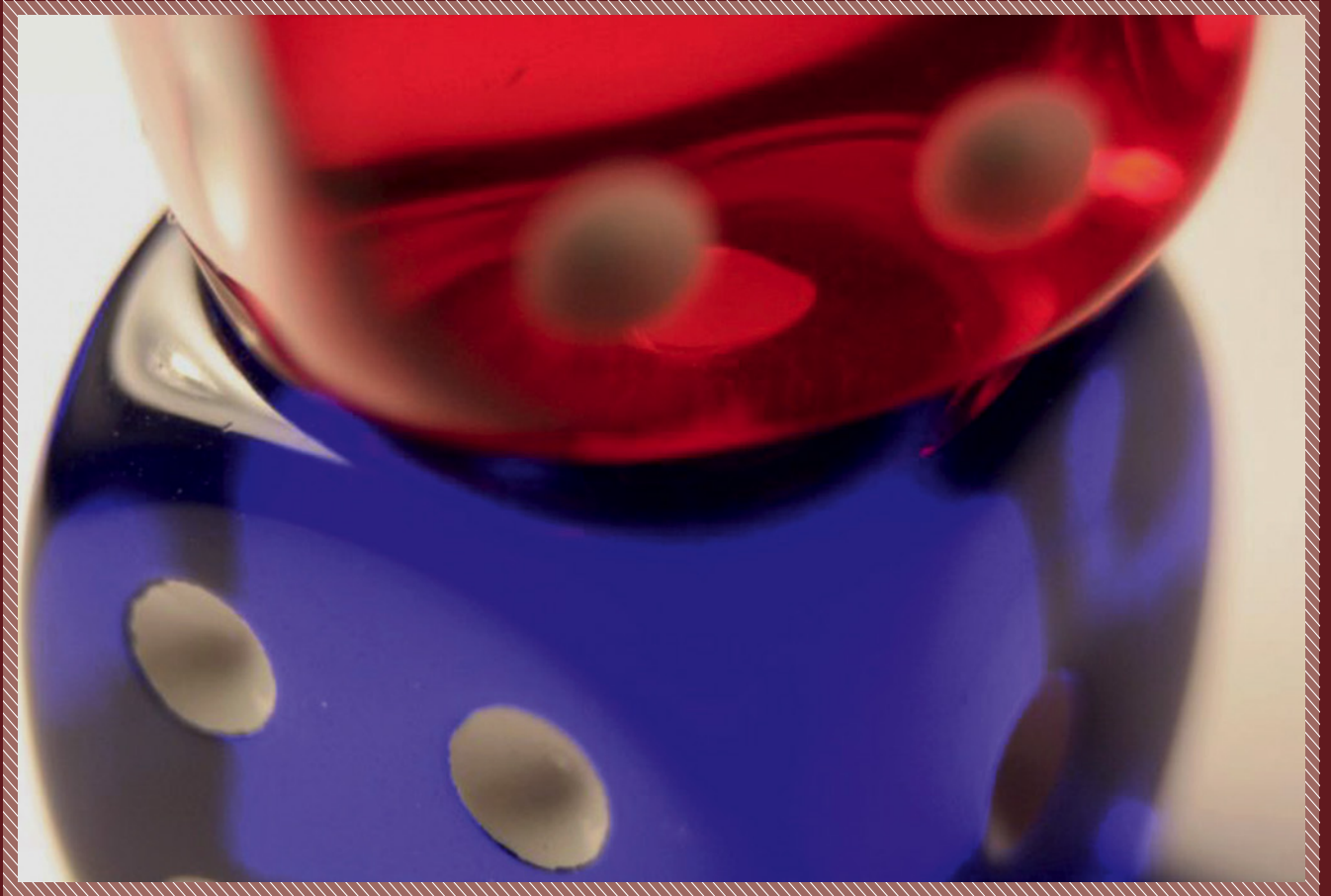


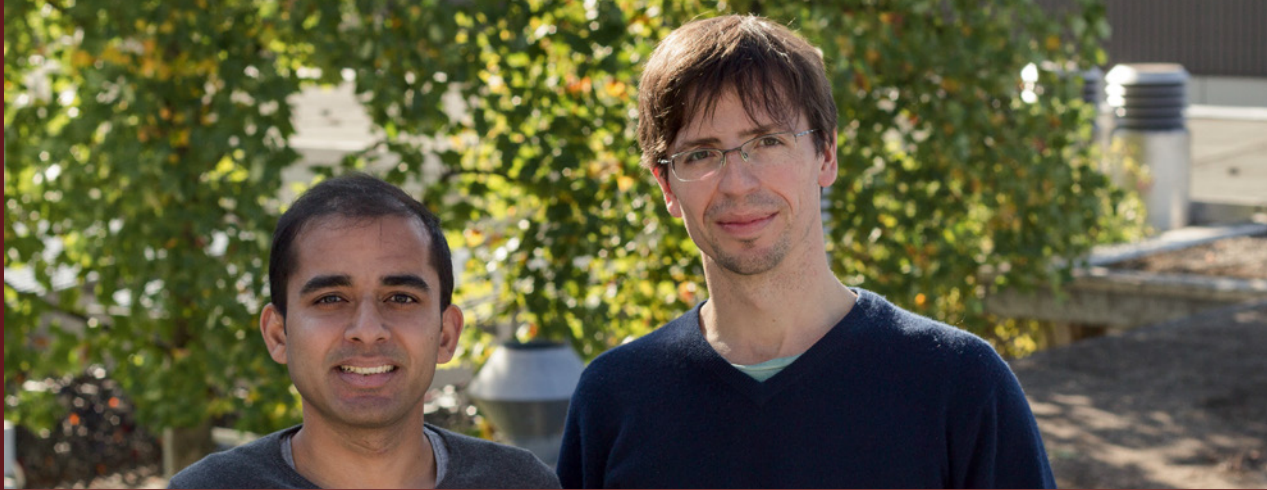
Figure 3 Subnuclear localization of Satb2 and scheme of the regulatory circuitry of Satb2 in ES cells.



🔍 LAB DOMINIC GRÜN

Do cells play dice? We are investigating the role of stochasticity of gene expression in cell fate decisions. We hypothesise that this so-called gene expression noise is utilized for the maintenance of cellular plasticity and stochastic cell fate transitions.

**Group
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Quantitative single
cell biology



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➤ **SELECTED PUBLICATIONS**

Grün D and van Oudenaarden A (2015). Design and analysis of single cell sequencing experiments. **Cell** (163), 799–810.

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Group Dominic Grün

QUANTITATIVE SINGLE CELL BIOLOGY

Investigating cell fate decisions with high resolution

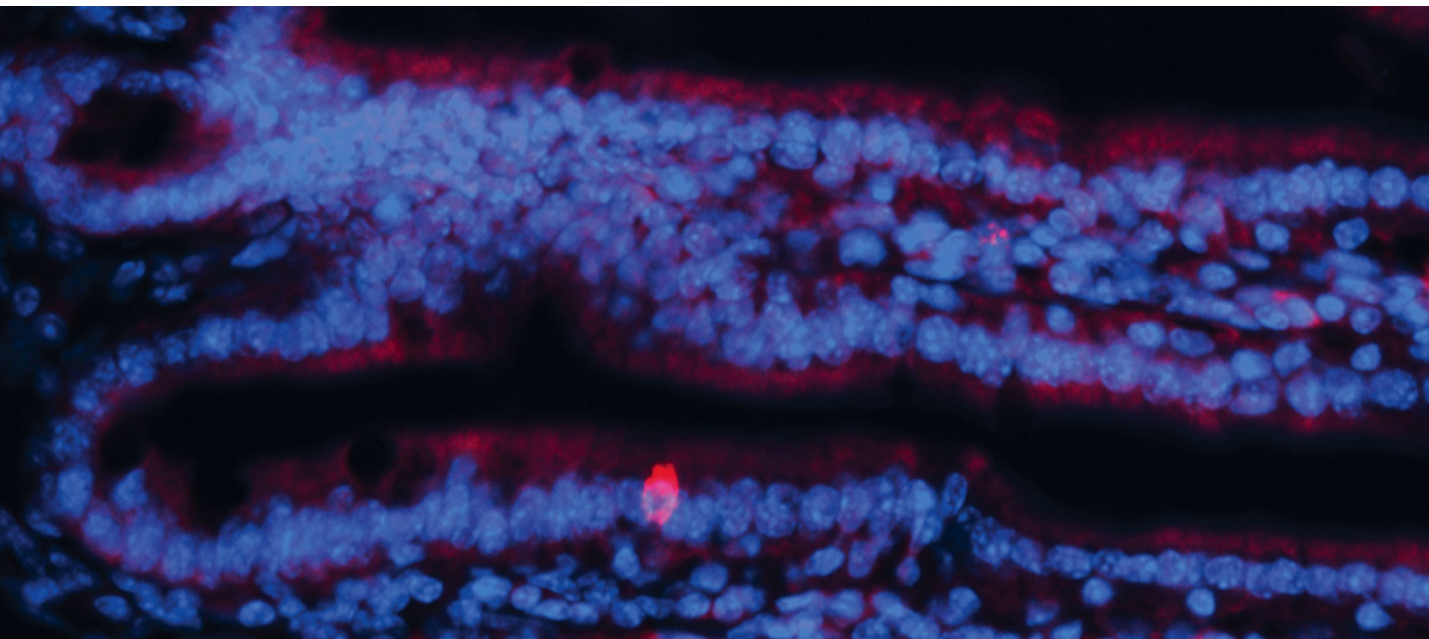


Figure 1 The image shows a staining of a marker for a rare intestinal cell type occurring at a frequency of ~1%. Single cell mRNA sequencing is a powerful tool for the detection of these rare cells.

➤ Substantial variability of mRNA levels across cells of the same type has been observed in any organism studied, ranging from bacteria and yeast to mammals. Gene expression variability can have diverse origins. Heterogeneity of cell states following extrinsic or intrinsic stimuli, e. g., due to cell cycle, metabolic fluctuations or differentiation events, can be one reason for cell-to-cell transcriptome differences. Moreover, many genes are presumably transcribed in bursts rather than at a constant rate, leading to substantially different transcript numbers in individual cells, commonly addressed as gene expression noise. Gene expression variability causes fluctuations in protein levels, and can entail physiological differences between cells.

In unicellular organisms, such as bacteria, yeast, and viruses, gene expression noise is utilized to increase heterogeneity of the population, permitting quick adjustment to fluctuating environments. In higher eukaryotes, the relevance and the regulation of gene expression noise are much less explored. Development of a multicellular organism requires robust execution of developmental transitions with spatial and temporal precision. Although the required cell differentiation events rely on coordinate execution of gene expression changes, mammalian genes have been found to exhibit strongly varying burst-

ing kinetics. The magnitude of gene expression noise in mammalian cells is comparable to unicellular systems, i.e., mRNA levels frequently differ more than twofold between identical cells. However, the role of gene expression noise and its regulation during cellular differentiation on a genome-wide level are largely unexplored.

In our lab we investigate how stem cells robustly maintain their state and reliably execute differentiation programs with spatial and temporal precision in the presence of gene expression noise. Key to this endeavor is the quantification of gene expression in individual cells. Traditional approaches based on mRNA sequencing in populations of cells mask the cell type heterogeneity, which is a particular problem for the identification of rare cell types (Figure 1).

Regulation of gene expression during cellular differentiation

To understand how gene expression is regulated during differentiation of a stem cell into all its daughter cell types one has to be able to discriminate distinct cell types and states in a complex mixture, such as a tissue or an organ. The established approach is profiling sub-populations of cells purified based on only a handful of marker genes.

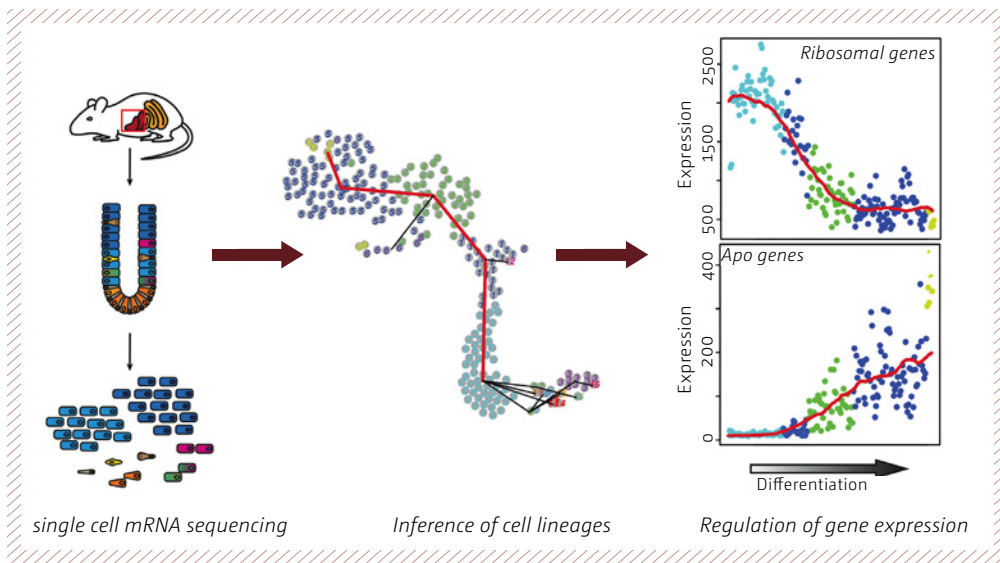


Figure 2 To understand organ differentiation cells are isolated from the tissue of interest, for example, the intestine, and profiled by single cell sequencing of all mRNAs expressed in a cell. The transcriptome of a cell can be interpreted as a fingerprint revealing its identity and computational methods are used to derive cell types and the lineage tree based on this information. This allows following gene expression dynamics on each branch of the lineage tree (example shown in red).

This pre-selection imposes strong constraints on the resolution of cell types. Moreover, measuring gene expression in populations of cells masks the true distribution of gene expression levels across cells.

Single cell profiling, on the other hand, reveals an unbiased sample of all cell types in a complex mixture. The transcriptome of a cell can be looked at as a fingerprint revealing its identity. We use single cell mRNA sequencing to investigate the transcriptome and other molecular readouts such as DNA methylation and genomic DNA of individual cells. Our lab develops computational methods to infer cell types and differentiation trajectories from these data with the goal to derive high-resolution lineage trees and to understand dynamics of gene expression during cellular differentiation (Figure 2). We are particularly interested in rare cell types, since those are oftentimes overlooked and can have crucial functions. For example, the stem cells themselves often occur at low frequencies. Our strategy will allow us to derive lineage trees de novo and to revise current models for cell differentiation in well-studied systems, such as the bone marrow. More-

over, marker genes for cell types and states can be identified with high specificity, which permits the purification of these cells and subsequent population based assays, e. g. ChiP-seq, to measure epigenetic marks and transcription factor binding. The ultimate goal is the derivation of a mechanistic model of gene regulation during differentiation by combining these population-based measurements with single cell gene expression data.

The role of biological gene expression noise during cellular differentiation

A specific focus of the lab is on the role of gene expression variability across single cells during differentiation. It has been shown that transcription is frequently not a continuous process but happens in bursts (Figure 3). This induces substantial cell-to-cell variability of mRNA levels, and the role of this so-called biological noise during cellular differentiation is not well understood.

Differentiation has to be a robust process with spatial and temporal precision and mechanisms for the control and utilization of gene expression noise are likely to exist.

Using single cell transcriptomics in conjunction with population based assays we try to elucidate how biological gene expression noise changes during differentiation and

how it is mechanistically regulated. Since single cell sequencing still suffers from substantial technical noise we also utilize microscopic imaging of individual mRNAs in single cells (single molecule FISH) to investigate gene expression variability with high specificity and sensitivity.

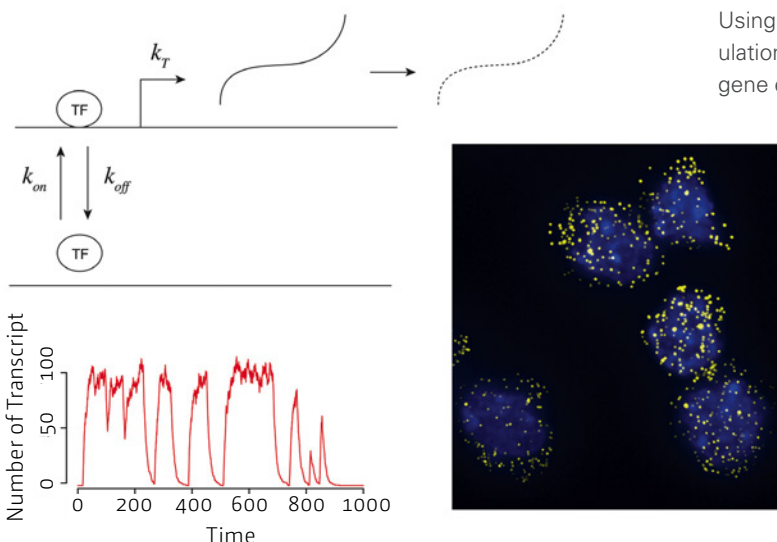
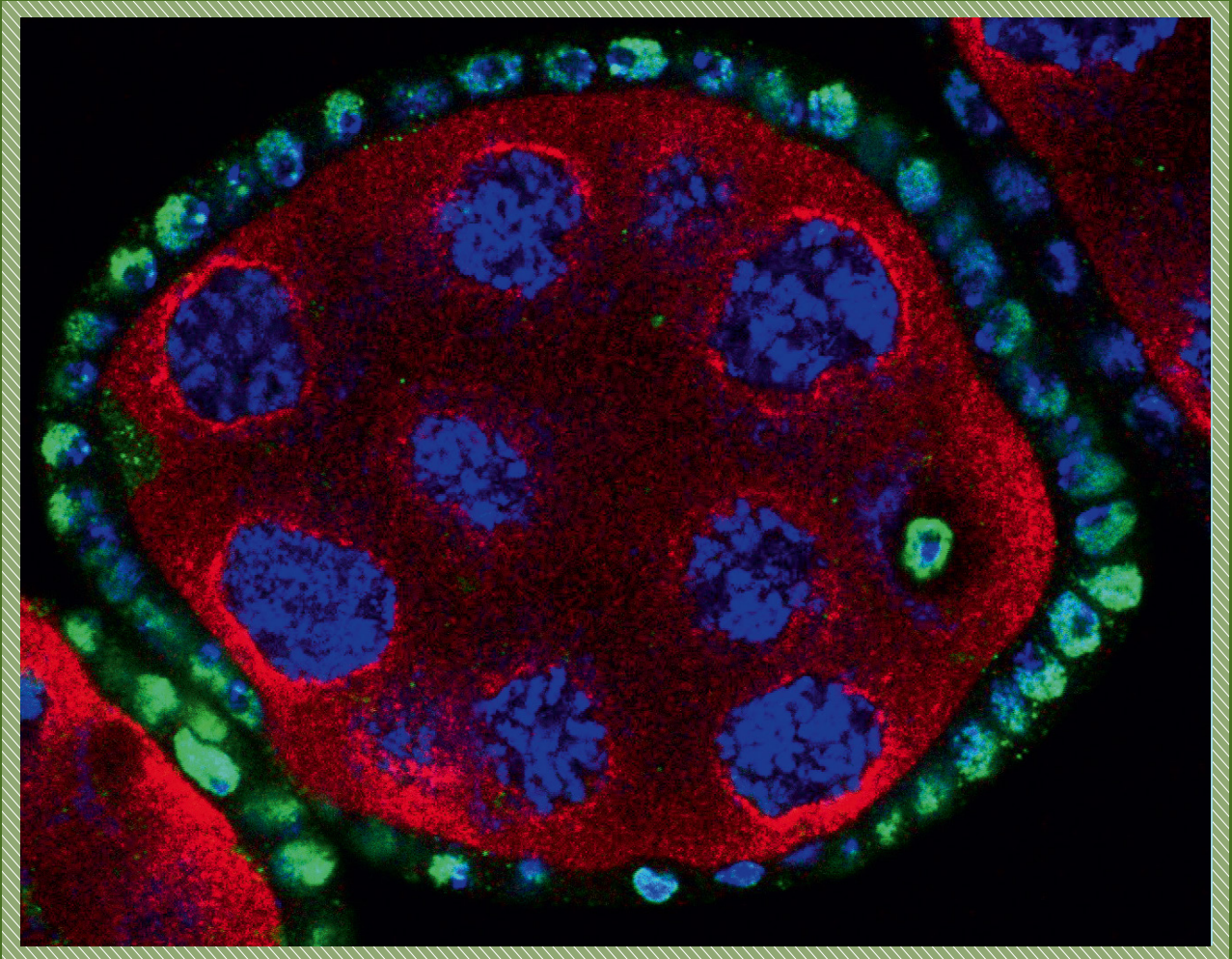


Figure 3 Gene expression noise arises from promoter switching between an active and an inactive state. Only in the active state large numbers of mRNA molecules are produced in a cell, giving rise to transcriptional bursts. We study gene expression noise with single cell mRNA sequencing and single molecule fluorescent in situ hybridization.



➔ LAB NICOLA IOVINO

Immunofluorescence image of a *Drosophila* egg chamber showing in blue a DAPI staining, in red a staining for Vasa and in green a staining for the histone modification H₃K27me₃.

Group Nicola Iovino

Epigenetic
reprogramming
and infertility



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➤ SELECTED PUBLICATIONS

Iovino N (2014). Drosophila epigenome reorganization during oocyte differentiation and early embryogenesis. **Briefing in Functional Genomics** **13**, 246-253.

Iovino N, Ciabrelli F and Cavalli G (2013). PRC2 controls Drosophila oocyte cell fate by repressing cell cycle genes. **Developmental Cell** **26**, 431-439.

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Group Nicola Iovino

EPIGENETIC REPROGRAMMING AND INFERTILITY

Epigenetic control of early embryogenesis

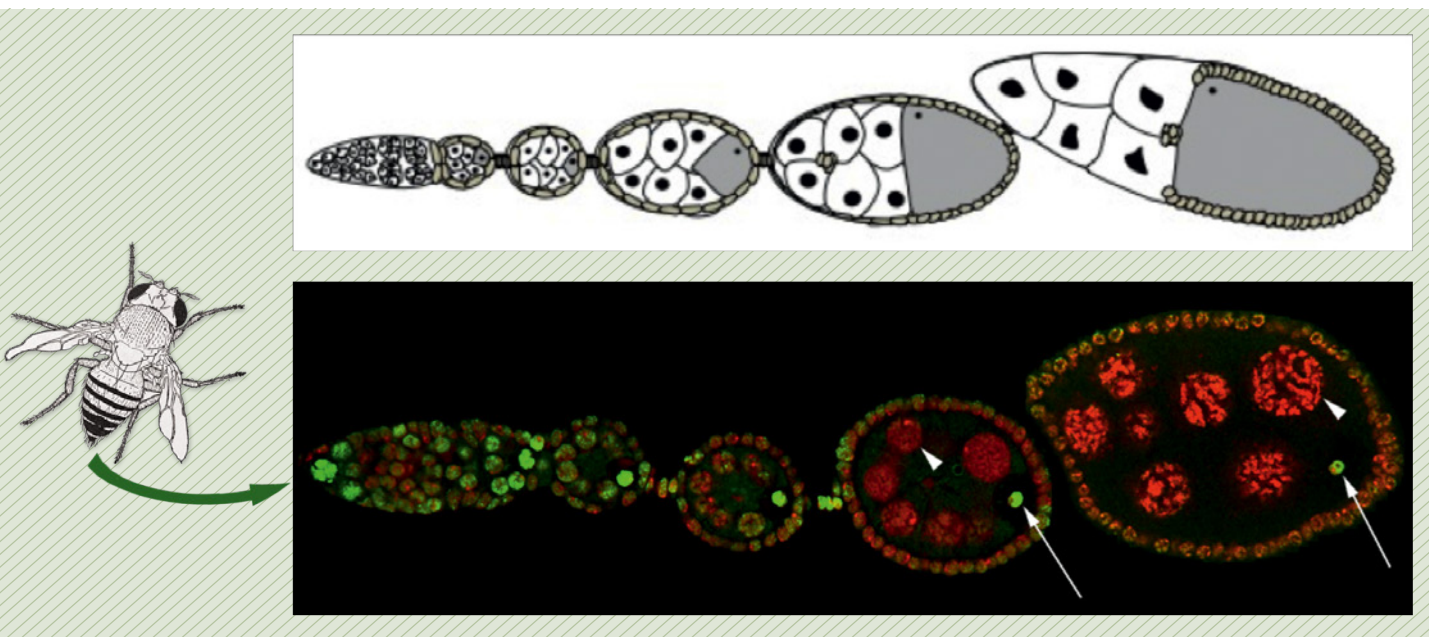


Figure 1 WT ovariole showing that H3K27me₃, a PTM associated with PRC₂, is specifically retained in the oocyte (arrow) and not in the nurse cells (arrowhead).

➤ Early embryo totipotent cells can differentiate in many cell types all sharing the same genome. How do these differentiated cells maintain their identity during development is still an open question. Memory of cell identity, reflected by the memory of transcriptional states, must be stored somewhere without affecting the primary genomic sequence. In these epigenetic phenomena, chromatin is supposed to play a central role. Moreover, a growing number of evidences suggest that stress-induced chromatin changes can be transgenerationally inherited through the germline, yet the mechanisms underlying epigenome inheritance are still unknown.

Our lab focuses on understanding the epigenetic events contributing to the formation of functional gametes, to the establishment of totipotency and to the conversion of the totipotent zygote's quiescent genome into a transcriptionally competent one. We use *Drosophila* germline and early embryogenesis as model systems and we employ functional genetics, imaging, genomics and proteomics approaches. The fundamental knowledge gained from this research will give important insights into the function of chromatin in developmental and physiological processes.

Given the evolutionary conservation of many epigenetic mechanisms between *Drosophila* and humans, understanding how epigenetic patterning occurs in the fly germline and during early embryogenesis may have profound implications for human health, for instance, for the prevention of heritable diseases, in the improvement of assisted reproductive technologies, somatic cell reprogramming and stem cell therapy.

Maternal gametes and epigenetic inheritance

Ova are generated by oogenesis in the female germline, the ovary. During the differentiation process, their chromatin undergoes profound condensation, transcriptional shutdown and reductive division to haploidy. Defects in any of these processes can lead to full sterility with complete lack of functional gametes. Chromatin condensation and transcriptional shut down in the ovum are mainly achieved through epigenetic mechanisms. Mutations in epigenetic modifiers affecting methylation of histone 3 at lysine 9 (H3K9) and at lysine 4 (H3K4) have been shown to cause defects in fertility in vertebrate and invertebrates. We have recently identified the Polycomb

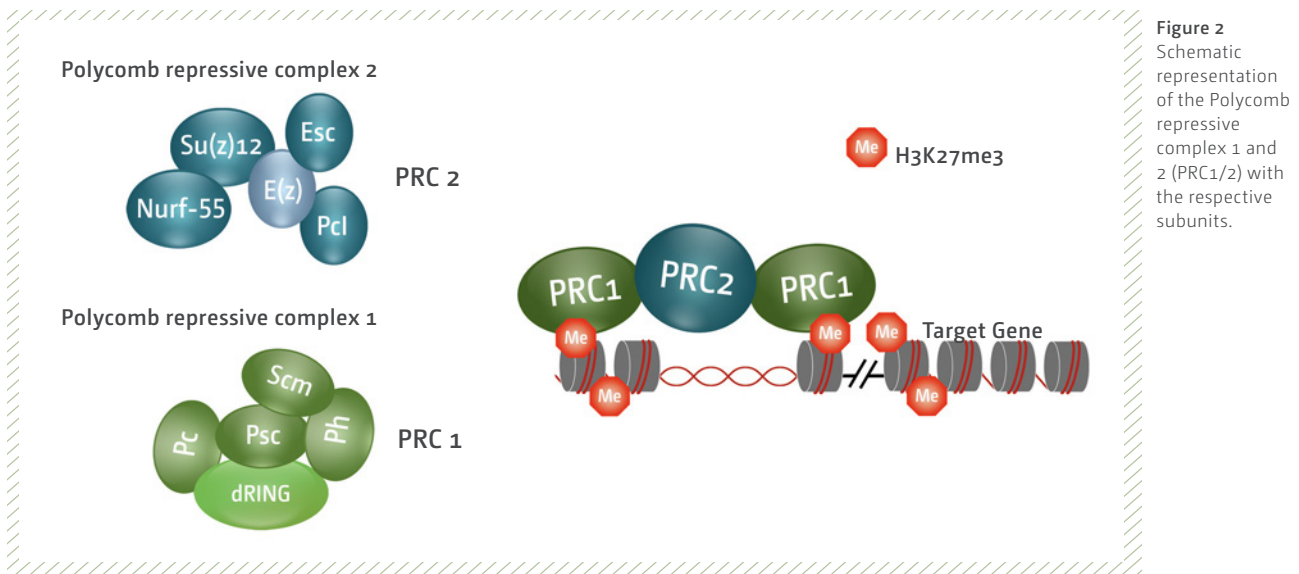


Figure 2
Schematic representation of the Polycomb repressive complex 1 and 2 (PRC1/2) with the respective subunits.

Repressive Complex 2 (PRC2) as a critical chromatin determinant of *Drosophila* oocyte cell fate (Figure 1). PRC2 is the major complex that catalyzes trimethylation of histone 3 at lysine 27 (H3K27me3), a mark usually associated with facultative heterochromatin and transcriptional repression (Figure 2). In the absence of PRC2 components, the oocyte undergoes a trans-determination process and becomes a polyploid cell. The resulting lack of a functional haploid gamete causes full sterility. Our lab is now currently exploring the role of this complex in controlling post-meiotic gametes transcription and its role in fertilization.

Paternal gametes, epigenetic inheritance and reprogramming

Male germ cells also undergo a radical transformation as they progress through spermatogenesis. In particular, they switch from a nucleosome to a mainly protamine-based chromatin structure that enables the male genome of the sperm to be deprogrammed and main-

tained in a quiescent state until it enters the oocyte. Protamines are small proteins rich in cysteine and the basic amino acids arginine, lysine and histidine. Protamines bind tightly to the phosphate backbone of DNA using the arginine-rich domain as an anchor and have intra and intermolecular disulfide bonds that contribute to the high degree of chromatin condensation and DNA stabilization of the sperm.

Upon fertilization, the sperm re-acquires a nucleosome-based structure in the male pronucleus and fuses with the female pronucleus. Although this series of events has been well characterized by light and fluorescence microscopy, very little is known at the mechanistic level about the early events that contribute to the unpacking of the chromatin of the sperm.

We are currently undertaking an *in vivo* reverse genetic screen aiming at identifying epigenetic factors required for paternal gamete reprogramming. We identified so far several factors that when mutated cause full infertility due to paternal gamete defects and we are currently dissecting the mechanistic function of these factors (Figure 3). ◀

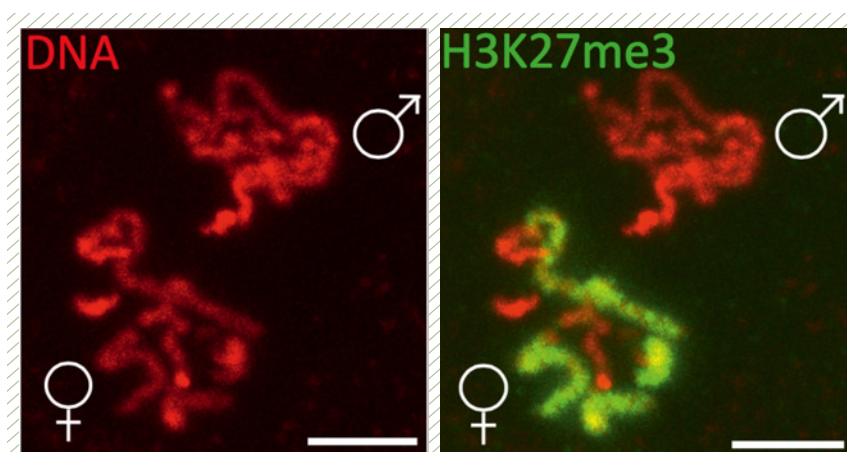
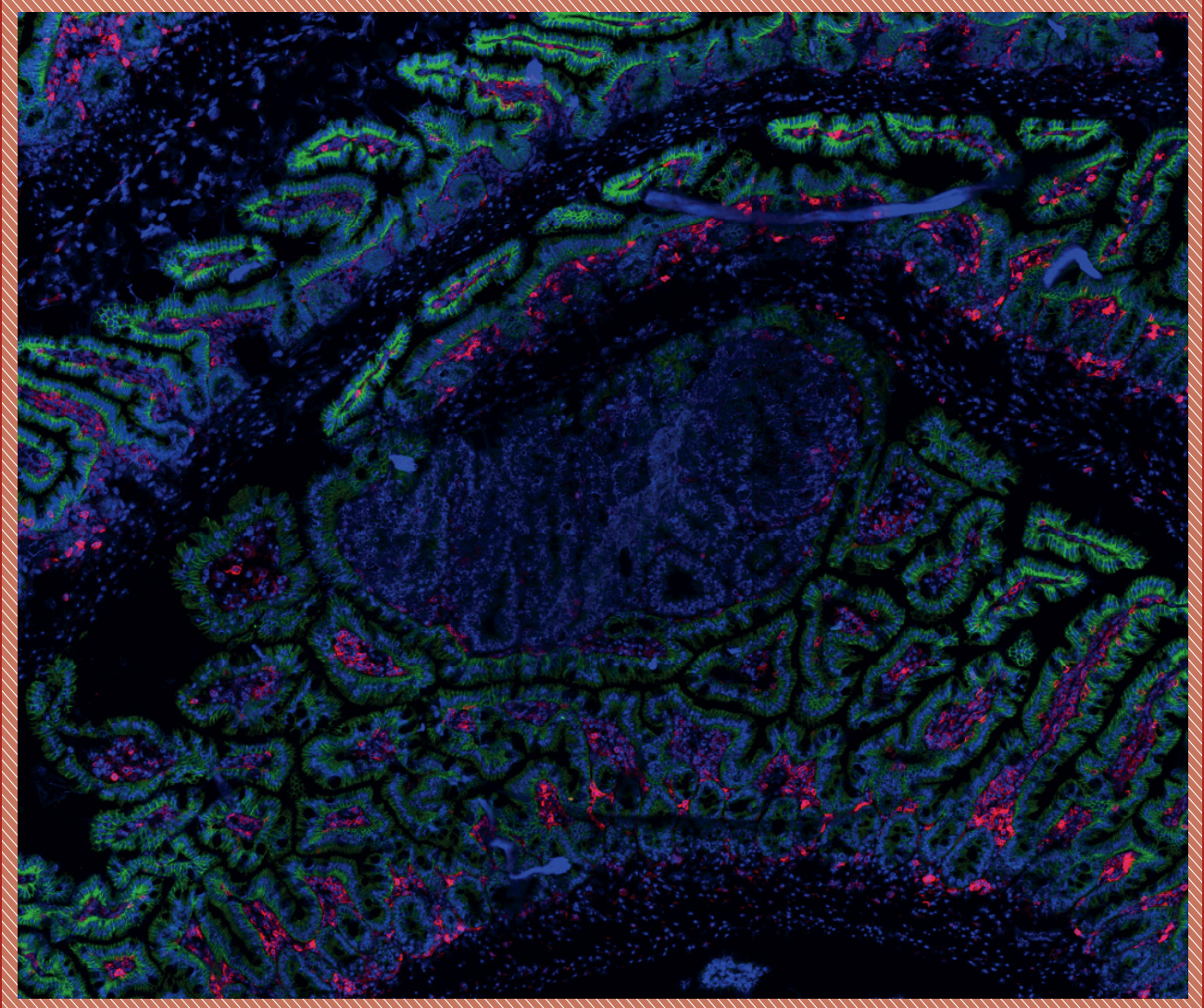


Figure 3 *Drosophila* early embryo nucleus showing asymmetric distribution for H3K27me3 between paternal (upper right) and maternal (lower left) chromosomes.



➤ LAB ANA IZCUE

Tumor in the intestinal immune environment. Blue: cell nuclei; green: E-cadherin as marker for normal epithelial cells; Red: CD45 as marker for immune cells.

Group Ana Izcue

Mechanisms of T cell tolerance



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➤ SELECTED PUBLICATIONS

Lupar E, Brack M, Garnier L, Laffont S, Rauch KS, Schachtrup K, Arnold SJ, Guéry JC and Izcue A (2015). Eomesodermin expression in CD4⁺ T cells restricts peripheral Foxp3 induction. **The Journal of Immunology** **195**, 4742-52.

Bremser A, Brack M and Izcue A (2015). Higher Sensitivity of Foxp3⁺ Treg Compared to Foxp3⁻ Conventional T Cells to TCR-Independent Signals for CD69 Induction. **PLoS One** **10(9)**, e0137393.

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Group Ana Izcue

MECHANISMS OF T CELL TOLERANCE

CD4⁺ Treg and tolerance to self- and exogenous antigens

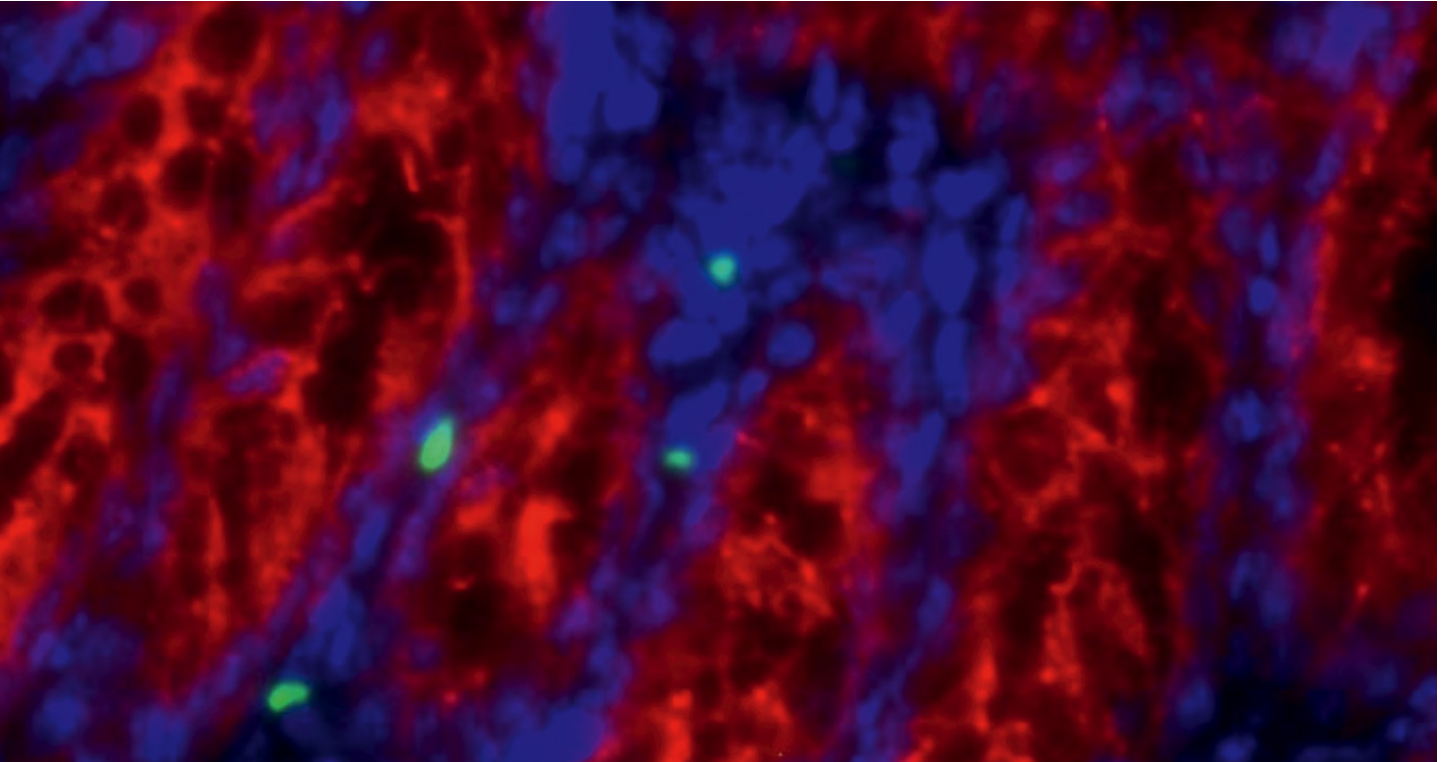


Figure 1 Foxp3⁺ T cells (Treg) in a section of mouse colon. Blue: cell nuclei; Red: E-cadherin as marker for epithelial cells; Green: Foxp3 as marker for Treg cells.

➤ 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 to 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) (Figure 1). 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 like 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 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. Understanding this crosstalk is crucial to understand and treat inflammatory bowel diseases, but it is also key for anti-cancer treatments. Indeed, it is known that Treg accumulate

in tumors and tumor-associated structures, where they are able to prevent anti-cancerous immune responses. We have found that epithelial changes that happen when normal epithelium turns cancerous promote the local accumulation of a subset of tissue-specific Foxp3⁺ regulatory T cells in the gut. This is true in mouse models where gut epithelial cells are modified, but no signs of tumors are visible, as well as in a mouse model of spontaneous intestinal tumor development. The identified pathways are conserved in mouse and humans, suggesting that this mechanism of Treg-mediated tumor protection may also be active in cancer patients. Our group aims to characterize the molecules mediating this interaction, which could prove a useful target in anti-cancerous therapy.

Control of immune responses in aged individuals

Immune responses become less efficient with age – this is a fact for human and mice. Among the factors involved in age-related immunodeficiency is the accumulation of Foxp3⁺ regulatory T cells with age. We have shown that mice lacking a certain transcription factor (Eomes) have a much stronger accumulation of Treg with age compared to normal mice (Figure 2). This observation indicates that under normal conditions Eomes restricts age-related Treg accumulation. We could prove that Eomes also reduces the induction of Treg from naive CD4⁺ T cells during immune responses. Based on this fact, we propose that immune activation during life

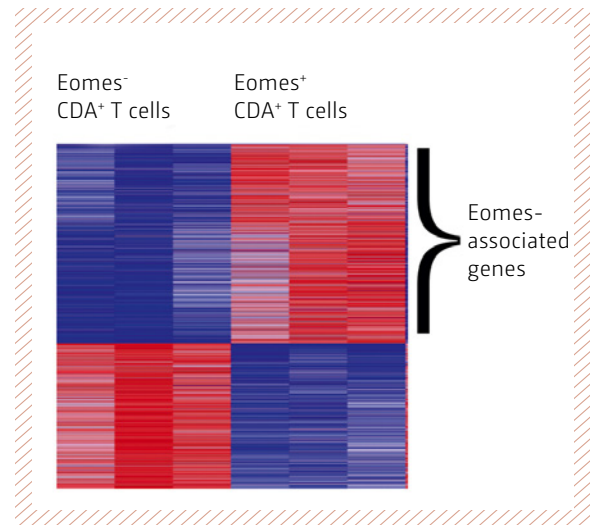


Figure 3 Comparison of the transcriptome of Eomes-expressing and Eomes non-expressing CD4⁺ T cells reveals genes associated with Eomes expression.

causes the accumulation of memory but also of anti-inflammatory lymphocytes that can lead to blunted immune responses in old age. The transcription factor Eomes is part of the mechanisms that limit the induction of Treg to ensure effective immune responses during the whole life span of an individual.

The question then arises whether this function of Eomes may play a role in human disease. In patients, the *EOMES* gene has been associated with susceptibility to multiple sclerosis. In collaboration with the group of JC Guery, we have shown that Eomes reduces the ability of T cells to prevent neuronal inflammation in a mouse model of multiple sclerosis. Hence, while Eomes may be useful in preventing age-related immunodeficiency, it can also be detrimental when immune tolerance is required, such as during autoimmune disease. Our studies focus now on the factors inducing Eomes expression and the function of Eomes-expressing CD4⁺ T cells. The analysis of genes specifically expressed by Eomes⁺ CD4⁺ T cells (Figure 3) will be instrumental to understand the function of these cells.

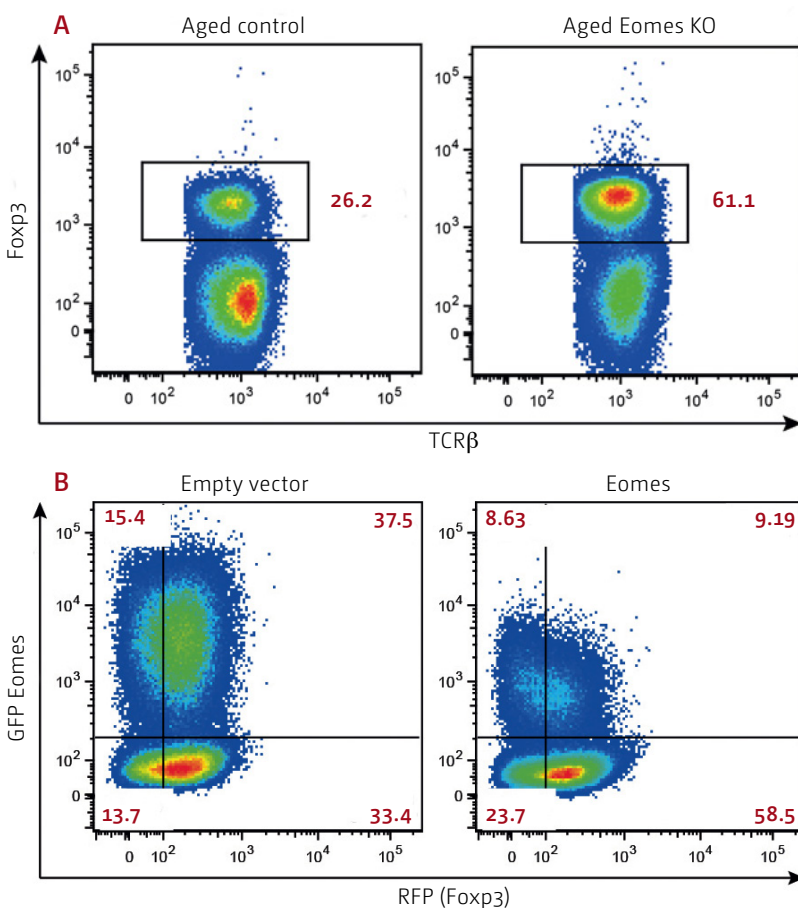
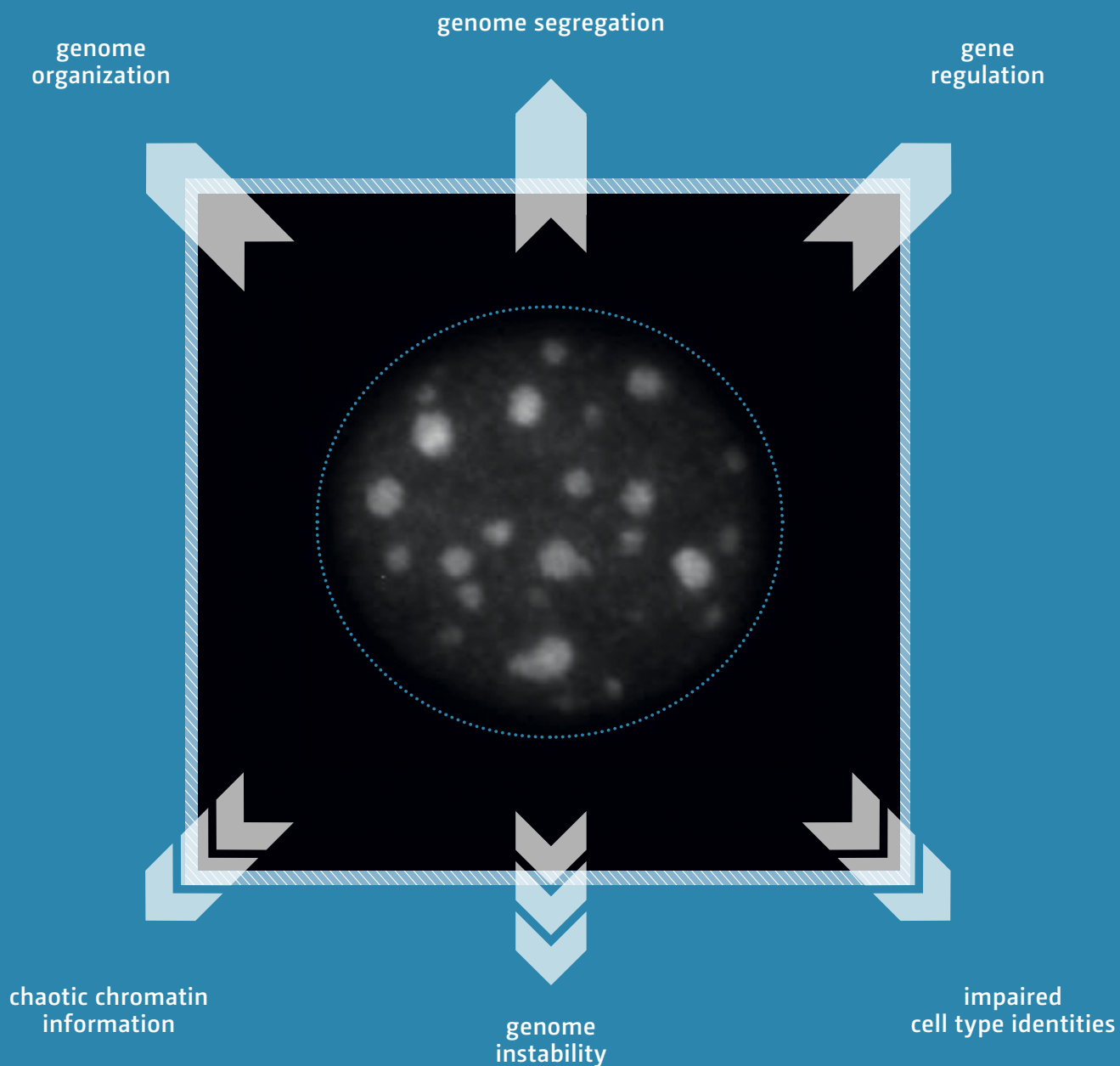


Figure 2 Eomes counteracts the appearance of Foxp3⁺ regulatory T cells.

A Eomes limits the age-related accumulation of Treg cells. Flow cytometry analysis of CD4⁺ splenic lymphocytes showing Foxp3 expression together with the T cell receptor (TCR) in aged control mice (left) or mice of the same age that lack Eomes in T cells (right). In the latter, more than half of the cells are anti-inflammatory cells.

B Eomes directly inhibits Foxp3 induction. Flow cytometry analysis shows that transduction with Eomes (right) reduces the transcription of a reporter for Foxp3 (RFP) when compared with transduction with a control empty vector (left).



⇒ LAB THOMAS JENUWEIN

The image shows the characteristic heterochromatic foci in a somatic mouse nucleus, as visualized by DAPI staining of the A/T-rich major satellite repeats. Intact heterochromatin (Top) has important functions in genome organization, chromosome segregation and gene regulation. Defective heterochromatin (Bottom) results in chaotic chromatin information, genome instability and impaired cell type identities.

Senior Group Thomas Jenuwein

Epigenetic Control
by Histone Lysine
Methylation



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➤ SELECTED PUBLICATIONS

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Senior Group Thomas Jenuwein

EPIGENETIC CONTROL BY HISTONE LYSINE METHYLATION

Establishment and maintenance of mammalian heterochromatin

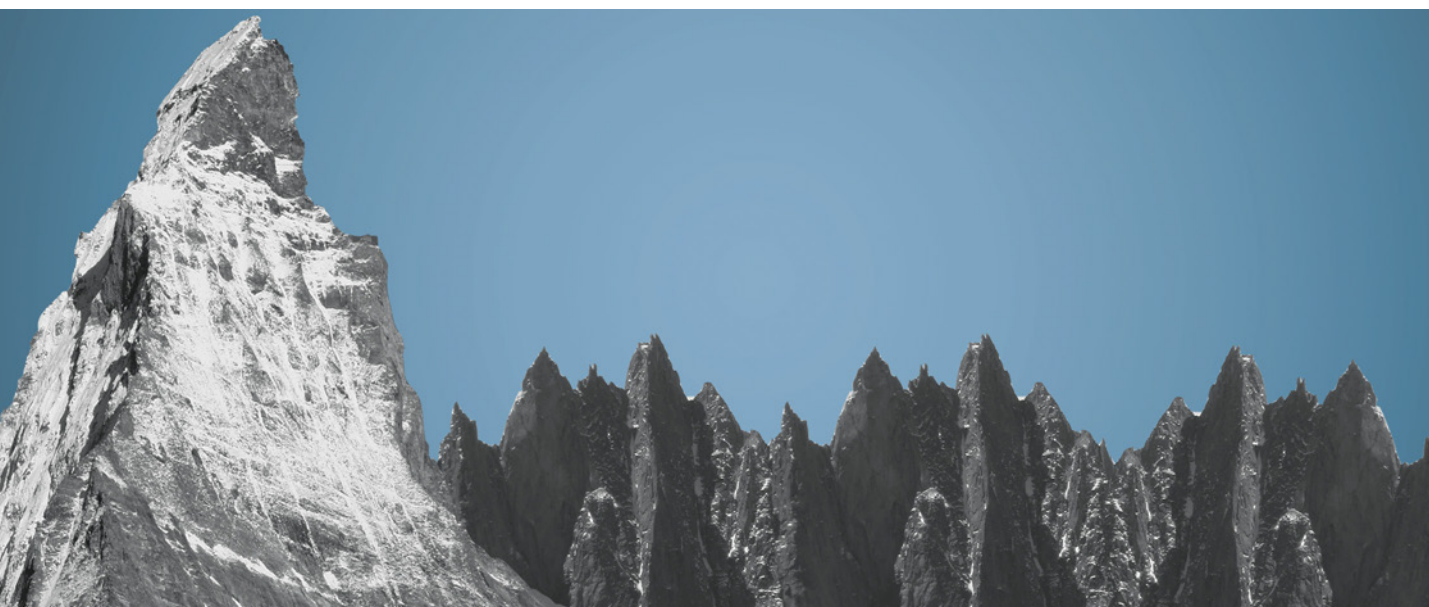


Figure 1 A transcription factor (TF) based model to discriminate euchromatin from heterochromatin is illustrated in analogy to Alpine mountains. While the Matterhorn resembles the pronounced synergy of TF binding found at promoters, the Aiguilles of the Mont Blanc massif are reminiscent of the reiterated TF binding within heterochromatic repeat sequences.

➔ In the nuclei of almost all eukaryotic cells genomic DNA is compacted with histone and non-histone proteins in a dynamic polymer called chromatin. Several epigenetic mechanisms, such as nucleosome remodeling, histone modifications, DNA methylation and non-coding RNA function together to organize chromatin into accessible (euchromatic) and inaccessible (heterochromatic) domains. We discovered the Suv39h enzymes as the first histone lysine methyltransferases (KMT) and have shown that Suv39h-dependent histone H3 lysine 9 (H3K9me3) methylation is a central epigenetic modification for a repressed chromatin state at heterochromatic regions. The goal of our research is to further dissect epigenetic gene regulation and to identify the underlying mechanisms that initiate and maintain heterochromatin in mammalian cells. Since heterochromatin has important functions in safeguarding genome integrity, in silencing of endogenous retroviruses and in stabilizing gene expression programs, our research is of crucial importance for a better understanding of normal and perturbed development and for new insights to protect from disease progression.

Initiation of heterochromatin by H3K9me1

The Suv39h KMT catalyze H3K9me3 at pericentric heterochromatin. Mouse embryonic fibroblasts (MEF) that are deficient for both Suv39h1 and Suv39h2 enzymes

(*Suv39h* dn cells), display impaired pericentric heterochromatin and chromosome segregation defects. These cells have lost most H3K9me3 methylation and instead accumulate H3K9 mono-methylation (H3K9me1) at pericentric heterochromatin. These data indicate that H3K9me1 partially maintains heterochromatin function and that there are other KMT that selectively induce H3K9me1.

H3K9me1 is present in the cytoplasm and the nucleus. Available data are consistent with a model to propose H3K9 mono-methylation in the cytoplasm, transport of H3K9me1 containing histone H3-H4 dimers to the nucleus, their incorporation into nucleosomes and the conversion of nucleosomal H3K9me1 to H3K9me3 by the Suv39h enzymes. Subsequently, H3K9 methylated heterochromatin is anchored to the nuclear lamina, which allows the organization of clustered heterochromatic foci. We will dissect the genetic and biochemical mechanisms that initiate H3K9me1-dependent heterochromatin by generating compound mutants for several H3K9 KMT (e.g. SetdB1/Eset and members of the Prdm family) in MEF and by identifying novel H3K9me1 binding factors.

Heterochromatin formation by DNA repeat sequences

Pericentric heterochromatin is characterized by its underlying repetitive DNA sequences, which in the mouse primarily comprise the major satellite repeats (MSR). The basic



unit of the MSR sequences is a 234 bp DNA element that is full of embedded transcription factor (TF) binding sites. We have identified the Pax3 and Pax9 TF as redundant regulators of mouse heterochromatin. Simultaneous depletion of Pax3 and Pax9 resulted in dramatic derepression of MSR transcripts, persistent impairment of heterochromatic marks and defects in chromosome segregation. Genome-wide analyses for Suv39h-dependent H3K9me3 showed enrichment at intergenic MSR only when these sequences retained intact binding sites for Pax and other TF. These data defined a general model in which reiterated arrangement of TF binding sites within repeat sequences is an intrinsic principle for the definition of heterochromatin (Figure 1).

Nearly half of the mammalian genome consists of repetitive elements. There are > 1.8 million of long intergenic nuclear elements (LINE) and endogenous retroviruses (ERV) in the mouse genome. However, only the ~ 70,000 transcriptionally competent LINE and ERV repeats with an intact regulatory unit at the 5' end are decorated with Suv39h-dependent H3K9me3 (Figure 2). This result confirms our previous conclusion, that only repeat elements with intact TF binding sites and the potential to become transcriptionally active will be targets for Suv39h-mediated repression. These data provide a novel function for the Suv39h KMTs in silencing intact LINE elements in mouse ES cells and reveal that only transcriptionally competent repeat elements (less than 5% of all the repeat elements in the mouse genome) are under epigenetic control. We will now reconstruct heterochromatin formation by CRISPR/Cas9 mediated insertion of repeat elements into inert genomic regions of the mouse genome. We will also disrupt distinct TF that have confirmed binding sites in the consensus MSR sequence. These analyses will allow the definition of functional TF binding sites within the MSR and uncover novel TFs that can direct the formation of mouse heterochromatin.

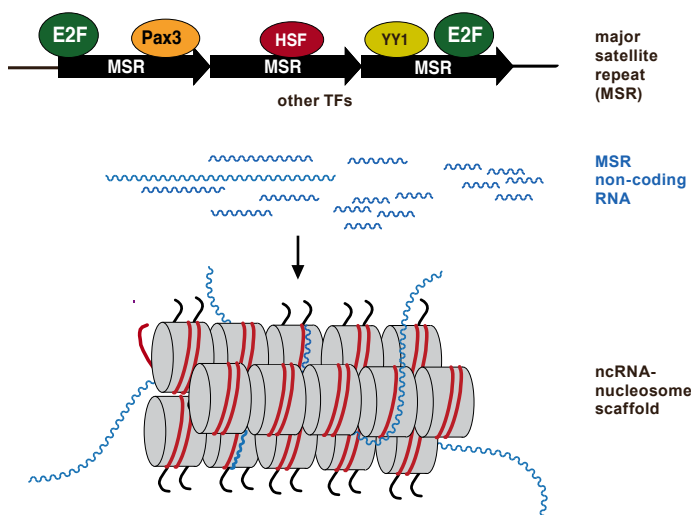


Figure 3 Major satellite repeat transcripts organize an RNA-nucleosome scaffold at mouse heterochromatin. Model depicting a higher-order RNA nucleosome scaffold that is formed by chromatin association of major satellite repeat (MSR) transcripts. Also shown are several TF that have known binding sites in the MSR.

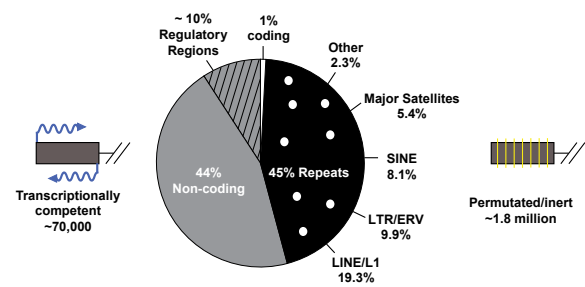


Figure 2 Only transcriptionally competent repeat elements in the mouse genome define epigenetic response. Pie chart showing that nearly half of the mouse genome consists of repeat elements. A transcriptionally competent repeat regulatory unit (tracRRU) with functional TF binding sites (e.g. 5'UTR of LINE or 5'LTR of ERV) is illustrated to the left. The great majority of repeat elements are inert DNA relics that have either lost their repeat regulatory units or contain highly permuted RRU, as indicated to the right.

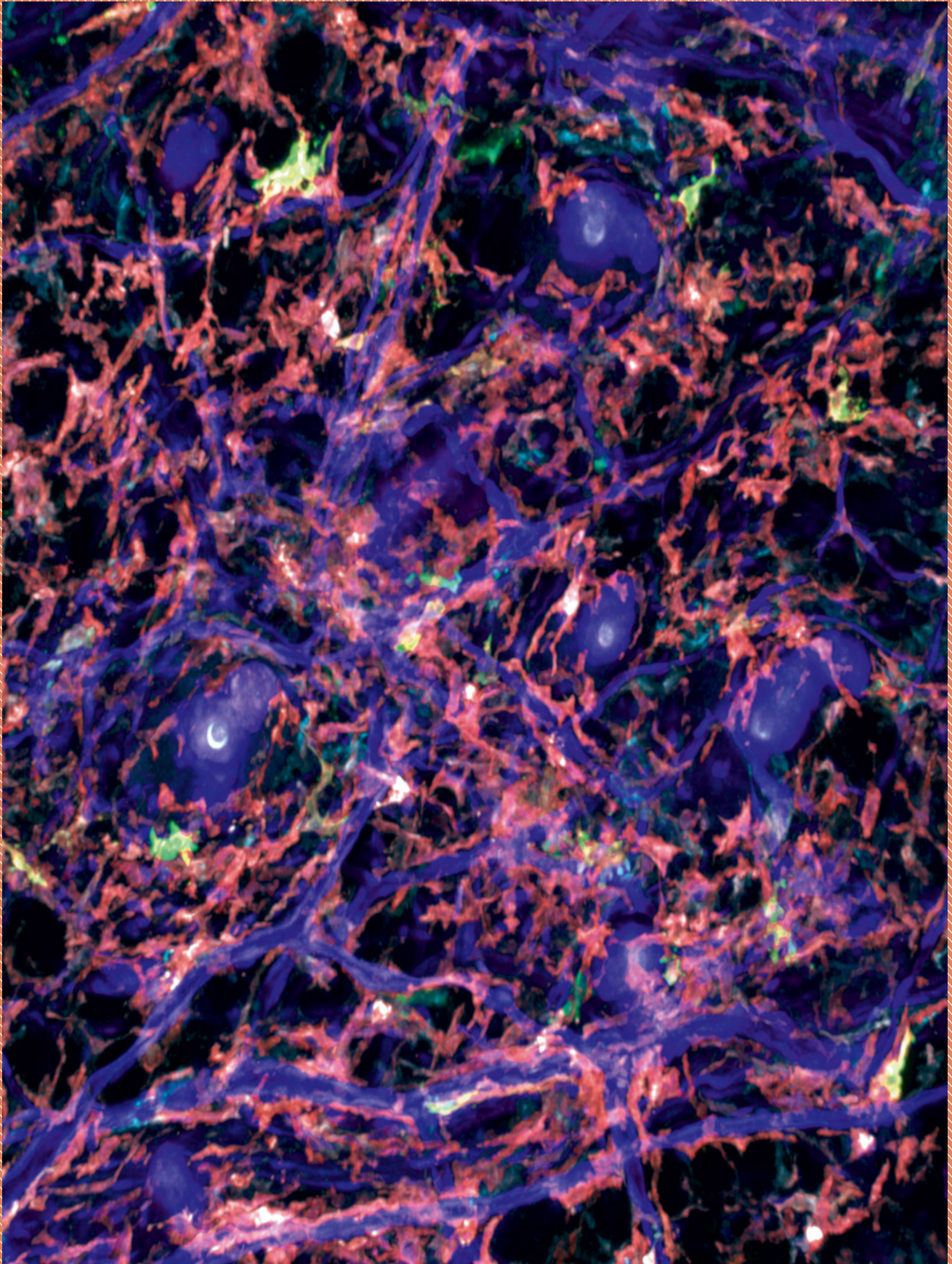
Repeat RNA organize a heterochromatic RNA-nucleosome scaffold

Another hallmark of heterochromatic regions is the occurrence of non-coding RNA, which originate from the repeat sequences. Attenuation and subsequent silencing of the initial repeat-derived transcription appears crucial for heterochromatin formation, as the absence or the excess of transcriptional activity fails to establish and maintain heterochromatic marks. Repeat-derived non-coding RNA could therefore facilitate recruitment of chromatin factors by serving as guide RNA and/or constituting a structural component of a distinct heterochromatin configuration. We investigated chromatin association of MSR transcripts at native nucleosomes that were prepared by micrococcal nuclease (MNase) digestion from mouse ES cells. RNA preparations that were purified from MNase-processed chromatin display significant sensitivity towards RNaseH, when they are examined with MSR-specific DNA probes. In addition, the exclusive association of Suv39h enzymes with poly-nucleosomal fractions was attenuated upon RNaseH incubation and entirely lost upon RNase digestion of the MNase-processed input chromatin. These data reveal RNA:DNA hybrids and other RNA components to be important for the recruitment of the Suv39h KMT and suggest that a higher-order RNA-nucleosome scaffold is the physiological template for the stable association of Suv39h enzymes to chromatin (Figure 3).

We are now extending these studies to examine secondary structures of MSR transcripts that allow the formation of RNA:DNA hybrids or fold into distinct stem loops for the possible RNA interaction of chromatin factors. Further, repeat-rich transcripts could also invade a DNA double helix and induce a DNA:DNA:RNA triple helix. Such non-canonical nucleic acid structures are poorly defined in vivo and require Hoogsteen bonds that are favored by 5-methyl cytosine DNA (or RNA) methylation. We will therefore also investigate 5-meC RNA methylation of repeat-derived transcripts. These approaches aim to identify non-canonical nucleic acid structures and 5-meC RNA methylation as novel epigenetic modifications for the organization of mammalian heterochromatin. ◀

➤ LAB TIM LÄMMERMANN

Innate immune cells as sentinels of our body periphery



Group Tim Lämmermann

Cellular dynamics
of innate immune
responses



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➤ SELECTED PUBLICATIONS

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Group Tim Lämmermann

CELLULAR DYNAMICS OF INNATE IMMUNE RESPONSES

Signals guiding immune cell movement and positioning

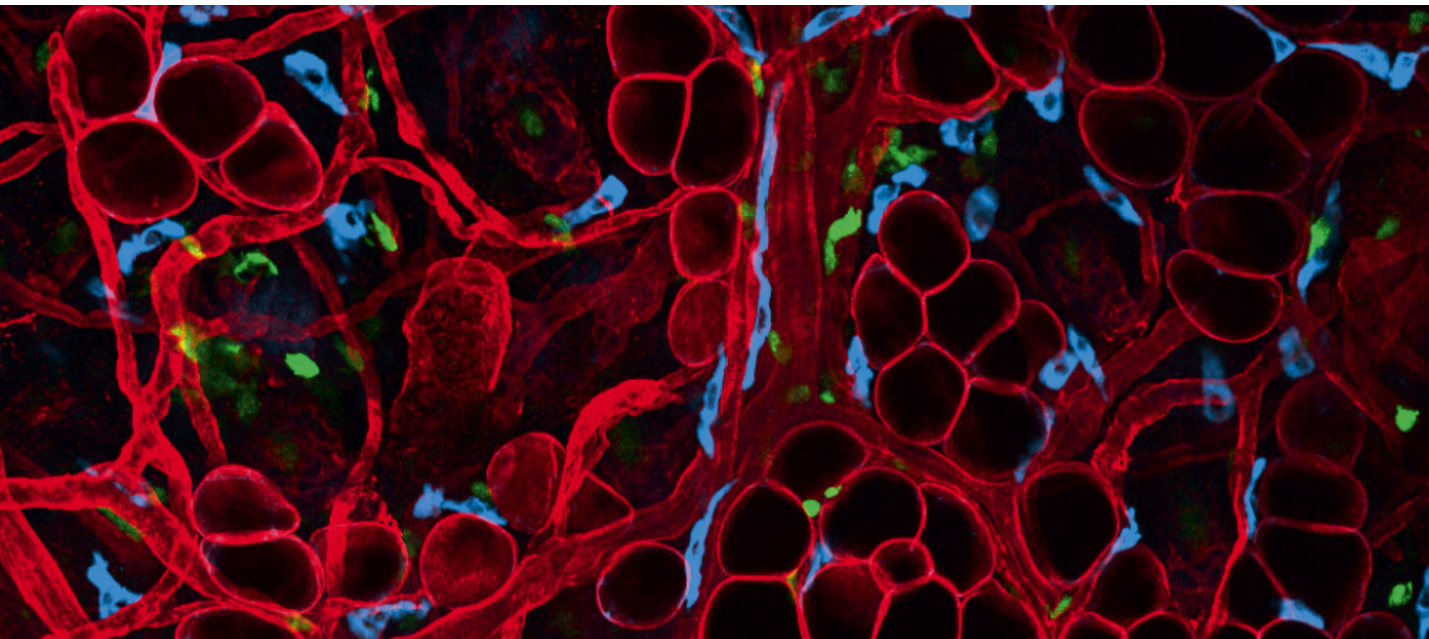


Figure 1 Strategically positioned immune cells in the heterogenous and complex tissue environment of the skin dermis, immunofluorescence staining of skin whole mount tissue.

➤ Immune responses are highly dynamic and require coordinated migration, tissue positioning and interactions of immune cells possessing diverse effector functions. Most cells of the immune system stand in contrast to other body cell types owing to their extremely fast movement and capacity to invade and migrate within diverse organs. In particular, within minutes of tissue damage or incipient infection, cells of our innate (non-specific) immune system undertake a coordinated, multi-cellular and multi-layered response to isolate sites of tissue damage and microbial invasion from healthy tissue. Phagocytes (neutrophils, macrophages, monocytes) are the major cell types involved in this front line of immune defense and *in vitro* studies have identified many factors that guide phagocyte migration and regulate their effector functions. While we have learnt from these *in vitro* studies how defined stimuli can alter immune cell function, we are only starting to discover how phagocytes integrate the plethora of signals arising in inflamed tissues to coordinate their dynamic behavior in physiologically complex *in vivo* settings. Our research addresses how immune cells coordinate and integrate multiple basic cell biological processes (directional sensing, cell polarization, cell adhesion, cell migration, phagocytosis, cell death, cell survival, cell-cell communication) that together shape the dynamic

immune response in complex tissue environments. We aim at gathering new insights how innate immune cells (a) sense, detect and eliminate damage in the tissue, (b) communicate with each other for optimal coordination of the innate immune response during wounding, inflammation, infection and anaphylaxis, and (c) strategically position themselves to initiate immune responses. Since most of our models are also applicable to human primary phagocytes, our new insights on the molecular regulation of phagocyte dynamics also promise to contribute new therapeutic strategies to modulate immune responses.

Neutrophil swarming at local sites of inflammation and infection

Neutrophils are indispensable effector cells of our innate immune response and regulators of adaptive immunity. As classic phagocytic cells, they engulf pathogens, release lytic enzymes from their granules, produce reactive oxygen species and are hence pivotal for clearing bacterial and fungal infections. Not only are neutrophils key cells for protecting the host from microbial invasion, but they also act as critical mediators of sterile inflammation in acute and chronic diseases. Upon local inflammation or infection, neutrophils undergo phases of highly directed

and coordinated migration, followed by neutrophil accumulation at sites of tissue injury or infection, a process termed neutrophil „swarming“. Neutrophils have evolved as true sentinel cells for detecting sites of tissue damage, but the molecular guidance signals that control neutrophil swarming *in vivo* have long remained unclear. In higher vertebrates, they express more than 30 cell surface receptors and are responsive to various chemically diverse chemoattractants including chemokines, lipids, complement factors, proteolytic ECM fragments, attractant alarmins, nucleotides or formylated peptides. Given that a mixture of various chemotactic factors is commonly present at sites of inflammation and infection, chemoattractants have long been considered overlapping in their function and redundant in guiding neutrophil migration. By using intravital two-photon microscopy, we could recently define a multistep attraction cascade that guides neutrophils at sites of local sterile injury (Figure 2) and identified key molecules controlling individual phases of the swarming response. One of our major findings revealed a critical role for intercellular communication among neutrophils mediated by the lipid leukotriene B4 (LTB4) of the swarming response, which acutely amplified local cell death events to enhance the radius of neutrophil recruitment within the tissue. When neutrophils accumulate and form cell clusters (mini-abscesses) at sites of tissue damage, adhesion receptors in concert with LTB4 and other chemoattractants promoted forming a tight wound seal. While our previous work provided an initial molecular map for neutrophil swarm formation, we are currently investigating the exact molecular mechanisms how the swarming response is initiated and also how immune cell clusters dissolve in the resolution phase of inflammation.

Positioning & migration patterns of other phagocyte types

For an optimal innate immune response, a variety of phagocytic cell types with diverse effector functions ex-

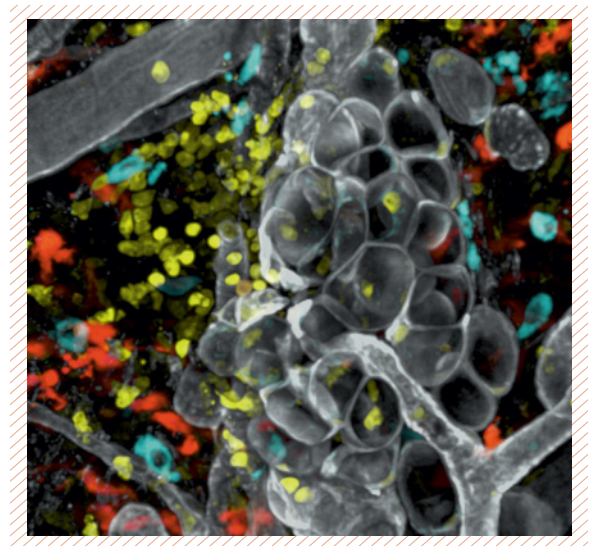
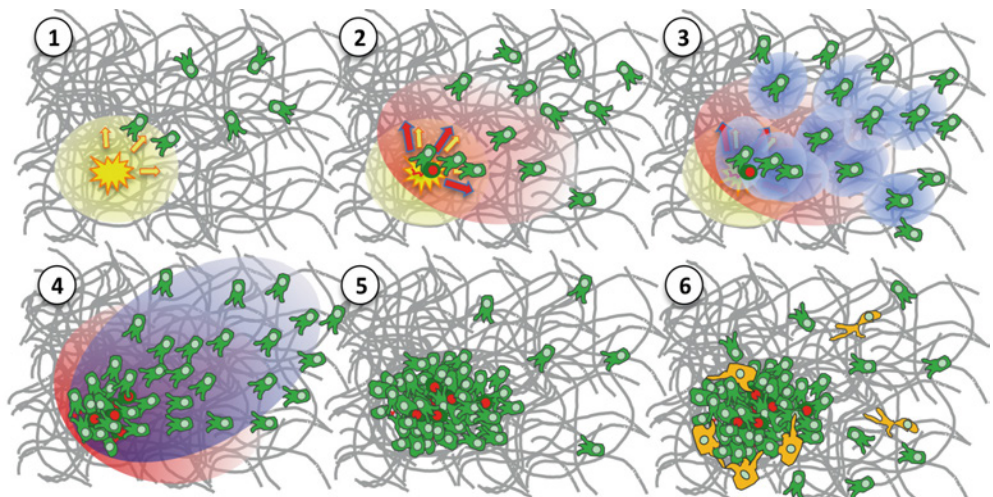


Figure 3 Coordination of immune cell functions between several immune cell types (differentially colored) of the innate immune response, immunofluorescence staining of skin whole mount tissue.

hibit coordinated cell migration, tissue positioning, and intercellular interactions (Figure 3). This includes neutrophils and monocyte subsets that are recruited from the bloodstream, which coordinate their function with sessile immune cell types (e.g. macrophages) that already seeded the tissue during embryonic development. How chemoattractants and adhesion receptors influence the motility patterns of diverse phagocyte subsets *in vivo* is largely unexplored. We are investigating how phagocytes influence the dynamics of each other and how this depends on the specific architecture of healthy and inflamed tissue compartments. To address inter- and intracellular immune cell dynamics under physiologically relevant conditions, we use advanced light microscopy techniques to characterize the behavior of live immune cells in mouse tissues and *in vitro* models as alternative mimics of physiological tissues. ↩

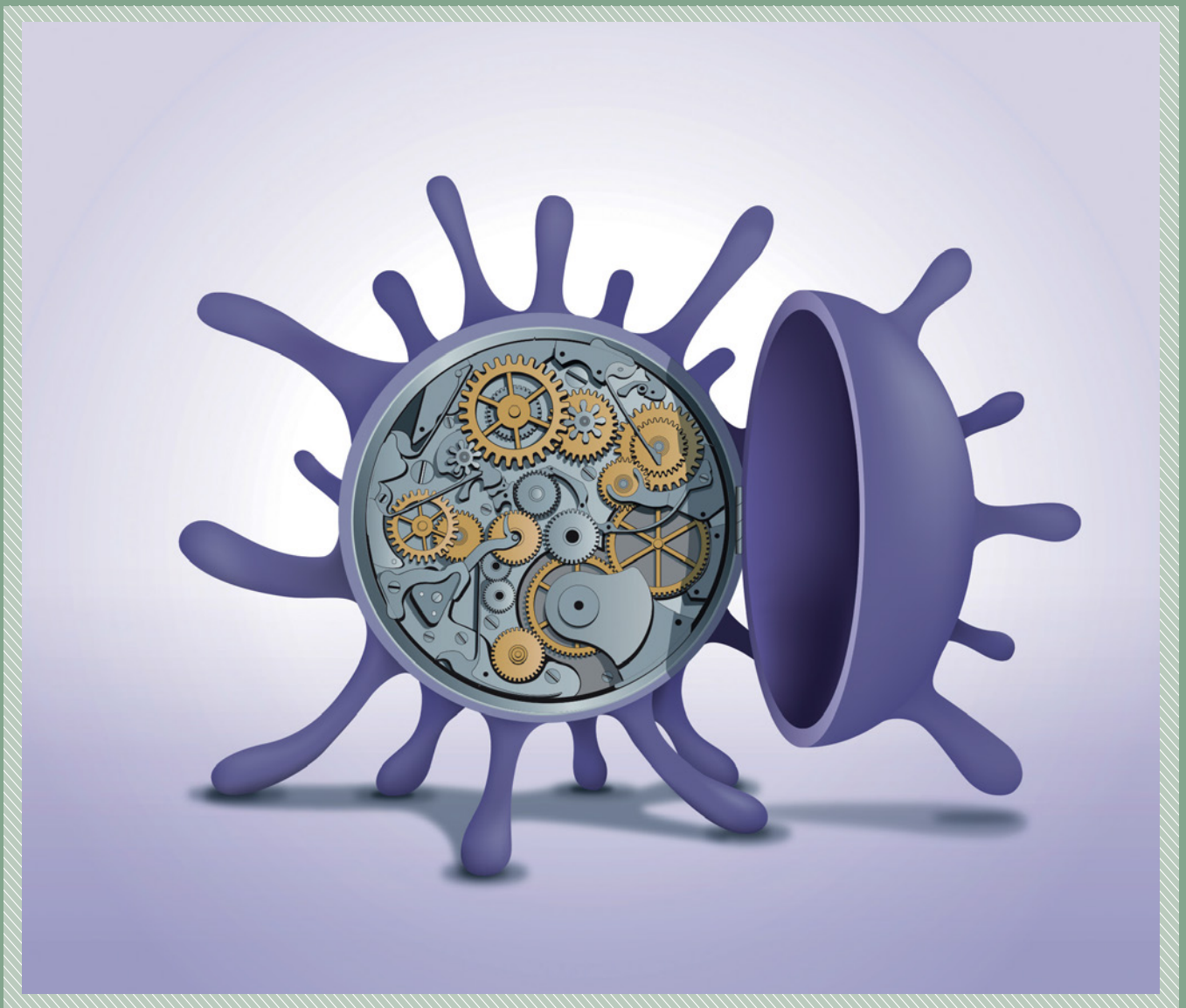
Figure 2 The multistep attraction cascade of neutrophil swarming upon local sterile injury (scheme): (1) Upon local tissue injury, neutrophils (green) close to the damage site sense short-range attractant factors (yellow). (2) Cell death (red) occurring at the injury site significantly correlates with amplified neutrophil recruitment from distant tissue regions. (3) Hereby, single neutrophils release the lipid B4 (LTB4, blue), which amplifies local cell death signals. LTB4 acts as a signal relay molecule between neutrophils, which (4) enhances the radius of neutrophil tissue recruitment. (5) Accumulating neutrophils form large cell aggregates that displace the surrounding collagen fibers (grey) of the connective tissue. These neutrophil clusters require LTB4 (and other attractants) in combination with adhesive forces (mediated by integrin receptors) to form a tight wound seal, whose borders cease in kinetic concert with the late recruitment of CX3CR1-positive macrophages and monocytes (orange) (6). Adapted from Lämmermann et al., Nature (2013).



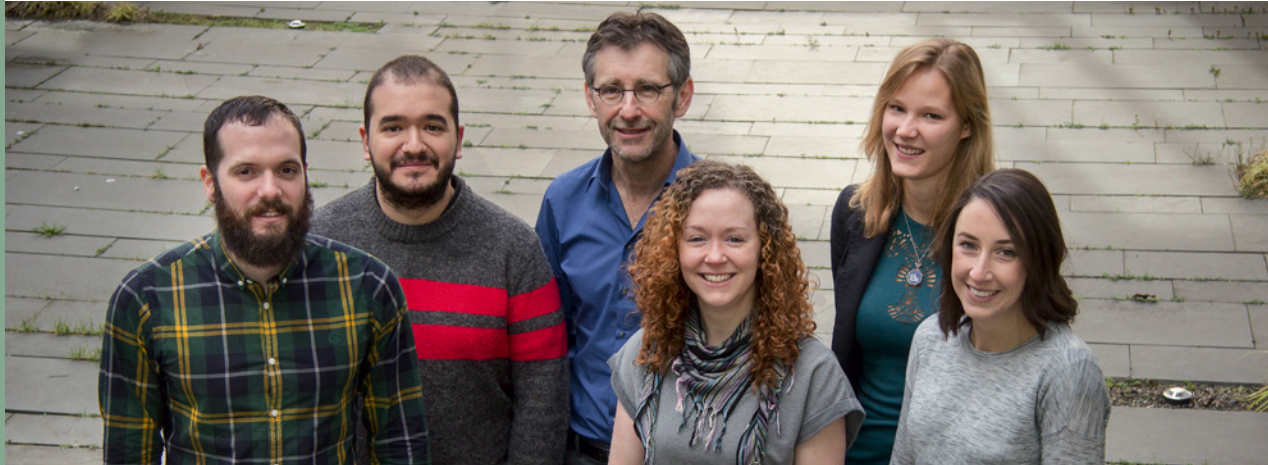
These neutrophil clusters require LTB4 (and other attractants) in combination with adhesive forces (mediated by integrin receptors) to form a tight wound seal, whose borders cease in kinetic concert with the late recruitment of CX3CR1-positive macrophages and monocytes (orange) (6). Adapted from Lämmermann et al., Nature (2013).

LAB EDWARD PEARCE

The lab is interested in the intricate pathways through which metabolic reprogramming affects cellular function and fate. The drawing was inspired by a review article on dendritic cell metabolism published by the laboratory in *Nature Reviews Immunology* and originally appeared on the cover of that journal.



'A Clockwork DC' by Simon Bradbrook; Reprinted by permission from Macmillan Publishers Ltd: *Nature Immunology Reviews* 15, copyright (2015).



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Everts B, Tussiwand R, Dreesen L, Fairfax KC, Ching-Cheng Huang S, Smith AM, O'Neill CM, Lam WY, Edelson BT, Urban JF, Murphy KM and Pearce EJ (2016). Migratory CD103+ Dendritic Cells suppress Helminth-driven Type 2 Immunity through Constitutive Expression of IL-12. *Journal of Experimental Medicine* **213**, 35-51.

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University/MPI-IE Senior Group Edward Pearce

METABOLIC REPROGRAMMING IN INNATE IMMUNE CELL ACTIVATION

Understanding how extracellular signals induce changes in cellular metabolism to support innate immune cell activation

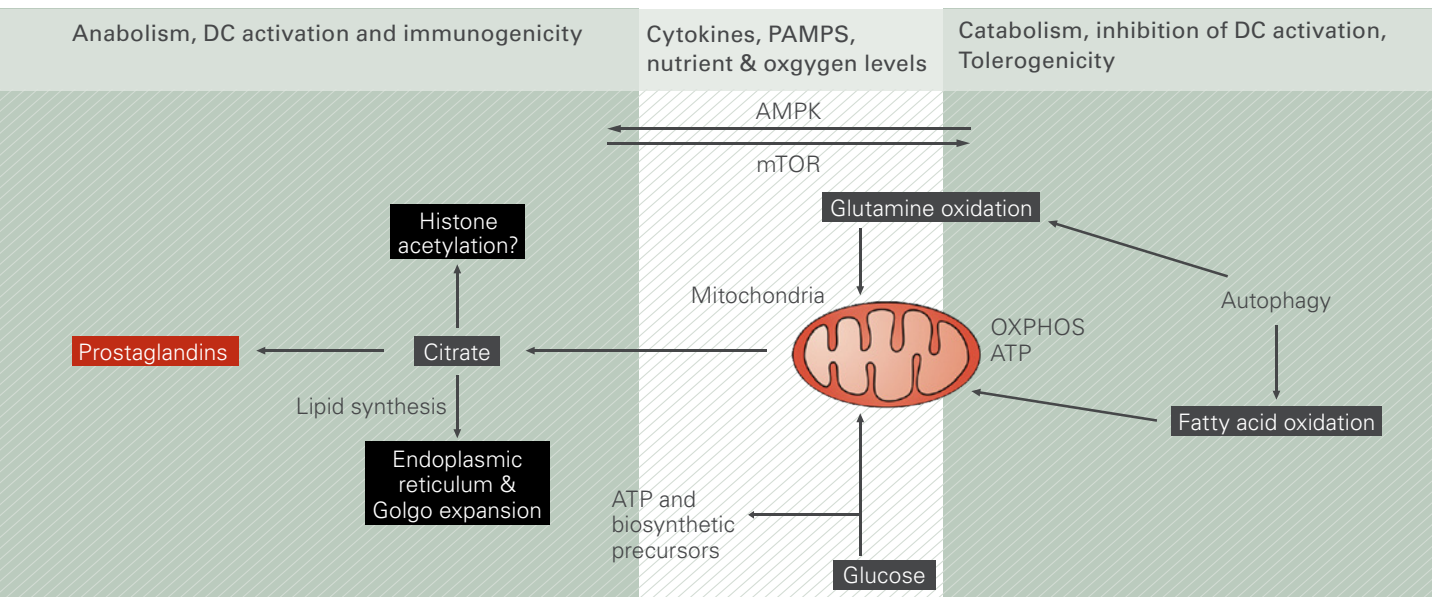


Figure 1 Anabolic metabolism vs catabolic metabolism and the control of dendritic cell immunogenicity vs. tolerogenicity. PRR agonists, cytokines and nutrient and O₂ levels can influence the balance of anabolic to catabolic metabolism, as shown. mTOR and AMPK are important regulators of this metabolic balance and their activation states are highly responsive to a broad array of intracellular and extracellular signals. Glycolysis coupled to the TCA cycle and citrate export from mitochondria supports an array of biosynthetic processes that are critical for DC activation. In contrast, autophagy and the oxidation of fatty acids and glutamine can create a state in which DCs are tolerogenic

➤ Innate immune cells play a role in rapid responses to infection or other danger signals and are pivotal in facilitating the development of adaptive immunity. Moreover, innate immune cells often mediate effector functions following instruction by cytokines or other signals from adaptive immune cells. Activation of innate immune cells is underpinned by altered expression of many genes. Recent studies have revealed that many of these genes encode core metabolic pathway components, and there is a growing realization that metabolic reprogramming is essential to support activation. We are interested in understanding how and why metabolic reprogramming occurs.

Metabolic reprogramming in dendritic cells

Dendritic cells (DCs) are a diverse group of related cell types that express various pattern recognition receptors (PRRs) which are able to bind molecular motifs that are characteristic of particular pathogens or that are associated with cellular damage. Ligation of PRRs initiates signaling pathways that lead to cellular activation and marked changes in gene expression and cellular biology. DCs activated via PRRs have central roles in innate immunity and in adaptive immunity (in which they drive the activation of antigen specific T cells). As such, DCs have a central role

in the immune system. It is becoming increasingly clear that different stages of immune cell activation coincide with, and are underpinned by, different types of cellular metabolism that are tailored towards the bioenergetic and biosynthetic needs of these cells. The metabolic requirements of an activated DC are distinct from those of a quiescent DC and, as such, changes in metabolism are integral to the successful activation of these cells. This realization has led to interest in the cellular metabolism of DCs, not least because it is possible that manipulation of the metabolic state of DCs could be used to modify inflammatory and immune responses for therapeutic purposes. We have found that in conventional DCs, stimulation through TLRs (a subset of PRRs) results in rapidly increased glycolysis, which serves to increase the amount of glucose-derived carbon available for fatty acid synthesis. Our findings indicate that this is necessary to allow expansion of endoplasmic reticulum and Golgi to support demands for increased protein synthesis associated with the production of cytokines for secretion (Figure 1). In ongoing work we are studying another subtype of DCs, plasmacytoid DCs (pDCs), in which activation through TLRs is accompanied by significantly increased fatty acid oxidation (FAO). We have found that this type of metabolic reprogramming is essential for full pDC activation, and is

in fact initiated by type 1 IFN, which is made in response to TLR agonists, and then signals in an autocrine fashion to alter metabolism. Collectively then, our data implicate DC metabolic reprogramming as an integral component of the activation process, and show that the nature of metabolic reprogramming differs between cDCs and pDCs. We are now focused on understanding the molecular basis for the requirement for metabolic reprogramming for activation (Figure 1). Our long term goal is to be able to manipulate metabolic processes in DCs in order to promote or inhibit their activation and/or longevity, and in so doing develop novel approaches for improving vaccination strategies, and limiting immune responses in chronic, pathological conditions.

Macrophage fatty acid metabolism in resistance and susceptibility to disease

Macrophages adopt different activation states depending on the overall immunologic context. Different types of infection/damage stimulate distinct types of immune responses, and therefore are associated with different types of activated macrophages. For example, in settings where interferon- γ is present, especially in combination with TLR agonists, macrophages become M1 (or classically) activated, whereas in conditions where IL-4 and IL-13 are produced, macrophages become M2 (or alternatively) activated (Figure 2). We are particularly interested in M2 activation, and study these cells in the context of helminth infections, where they play a host protective role (Figure 3), and in cancer, where they promote tumor progression and metastasis.

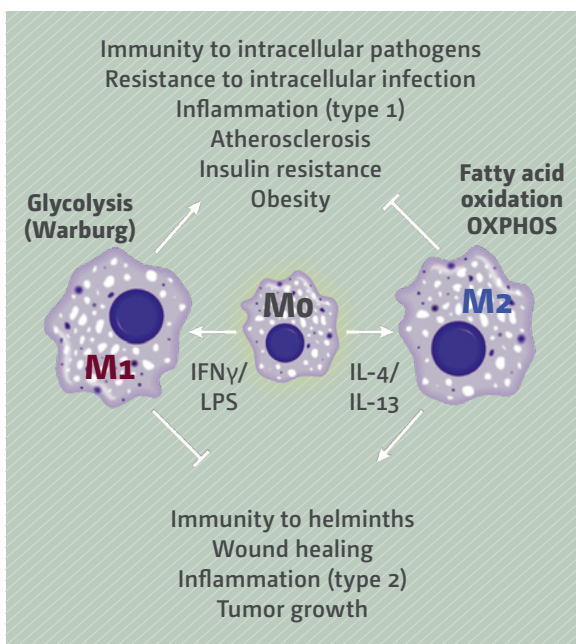


Figure 2 Differential macrophage activation. Macrophages can be activated by different signals to attain distinct activation states which, depending on the situation, can be beneficial or detrimental, as indicated. Different activation states can antagonize the effects and development of each other, and are supported by distinct types of metabolism.

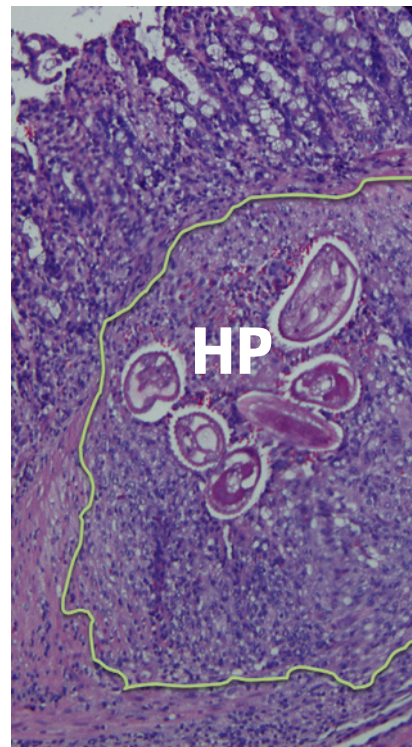

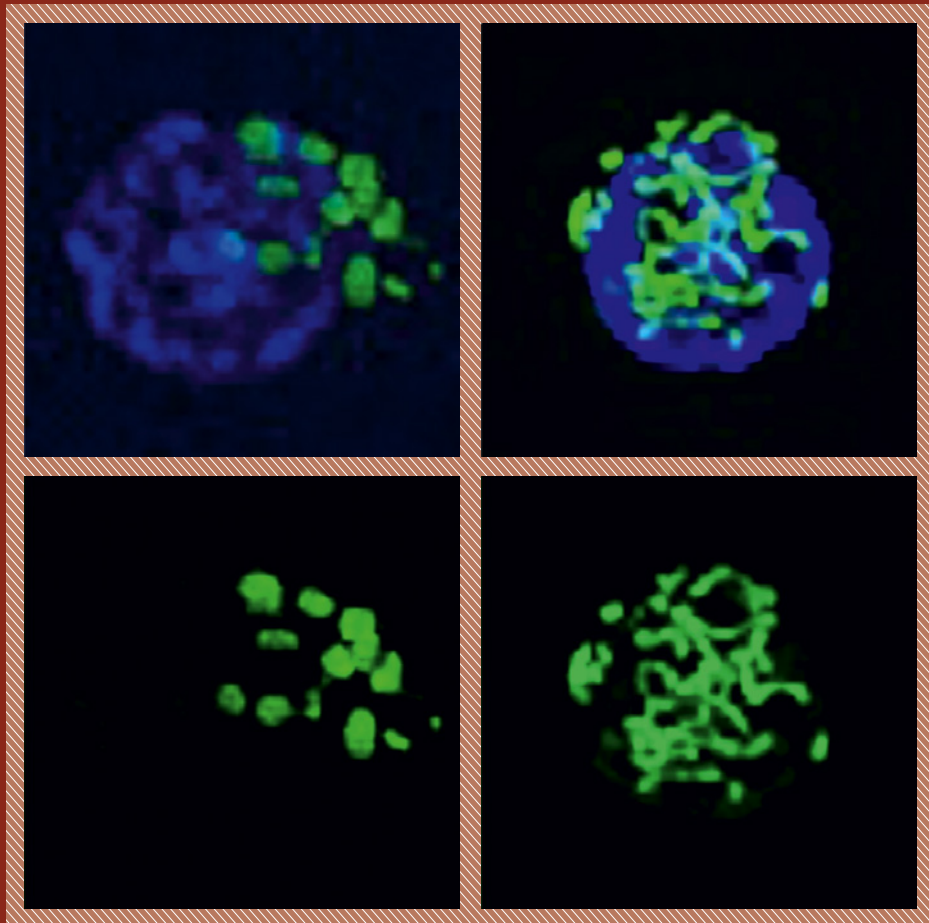


Figure 3 M2 macrophages play a role in immunity to helminth parasites. A granuloma enriched in M2 macrophages (outlined in green) trapping invading *Heligmosomoides polygyrus* (HP, a nematode parasite of mice that is used to model human intestinal nematode infections) larvae in the mouse intestine.

Differential functions in M1 and M2 macrophages are supported by distinct core metabolisms, with M1 cells committing to aerobic glycolysis, and M2 macrophages utilizing FAO and mitochondrial oxidative phosphorylation (Figure 2). Indeed, FAO has been shown to be necessary for M2 activation. This realization has focused our attention on macrophage fatty acid metabolism, which since it is essential for M2 activation must, by definition, be an essential facet of protection against helminth infections, and susceptibility to cancer progression. We have shown that fatty acids for FAO are derived from the lysosomal lipolysis of triacylglycerols, which are sourced either from the exterior or through endogenous synthesis of fatty acids. Despite the fact that it is clear that FAO is essential for M2 activation, the underlying reasons for the importance of this type of metabolism for M2 activation are unclear. We hypothesize that FAO serves as an efficient source of citrate for export into the cytosol where it can be used for processes that are critical for M2 activation such as synthesis of fatty acids for FAO and to act as ligands for PPARs, and the production of acetate for the acetylation of histones to permit the expression of M2 genes, and our current research is exploring these possibilities.

In addition to their role in immunity to helminths, M2 macrophages play roles in wound healing, and in whole body metabolic homeostasis. Remarkably, helminth infection has been reported to be capable of mitigating the metabolic consequences of a high fat diet. We hypothesize that strong type 2 immunity induced by helminth infection is able to maintain metabolic homeostasis by broadly supporting M2 activation in tissues distal to the site of infection. We plan to explore this possibility in the near future. 

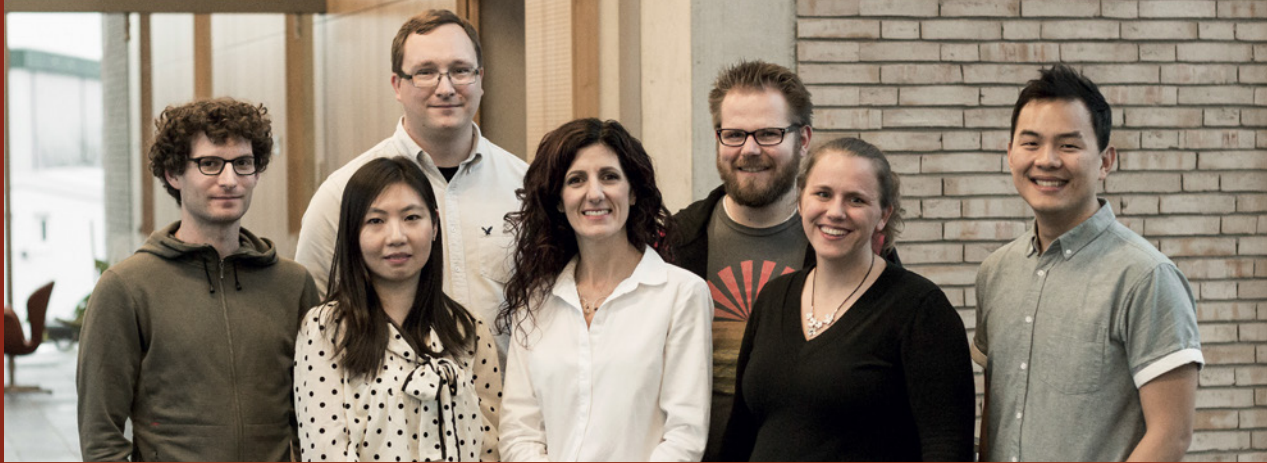


➤ **LAB ERIKA PEARCE**

Effector T cells (left) and memory T cells (right) have distinct mitochondrial morphologies. These differences in mitochondria underlie the unique functional capacities of each of these cell types. (Image taken by Angelika Rambold).

Senior Group Erika Pearce

Immunometabolism



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Senior Group Erika Pearce

METABOLIC REGULATION OF T CELL FUNCTION AND FATE

Understanding metabolic reprogramming in T cells during infection and cancer

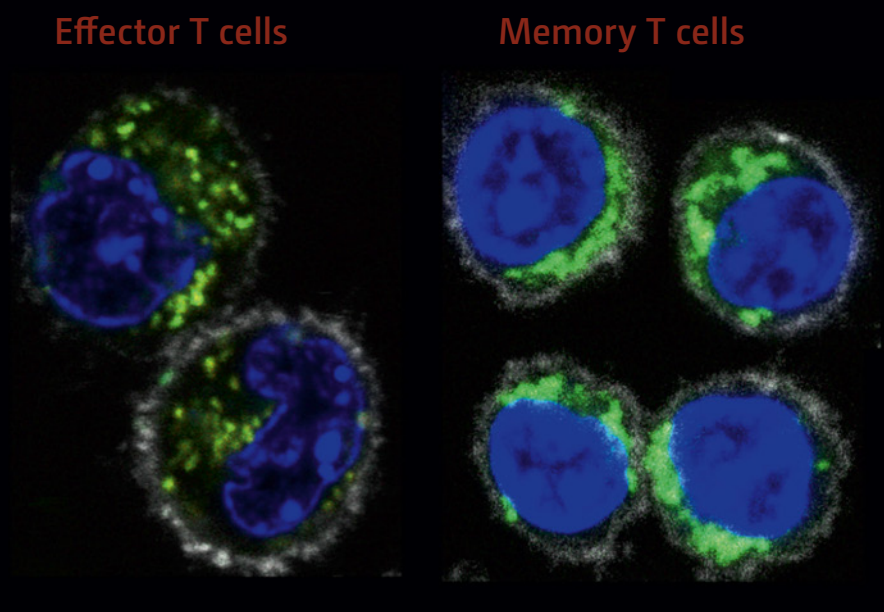


Figure 1 Effector and memory T cells have distinct mitochondrial morphologies and metabolic phenotypes that support their function and fate.

➤ Immunometabolism is an emerging field that investigates the interplay between immunological and metabolic processes. Interest in this field is gaining momentum due to the realization that incorrect metabolic remodeling underlies many aberrant immune responses, and that manipulating cellular metabolism can beneficially enhance or temper immunity.

Our genetics and environment dictate how we metabolize the nutrients we consume and shape our growth, function, appearance, and overall health. The same principles hold true on the cellular level. Just as track runners quickly engage their muscles to propel themselves from rest to sprint after a gun signals the start of the race, pathogen-derived or inflammatory signals drive T lymphocytes of the immune system out of quiescence to rapidly modulate the expression of genes allowing them to acquire new functions. These changes range from the acquisition of effector functions marked by increased production of cytokines and cytolytic molecules, to the ability to undergo very rapid cell division and migration. Intimately integrated into this program of activation is the regulation of cellular metabolism.

The goal of our research is to define underlying molecular mechanisms that control T cell responses to infection and cancer, with a particular emphasis on how cellular metabolic pathways govern these events.

Metabolic reprogramming in memory T cells

T cells are maintained in fairly constant numbers, but upon activation undergo a developmental program characterized by distinct phases encompassing the expansion and then contraction of antigen-specific effector T cells, followed by the persistence of long-lived memory T cells (Figure 2). Although this predictable pattern of a T cell response is well established, the mechanisms regulating how T cells generate these different fates are not well understood. Our work has shown that specific metabolic programs must be in place to support the development of memory T cells. We have found that memory T cells rely on mitochondrial metabolism, and in particular, require the oxidation of intracellular fatty acids for development. New work in our lab is focused on how mitochondrial phenotype and morphology dictate metabolic pathway engagement and as such enforces either the effector or memory fate of a T cell (Figure 1).



Post-transcriptional regulation of T cell function by metabolic enzymes

Resting T cells use oxidative phosphorylation to generate energy, and greatly promote aerobic glycolysis upon activation. It was thought that this engagement of aerobic glycolysis was required to meet the metabolic demands of proliferation. However, why proliferating T cells would adopt this less efficient energy metabolism, especially in an oxygen-replete environment, remained incompletely understood. During the course of our studies we found that aerobic glycolysis is actually required for effector function in T cells, in particular, for optimal production of effector cytokines, but that this pathway is not necessary for their proliferation or survival. We found that this defect in effector function was translational, and regulated by the binding of the glycolysis enzyme GAPDH to AU-rich elements within the 3' UTR of cytokine mRNA. We demonstrated that GAPDH, by engaging/disengaging glycolysis, controlled cytokine production in a posttranscriptional manner (Figure 3). One of our future goals is to define how the RNA binding function of metabolic enzymes is altered by the engagement of particular cellular metabolic pathways, and how this ultimately influences T cell function in different conditions in vivo.

Immune cell metabolism in the tumor microenvironment

During a productive immune response to cancer, naive tumor antigen-specific T cells become activated and produce a variety of effector molecules that mediate tumor clearance. However, T cells often experience a progressive decline in function and responsiveness during can-

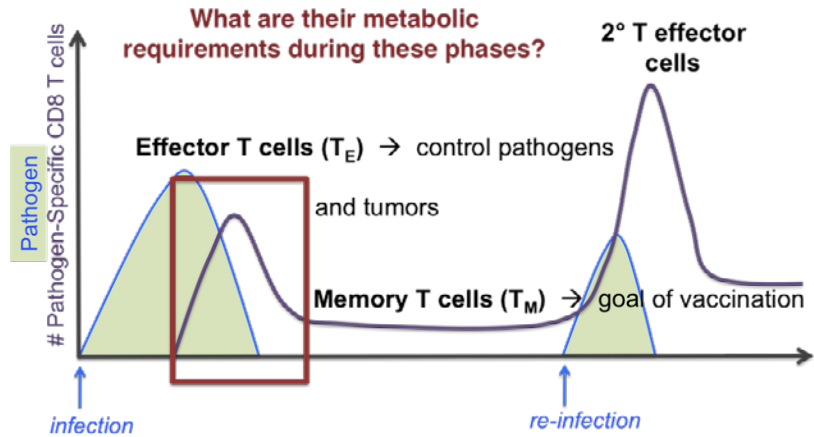


Figure 2 In response to antigen and costimulation, T cells become activated, proliferate, and gain effector functions important for clearing pathogens or tumors. Long-lived memory T cells are also generated during an immune response and are important for protecting the host against future re-infection. T cells must adapt to a wide array of environmental stressors as part of their normal development and undergo dramatic metabolic remodeling in the process.

cer, and without properly functioning T cells, tumors will continue to grow. We have recently shown that this T cell dysfunction, or exhaustion, in cancer can result from a metabolic competition between tumors and T cells, which compete for the same nutrients in the tumor microenvironment. Competition for glucose alone can dampen the ability of tumor infiltrating T cells to engage aerobic glycolysis, which is required for their acquisition of full effector function and their ability to control tumor progression. We also found that several checkpoint blockade antibodies, which are used clinically to treat cancer patients, can restore glucose in the tumor microenvironment, permitting T cell glycolysis and effector cytokine production. These findings suggest that new efforts to target cancer should incorporate the idea that metabolic competition occurs in tumors and greatly influences tumor progression. Our new work focuses on understanding whether even transient nutrient restrictions can lead to permanent states of exhaustion in T cells.

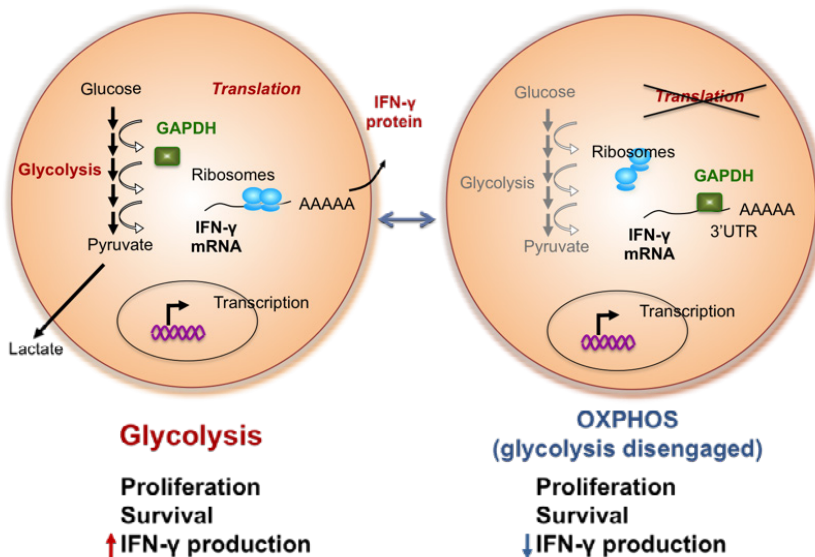
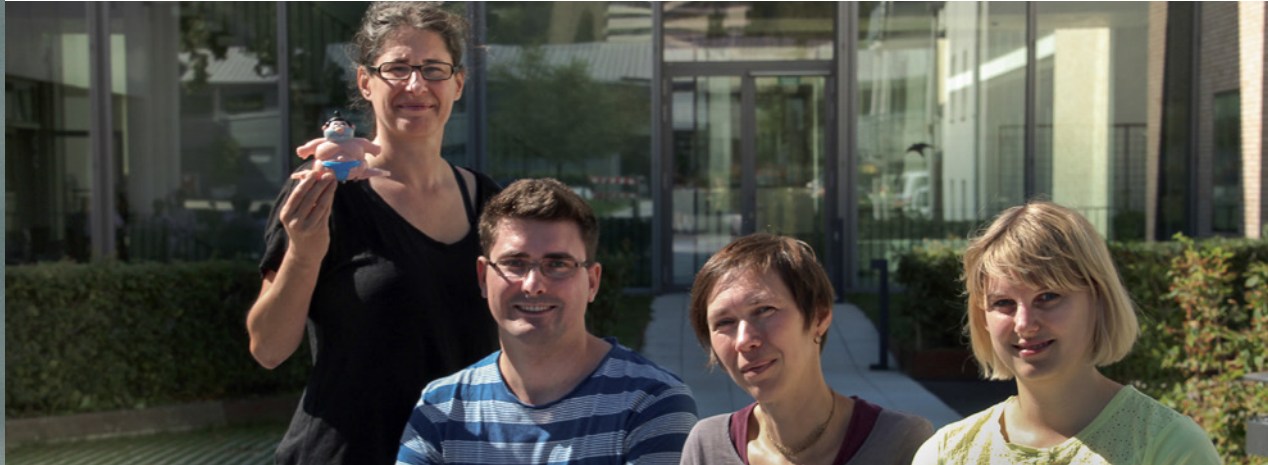


Figure 3 The metabolic enzyme GAPDH, by engaging/disengaging in the glycolysis pathway, specifically controls cytokine production in effector T cells in a posttranscriptional manner. We are interested in determining how bifunctional metabolic enzymes connect metabolism and gene regulation in T cells.

➤ LAB ANDREA PICHLER

SUMO chain assembly. Workman (red) represents the conjugating SUMO enzymes.





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⇒ SELECTED PUBLICATIONS

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Group Andrea Pichler

REGULATION OF SUMO CONJUGATION

Unexpected molecular insights into E2, E3 and E4 enzyme activity and how they assemble SUMO chains.



Figure 1 SUMO chain assembly. Workman (red) represents the conjugating SUMO enzymes.

➤ Posttranslational protein 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 SUMO (small ubiquitin related modifier) are amongst the most frequently used reversible modifications.

SUMO is a small protein that regulates protein functions like stability, activity, intracellular localization etc. The covalent attachment of SUMO to its substrate, called sumoylation, is essential for viability in most organisms. Sumoylation is executed by the hierarchical action of E1, E2 and E3 enzymes that results in mono- or multi-sumoylation of a target protein or attachment of a SUMO chain (Figure 1 and Figure 2, upper panel). Deregulation of this system is implicated in various diseases ranging from diverse types of cancer to several neuropathological diseases. We aim to understand the molecular mechanisms of how conjugation of SUMO is regulated. 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 SUMO enzymes.

Regulation of sumoylation by E2 enzyme (Ubc9) sumoylation

Sumoylation is primarily regulated via E3 ligases and SUMO specific proteases because these enzymes mainly ensure substrate specificity. We characterized an alternative mechanism of regulation on the E2 level: E2 enzyme (Ubc9) sumoylation. This modification is conserved from yeast to mammals but involves structurally different sites of modification suggesting distinct enzymatic consequences.

In mammalian cells, we found that N-terminal Ubc9 sumoylation enhances the affinity and modification of selected substrates in dependence of a non-covalent SUMO interaction motif (SIM) (Knipscheer, Klug et al., Mol Cell 2008, Figure 2, middle panel).

By contrast, C-terminal Ubc9 sumoylation in *Saccharomyces cerevisiae* results in E2 inactivation but turns this inactive Ubc9*SUMO into a cofactor for the unmodified Ubc9. Together, these enzymes cooperate in SUMO chain assembly, which is important for successful synaptonemal complex (SC) formation in yeast meiosis (Klug et al, Mol Cell 2013, lower panel).

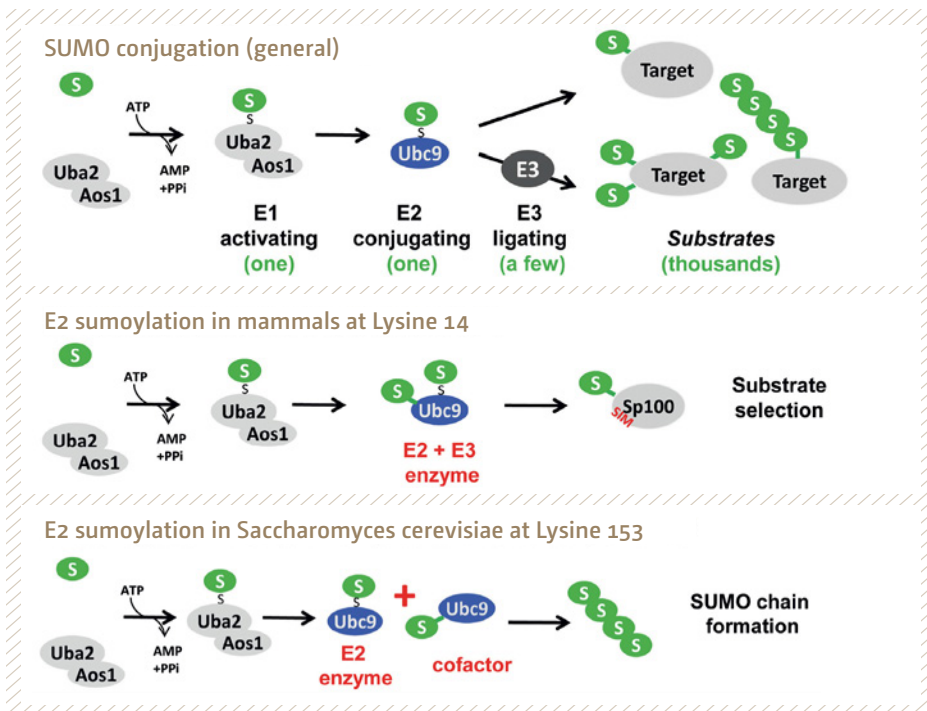


Figure 2 SUMO conjugation is regulated via E1, E2 and E3 enzymes (upper panel). E2 (Ubc9) sumoylation contributes to regulation of SUMO conjugation: in mammals Ubc9 is modified at Lysine 14 and this contributes to substrate selection (middle panel), in yeast Ubc9 is modified at Lysine 153, which inactivates the enzyme and turns it into a cofactor for SUMO chain assembly (lower panel).

The ZNF451 family, a novel class of SUMO enzymes

Very recently, we discovered a novel family of SUMO conjugating enzymes with E3 ligase and E4 elongase (specialized E3s for SUMO chain elongation) functions (Figure 3, upper panel). We show that ZNF451, a mainly uncharacterized zinc finger protein, has SUMO E3 ligase activity and efficiently assembles SUMO2/3 chains. Detailed biochemical analysis demonstrates that ZNF451 functions distinct to all known E3 ligases described for SUMO and ubiquitin conjugation: ZNF451 executes catalysis via a tandem-SIM and its interSIM region. One SIM orients the donor-SUMO, while a second SIM binds

SUMO on the backside of the E2 enzyme. Intriguingly, this short tandem-SIM region is sufficient to extend a backside-anchored SUMO chain (E4 elongase activity) in contrast to chain initiation, which in addition requires a zinc finger region to recruit the initial acceptor SUMO (E3 ligase activity, Figure 3, lower left panel). Four human proteins share this E4-elongase activity (Figure 3, lower right panel) and are involved in stress-induced global sumo(2,3)ylation after DNA double strand break induction or proteasome inhibition in vivo (Eisenhardt, Chau-gule et al 2015 and Cappadocia et al 2015).

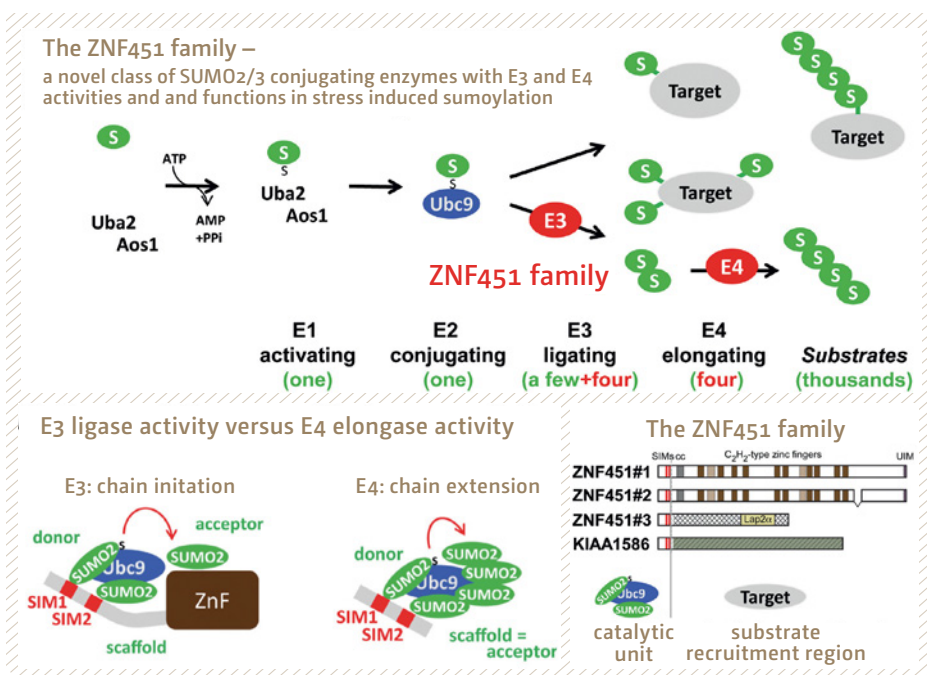
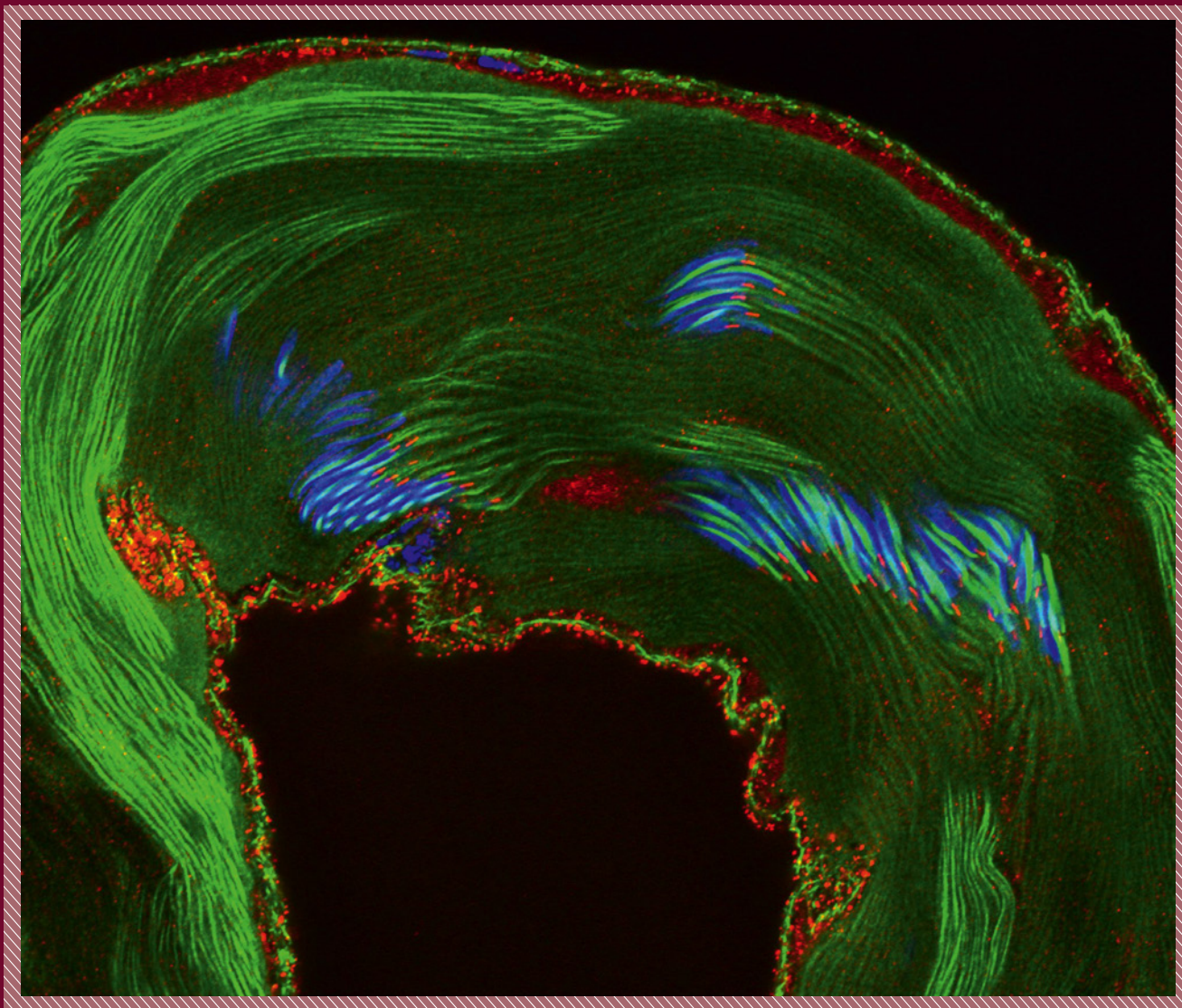


Figure 3 The ZNF451 family presents a novel class of SUMO conjugating enzymes with E3 and E4 activities (upper panel). A tandem SIM and the interSIM region display the catalytic unit of the enzymes. E3 and E4 activities involve distinct substrate (acceptor) binding interfaces (lower, left panel). The ZNF451 family has four family members which share the N-terminal catalytic unit but differ in their substrate recruiting C-termini (lower, right panel).



➤ LAB J. ANDREW POSPISILIK

Immunofluorescence of *Drosophila* sperm bundles inside the testis. Blue shows DNA (elongated sperm nuclei), green shows acetylated alpha-tubulin (sperm tails) and red shows piwi. In addition to delivering the DNA code, sperm appear able to carry additional epigenetic information capable of stably influencing disease phenotypes lifelong in the offspring.

Group J. Andrew Pospisilik

Epigenetic Control
of complex disease



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➤ SELECTED PUBLICATIONS

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Group J. Andrew Pospisilik

EPIGENETIC CONTROL OF COMPLEX DISEASE

Chromatin plasticity in metabolic disease

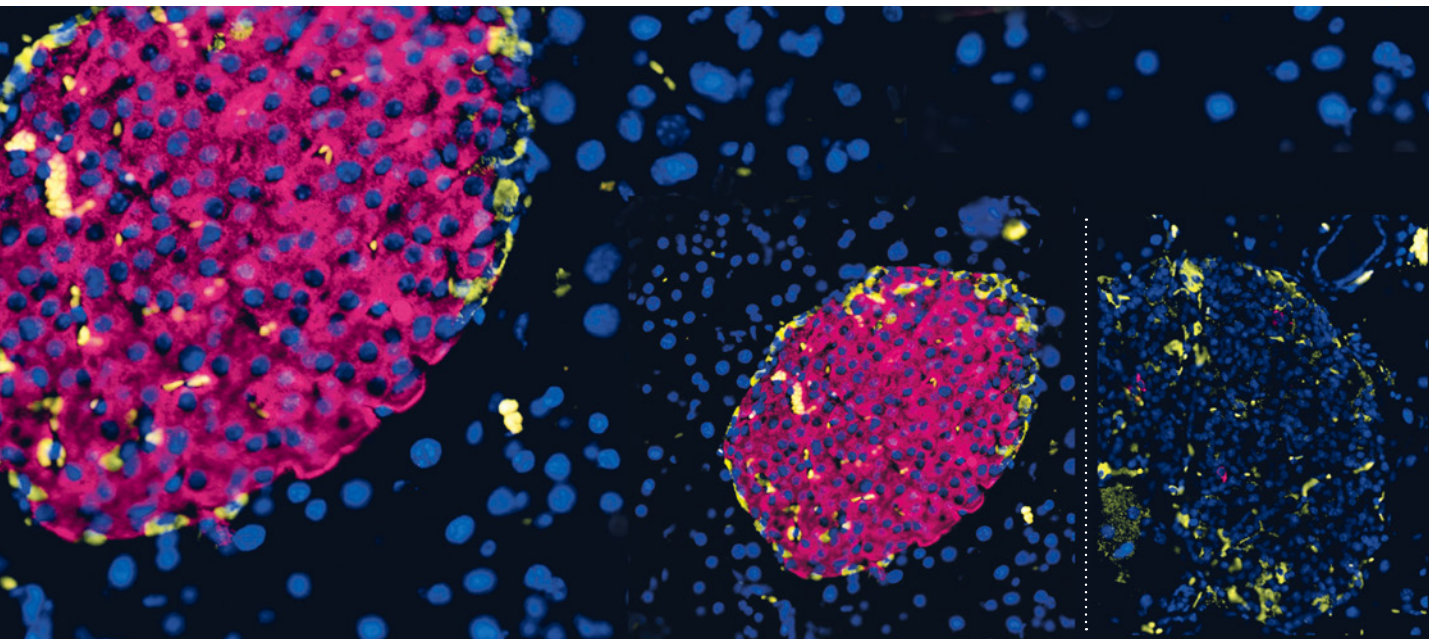


Figure 1 Recent studies have shown beta-cell de-differentiation to be a hallmark of diabetes progression. We find that PcG disruption can induce normal islets (left) to de-differentiate (right), without altering key cellular processes such as proliferation or apoptosis.

➤ 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 the nature and mechanistic basis for 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:

Epigenetic mapping of mammalian disease

The first comprehensive attempts to provide an epigenetic framework on a genome wide scale are now being realized en masse (eg. NIH Roadmap). These efforts, many of which were initially performed on in vitro, have provided the seminal cataloguing system from which to organize and compare information of transcription, chromatin state and phenotypes (for example disease states). A significant body of evidence supports the existence of a robust layer of epigenetic control in the establishment of robust metabolic homeostasis mechanisms as well as in complex metabolic diseases such as obesity and diabetes. Our experimental goals intersect genetically and epigenetically founded human, mouse and fly models systems with next generation sequencing approaches to characterize the plasticity dysregulation of chromatin-based transcriptional circuitry for health and disease. Ultimately, we intersect the model organism findings with molecular analyses of clinical biopsies from highly characterized human patients, placing the findings directly into human context.

Current studies of this nature include generating reference Epigenomes for mouse and human white adipocytes from lean and obese individuals, from polycomb mutant (de-differentiating) pancreatic beta-cells, and in the fruit fly, where we are mechanistically dissecting how physiological changes in a parent can have life-long impacts on the health and vigor of offspring. These projects are funded by the Max Planck Society, the ERC, as well as larger-scale consortia including Epigenesys – an EU network of excellence on Systems Biology and Epigenetics; Medep – DFG funded collaborative research center focusing on Medical Epigenetics; and DEEP / EpiTriO – BMBF funded Epigenome contributions to the International Human Epigenome Consortium (IHEC). The long-term goals thus include building up an international resource for understanding the interplay between genetics, epigenetics, gene expression, chromatin plasticity, DNA accessibility 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 alterations in vivo. These studies capitalize on the integration of targeted mouse genetics and systems biology approaches and address causality and mechanism of action. Experimental control of gene expression in fruit flies is helping us dissect the genetic requirements of parental effects (Oest, Lempradl et al. 2014). We find, for instance, that the well known Polycomb and H3K9me3 silencing factors are absolute requirements to mediate intergenerational obesity effects and that these are necessary in two distinct time-windows, one in the germline (Figure 2), and one in the embryo.

Experimentally targeting these same two systems in the mouse has revealed a critical requirement for Polycomb in maintaining beta-cell identity and function (Figure 1) and in canalizing development towards a single developmental outcome. Experiments focusing on Trim28, a chromatin associated protein that ensures proper imprinted gene control during and after development, have provided what we believe to be the first mammalian ev-

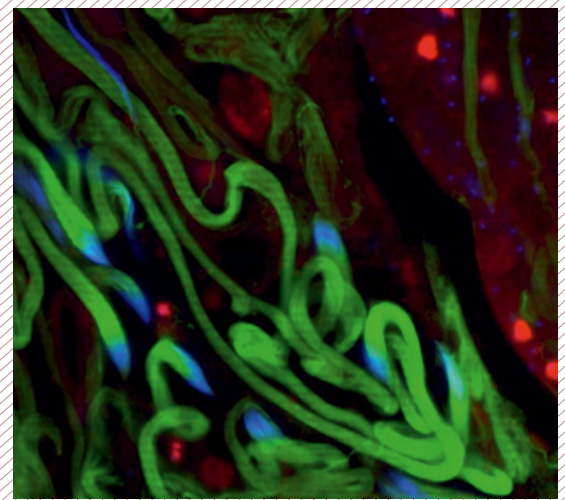


Figure 2 The fruit fly, *Drosophila melanogaster*, has some of the longest sperm cells of any species. Here one can see bundles of blue headed, green tailed sperm during late sperm development. These single cells are able to carry epigenetic memories of parental experience or stress and re-shape complex phenotypes of the offspring.

idence for a phenomenon known as polyphenism. Polyphenism describes the potential for a single genetic template to evoke multiple channeled phenotypic outputs. A famous example for polyphenism is the ant: Despite being born of nearly identical genetic and environmental settings, the genome can be channeled to generate distinct adult phenotypic states including soldier, worker and queen ants. In our studies, we find near-identical genetic and environmental scenarios can trigger lean or obese phenotypes, but nothing in between. Importantly, we find molecular signatures indicating that parallel processes may underlie human variation and disease.

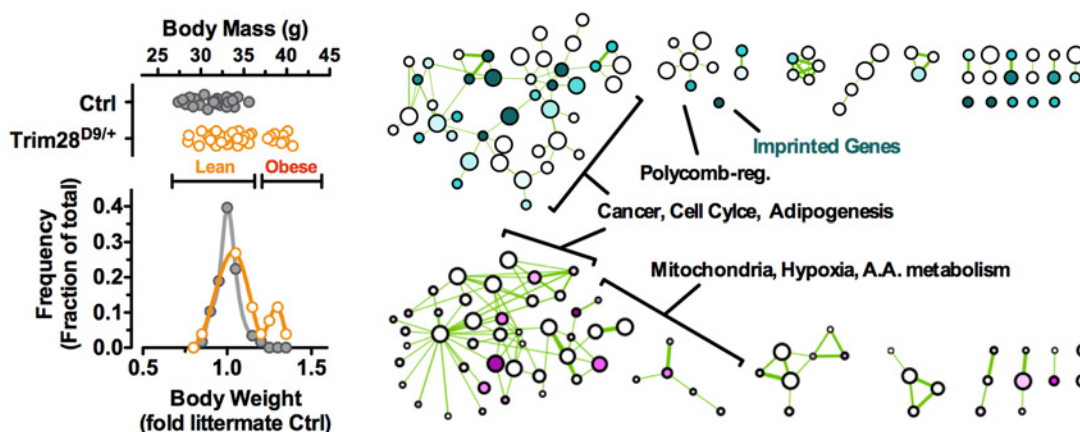
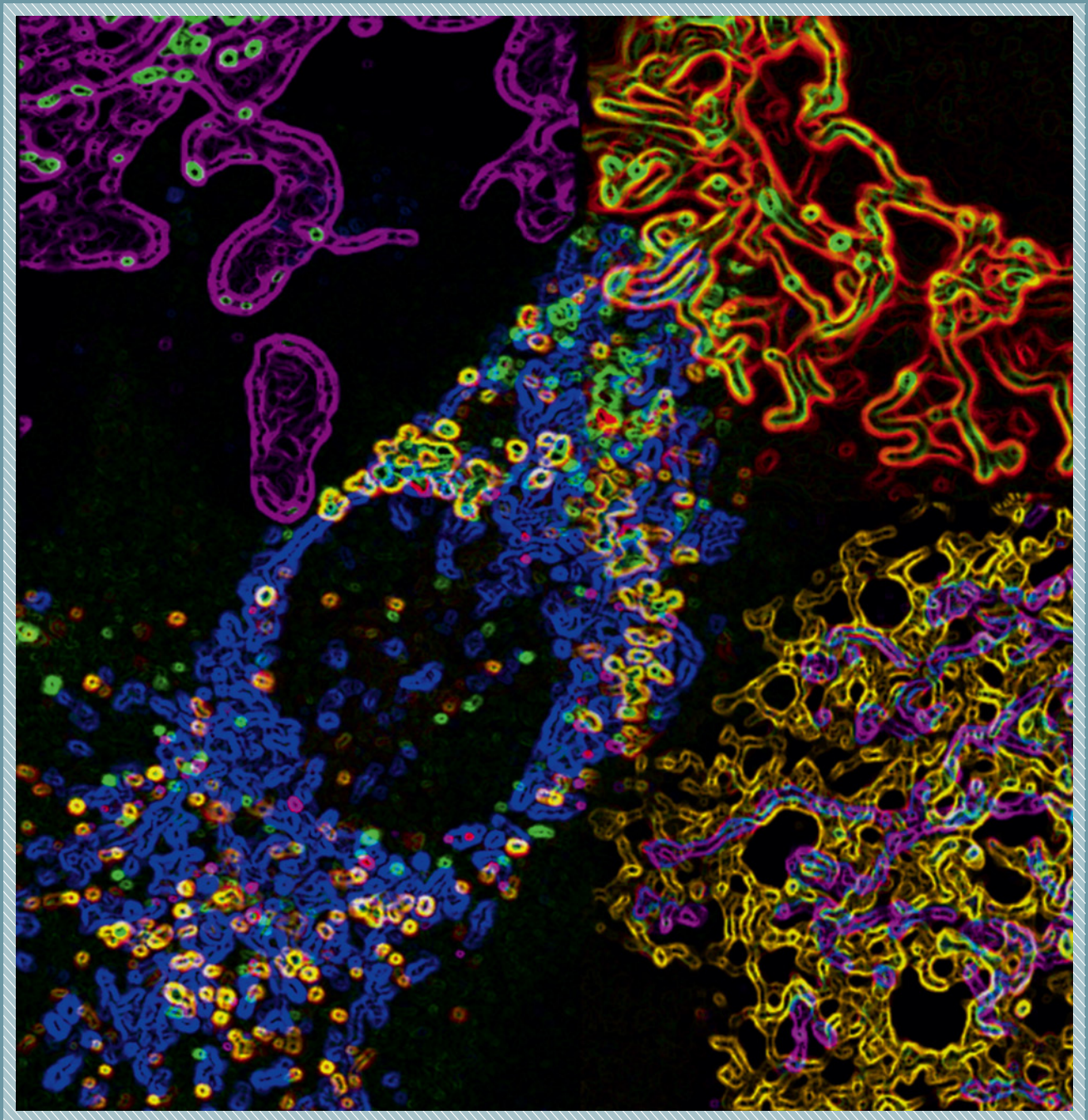


Figure 3 By examining epigenetically sensitized mouse strains, we have found evidence of polyphenism in mammals. Polyphenism is a phenomenon where genetically identical individuals can emerge from development in one of two or more forms. In the case shown, mice emerge into either Lean or Obese states (left panel) with high-dimensional rearrangement of their gene expression (right panel).



➤ LAB ANGELIKA RAMBOLD

Inside cells multiple organelle systems form elaborate communication networks that rapidly adapt to the cellular need. These networks rely on dynamic changes in organelle mass, size, morphology, position and inter-organelle interaction. Dynamic live cell imaging allows us to decipher such dynamic organelle networks (see image) and their importance to mount a proper immune response.



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ORGANELLE NETWORKS IN CELLULAR IMMUNITY

Communication between cell organelles shape the function of immune cells

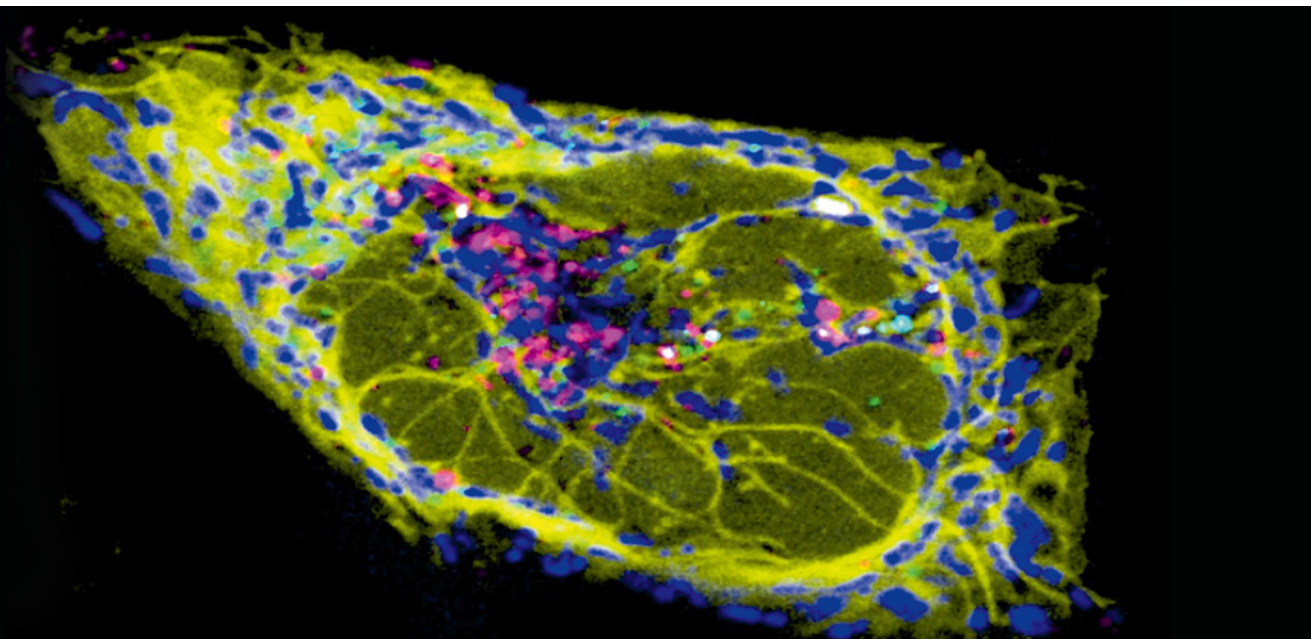


Figure 1 Organelles (differentially colored) selectively arranging their position and functional interaction in the dense interior of the cell.

⇒ Organelles are membrane-bound cell compartments characteristic for all eukaryotic cells. They enable and segregate specialized biochemical reactions, which otherwise could not coexist inside a cell. As highly dynamic structures, organelles adapt their function, morphology, position and mass to the acute cellular state (proliferation, differentiation, stress). Defects in the biogenesis, activity or dynamics of cell organelles have been linked to various diseases, including several immune disorders. Mitochondrial defects cause altered immune cell homeostasis and lead to severe combined immunodeficiencies (SCID). Gene polymorphisms impairing autophagosomal homeostasis underlie Crohn's disease and systemic lupus erythematosus. Most prevalent, however, are immune disorders linked to defects in lysosomes and lysosome-related organelles, including the Chediak Higashi Syndrome, Gaucher disease or α -mannosidosis.

Organelle networks as drivers of metabolic cell activation

Recent studies in non-immune cells have highlighted that organelles do not act as separate entities, but work as functionally interconnected networks. We have contribut-

ed to the current concept that lysosomes and autophagosomes interact and work in dynamic concert with other organelles (mitochondria, lipid droplets) to sustain metabolic reprogramming and cell survival in non-immune cells. Lysosomes, autophagosomes, lipid droplets and mitochondria synergize their dynamics to adapt their activity and form selective interactions to direct the efficient exchange of fatty acids, sugars and amino acids between them.

Communication between organelles can also take place without physical contact by exchanging bioactive molecules, such as peptides or ATP, or regulating the activity of metabolic kinases and transcription factors. While evidence of such organelle communication is arising from the field of basic cell biology and metabolism, the functional significance of such multi-organelle networks for immune cell function and their contribution to organelle-related immune disorders remains unclear. Our future research aims to gain novel insight into how organelles use dynamic changes in their morphology, shape, position and interaction to modulate spreading effects to other organelle systems and how this impacts immune cell function.

Understanding organelle-related immune disorders

The Chediak Higashi Syndrome (CHS) is a classic lysosome-related immunodeficiency. CHS is caused by mutations in the gene encoding the lysosomal trafficking regulator *Lyst*, inducing aberrant lysosomal fusion/fission dynamics and resulting in a striking morphological phenotype, the giant lysosome. CHS patients suffer from life-threatening and recurrent bacterial infections and can show partial albinism, progressive neurological dysfunction and uncontrolled T-cell and macrophage activation. While lysosomes are mostly known for their roles in intracellular protein degradation or release of cytotoxic molecules (lytic granules), the recent advances from studies in non-immune cells have highlighted them as integral parts of organelle networks, regulating cell metabolism, energy production and survival. We are using two different approaches, focused on the lysosomal regulation of central metabolic transcription factors and the integration and formation of lysosome-directed metabolic organelle networks. Our current research addresses how lysosomes are embedded in organellar networks and, through them, control the metabolism and effector functions of different immune cells (T cells, macrophages). Integrated into the Center for Chronic Immunodeficiency (CCI) of the University of Freiburg, we investigate if metabolic alterations contribute to the onset and progression of lysosome-related immune disorders, such as CHS.

To gain an in-depth understanding of how organelle dynamics and the organelle network infrastructure dictate

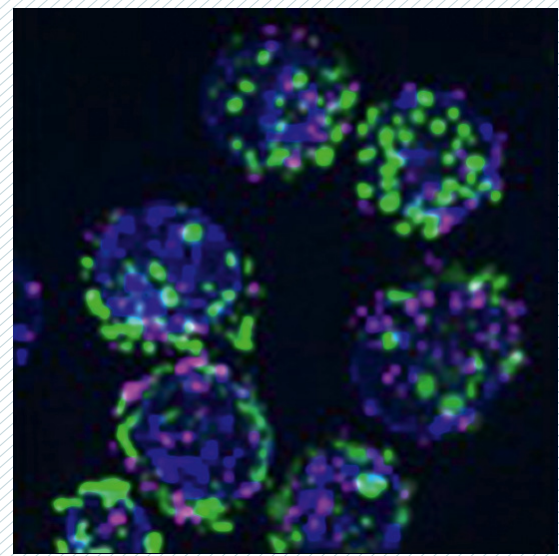


Figure 2 During an immune response different organelles (differentially colored) adapt their morphology and inter-organelle interaction position and functional interaction in the dense interior of the cell.

immune function in a spatiotemporal manner, we have a strong focus on state-of-the-art high-resolution microscopy and advanced live cell imaging techniques in combination with metabolic analysis and transcriptome profiling. Our overall goal is to identify novel therapeutic intervention points for organelle-related immune disorders, with the aim to extend our studies to human patient material. ↩

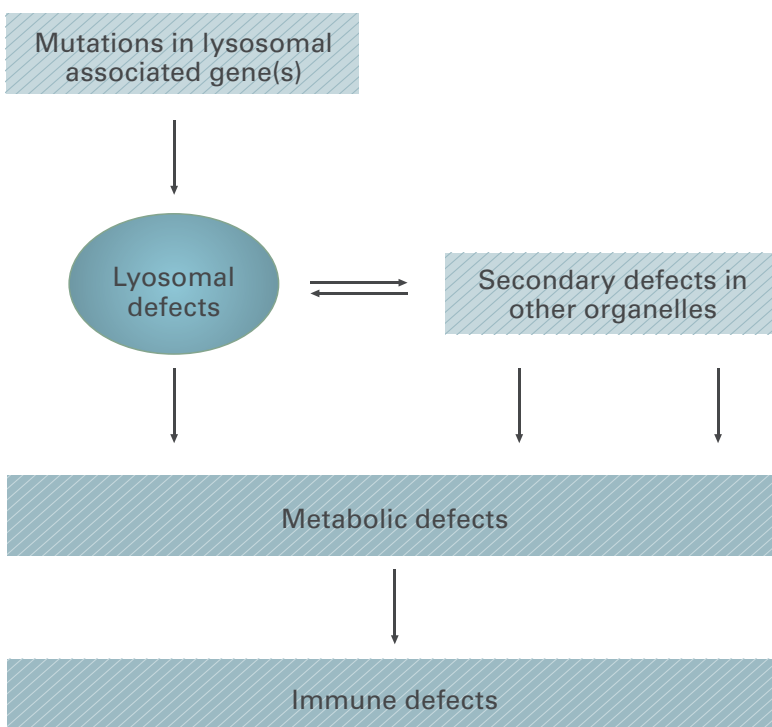
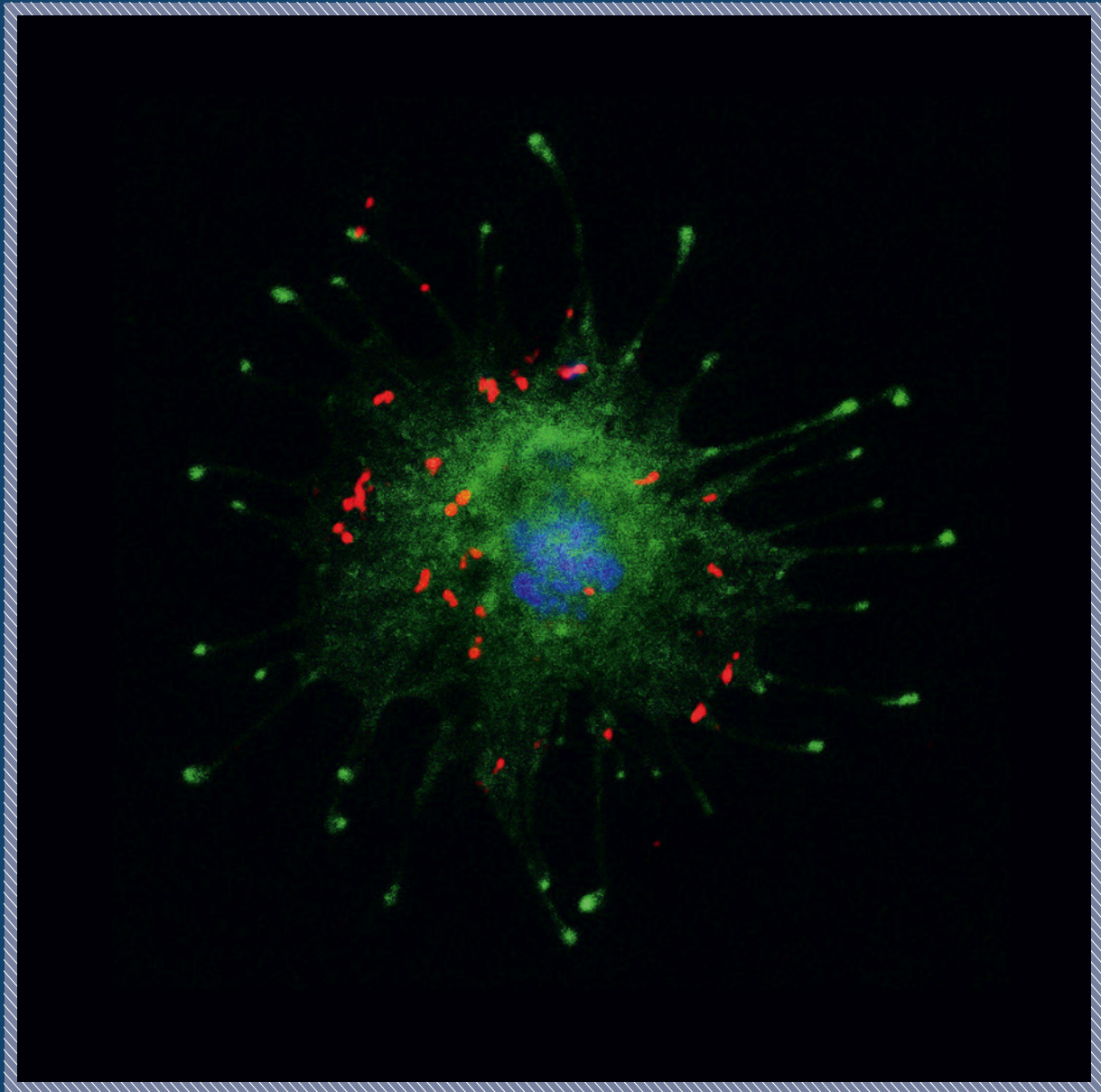


Figure 3 Model of organelle networks contributing to the onset and/or progression of organelle-related immunodeficiencies. Defects in metabolically active organelles, such as lysosomes, can affect the cell's metabolic flexibility, a process essential for the proper function of most immune cells. Organelle-driven changes in cellular metabolism can be initiated either directly by the damaged organelle or through spreading effects to secondary organelles along their functional network. Gaining insight into such network-spreading effects we hope to contribute to the mechanisms underlying organelle-related immune disorders and identify novel intervention points for patient care and treatment.

▶ LAB MICHAEL RETH

The power of synthetic biology: Study of the function of the protein tyrosine kinase SYK in the S2 Drosophila system. In this synthetic biology rebuilding approach we coexpressed the BCR (indicated by the red PLA signal) together with a GFP-Syk (green) fusion protein in S2 cells with a blue nuclear staining. In the presence of the BCR the GFP-Syk fusion protein becomes activated and then interacts with the cytoskeleton to form focal adhesion structures (green dots) that drastically alter the cellular appearance of the S2 cells.





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➤ SELECTED PUBLICATIONS

Maity PC, Blount A, Jumaa H, Ronneberger O, Lillemeier BF and Reth M (2015). B cell antigen receptors of the IgM and IgD classes are clustered in different protein islands that are altered during B cell activation. *Science Signaling* **8**, ra93.

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University/MPI-IE Senior Group Michael Reth

GENETIC AND NANOSCALE ANALYSIS OF B CELL SIGNALING PROCESSES

Study of the structure and function of the B cell antigen receptor with novel genetic and super-resolution techniques

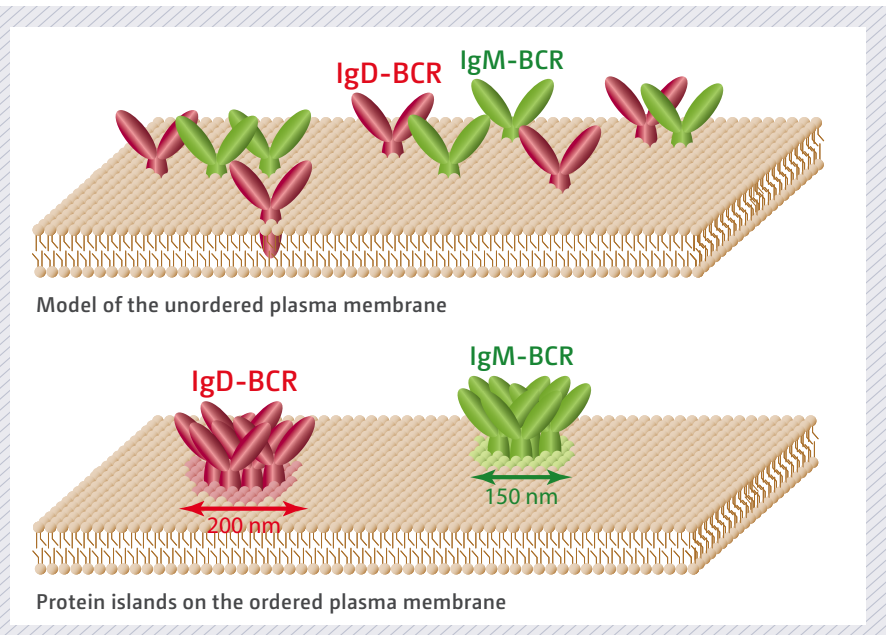


Figure 1 Previously it was thought that the two classes of the BCR on mature B cells namely the IgM-BCR (green) and IgD-BCR (red) are randomly distributed on the surface of resting B cells. Our super-resolution analysis showed that these receptors are clustered in separated, class-specific protein islands of roughly 150 nm and 200 nm diameters.

➔ The goal of the Department of Molecular Immunology is to gain 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. With the dissociation activation model (DAM) we have proposed a new model for the structure and activation of the B cell antigen receptor (BCR) and we have discovered new signalling components in activated B cells. Several of our findings have contributed to a better understanding of human diseases such as leukemia and autoimmunity. Furthermore, we are among the first groups to adopt synthetic biology approaches for studying signalling in mammalian cells. The department also organizes a basic and advanced teaching program for molecular immunology as part of the Bachelor/Master study in biology of the University Freiburg. This popular program attracts students from all over Germany and other parts of the world.

Gain-of-function and Synthetic Biology approaches in signalling research

Over the last two decades, more than 100 components have been discovered to be part of the diverse signal-

ling 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? Using only loss-of-function (knock-out) approaches it is difficult to gain a deeper insight into the function and signalling mechanisms of these elements. We thus have developed a method allowing the transient and inducible co-expression of up to 12 genes in the S2 *Drosophila* cell line. With this gain-of-function and synthetic biology approach, we have rebuilt the BCR and its proximal signalling elements and gained insight into the working principles of these molecules. In this way, we discovered that the BCR oligomer is opened by the protein tyrosine kinase Syk via an inside-out signalling and positive feed-back mechanism that results in a strong amplification of the BCR signal. We are currently using the same methods to study how the cytoskeleton is associated with the resting and activated BCR.

BCR conformation and nano-scale organization of the B cell membrane

Due to the diffraction barrier of 250 nanometers (nm) of visible light the nano-scale organization of proteins and re-

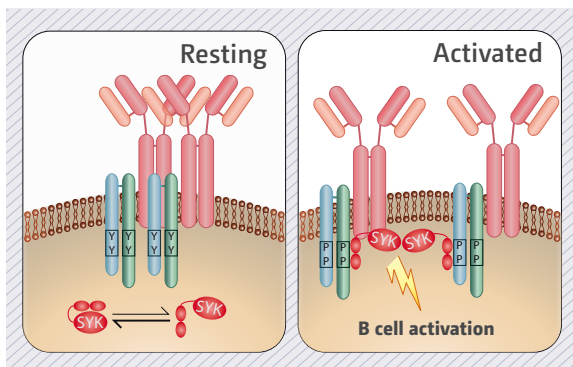


Figure 2 On resting B cells the BCR forms closed oligomers that cannot interact with the protein tyrosine kinase Syk. Upon B cell activation the BCR oligomers are opened and form a BCR/Syk complex that transmits signals into the cytosol.

ceptors on the membrane of living cells cannot be studied with the light microscope. A better knowledge of this topic is of great importance for biological research and medical applications that often target membrane structures. We have recently employed three different super-resolution techniques, namely two-color direct stochastic optical reconstruction microscopy (dSTORM), two-marker transmission electron microscopy (TEM) and the in situ proximity ligation assay (PLA) for the study of the organization of the B lymphocyte membrane.

We found that IgM-BCRs and IgD-BCRs reside on the plasma membrane in separated protein islands of approximately 150 and 240 nm dimensions, respectively. Upon B cell activation, the IgM and IgD protein islands become smaller and the two classes of receptors are now found in close proximity to each other.

These studies provide direct evidence for the nanoscale compartmentalization of the lymphocyte membrane. Furthermore, they suggest that upon B cell activation, the different IgM and IgD protein islands form nano-synapses which allow the exchange of lipids and proteins. This could explain how the IgM class antigen receptors find contact to Raft-associated lipids and proteins. The association of IgM with these lipids is a well-known hallmark of B cell activation. Together, these findings provide evidence for a preformed multimeric organization of BCRs

on the plasma membrane that is remodeled after B cell activation.

Studying B cell signalling with the Cre/loxP and CRISP/Cas9 techniques

Our department was one of the first to develop a tamoxifen-inducible Cre recombinase system allowing the remote regulation of genes in living organism. To date, we have sent our vectors and B cell specific Cre mice (mb1Cre and mb1CreERT2) to more than 200 labs all over the world. Currently, with the help of our Cre mice we are studying the role of the BCR and its signalling components such as the kinase Syk for the proper development and survival of B cells. We found that early deletion of the Syk gene results in an arrest of B cell development at the pre-B cell stage. More than 30 % of the mature B cells, however, survive for longer times in the mouse without Syk. Our further studies suggest that in the absence of Syk, the BCR co-receptor CD19 and the BAFF receptor can provide essential pro-survival signal for Syk-negative mature B cells.

Phosphatases also play an important role in suppressing hyperactive B cells and are 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.

With the CRISPR/Cas9 techniques one can rapidly delete or mutate genes of cells in culture, in a way that was not possible just a few years ago. We are using this technique to study the signalling processes in the human B cell line RAMOS. We have already generated more than 10 signalling RAMOS mutants. This mutant study is accompanied by a transcriptome and proteome analysis. ↩

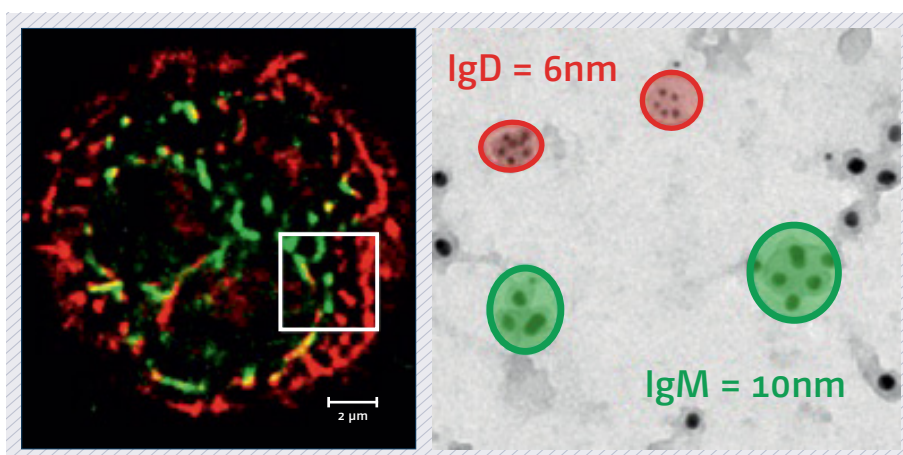
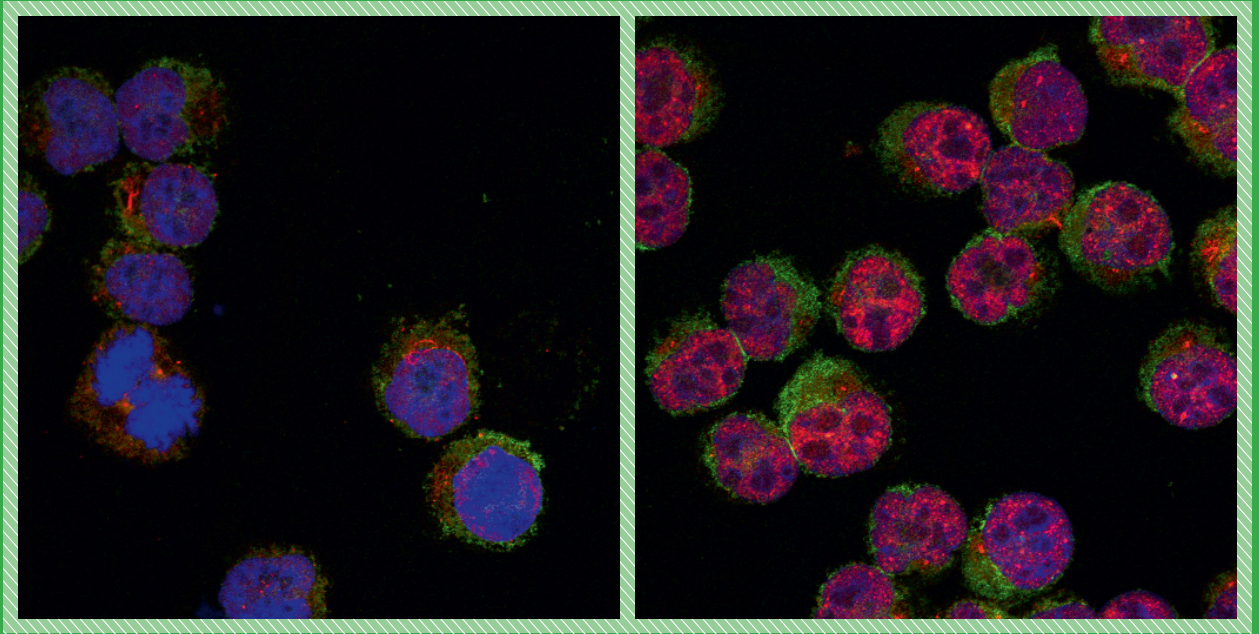


Figure 3 A two-color dSTORM (left) and two-marker TEM (right) super-resolution analysis of the nanoscale distribution of the IgM-BCR (green) and IgD-BCR (red) on the surface of resting B cells.

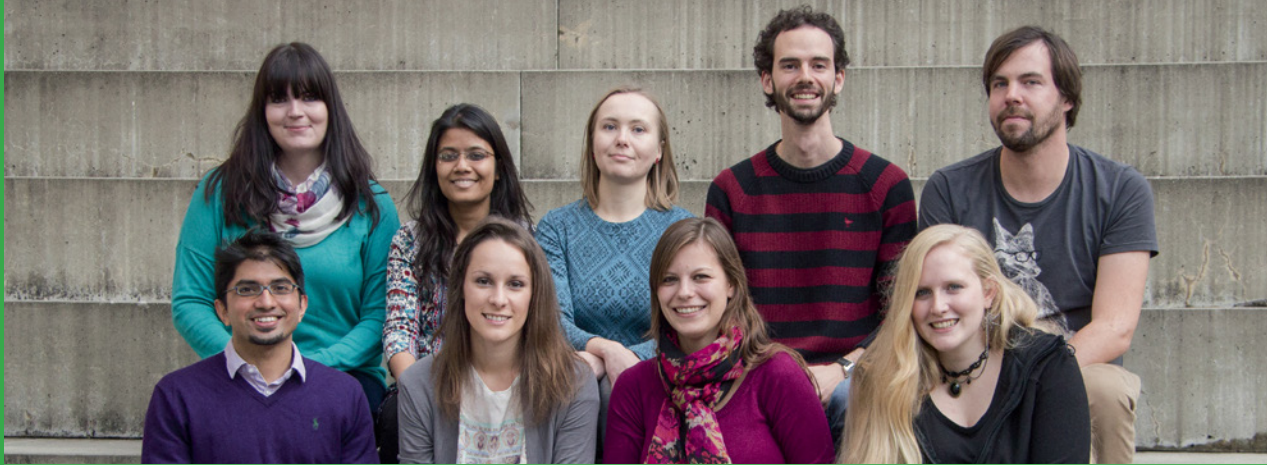


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➤ LAB RITWICK SAWARKAR

Increase in nuclear ubiquitination upon thermal stress in leukemia cells. Immunofluorescence image of K562 erythroleukemia cells stained with DAPI (blue), a cytosolic marker (green) and ubiquitin mark for degradation (red). Upon thermal stress (right image), an increase in nuclear ubiquitination is seen.

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Regulation of
transcription by
protein homeostasis



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➤ **SELECTED PUBLICATIONS**

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Group Ritwick Sawarkar

REGULATION OF TRANSCRIPTION BY PROTEIN HOMEOSTASIS

The role of molecular chaperones and proteasomes in gene expression

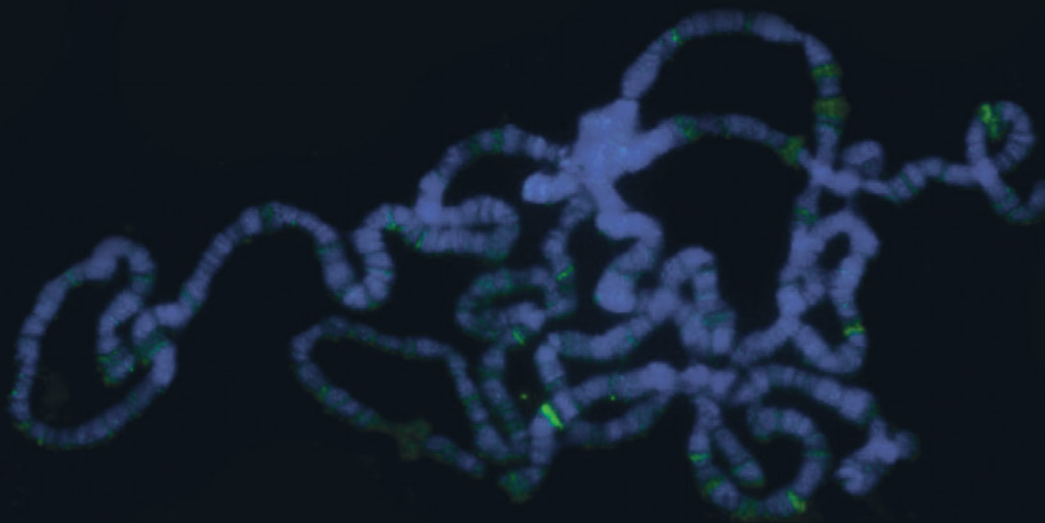


Figure 1 Activity of individual genes can be visualized by staining the giant chromosomes of salivary glands of *Drosophila*. In this picture, Cdc37 co-chaperone of heat-shock protein 90 (Hsp90) is shown to bind to specific loci on polytene chromosomes. This co-chaperone helps Hsp90 in stabilizing protein kinases. Which kinases are substrates of Cdc37-Hsp90 complex is not known.

➔ Protein homeostasis, or proteostasis, is orchestrated through coordinated activities of protein stabilization by chaperones and degradation by the proteasome forming the proteostatic network. By directly influencing the half-life of most proteins in the cell, this network plays the role of an essential quality controller in maturation of proteins and their complexes. Neurodegenerative diseases, ageing and cancers exhibit heightened proteotoxicity and hence are critically dependent on this network – inhibitors of chaperone heat-shock protein 90 (Hsp90) and proteasomes are successful cancer therapeutics. While the mechanisms and regulation of proteostasis occurring in the cytosol have been described in great detail, its function at chromatin, at sites of gene expression, is only now being deciphered.

Our lab focuses on chromatin-based protein homeostasis. Using the mammalian cells as a model, we aim to describe the proteostatic network operating at chromatin and elucidate how it helps reorganize chromatin during stress and disease.

Chromatin-based proteostatic network

Molecular chaperones and proteasomes mainly operate in the cytosol of mammalian cells. In recent pioneering work, others and we have shown that several components of the proteostatic network bind chromatin at a subset of enhancers and promoters (Figure 1). These studies pointed out that unlike the diffuse cytosol and nucleoplasm, proteostasis needs to function in a locus-specific manner in the chromatin context. This presents different biochemical challenges to the network of chaperones and proteasomes. How is the primarily cytosolic proteostatic machinery adapted to optimally execute its actions in the context of chromatin? By systematic analysis of chaperones and proteasomes bound to chromatin, we will address two specific questions:

(i) What are the mechanisms that target chaperones and proteasomes to specific genomic loci? By defining chromatin-binding profiles of these proteins in conjunction with their chromatin-based interactomes, we will outline the molecular basis of their chromatin recruitment in human cells. Our studies will provide a conceptual



framework to understand how cytosolic proteins reach specific chromatin locations.

(ii) How does local proteostasis at chromatin influence transcription? Stabilization of a repressor bound to promoter can lead to sustained transcriptional repression of the corresponding gene, whereas degradation of the same repressor bound to another promoter could cause activation of the cognate gene. Thus the context-dependent proteostasis can control transcription, which may be subject to environmental stimuli. By using specific inhibitors and knock-down approaches, we will delineate the functional links between proteostasis and transcription. Our recent exciting work has highlighted the role of proteostasis in repression of transposons in the mammalian genome (Figure 2).

Response of chromatin to environmental stress

Both chaperones and proteasomes help the cell during conditions of external stress. Cancer cells also exhibit enhanced proteotoxic stress owing to rapid proliferation, aneuploidy and genomic rearrangements. Consequently the normal functioning of the proteostatic machinery in cytosol and at chromatin is severely compromised. How does chromatin react to this situation? We employ the universal and highly conserved model of thermal stress on human cells to understand the proteome changes at chromatin induced by stress. We aim to elucidate how these changes in chromatin composition are brought about, and how they alter the transcriptional output of the cell during stress (Figure 3). By careful quantitation of the

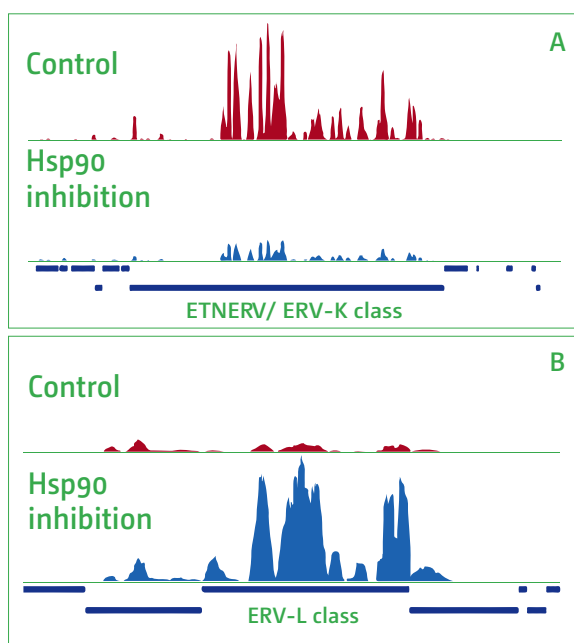


Figure 2 Genome browser snapshots of RNA sequencing data from mouse cells treated with or without a highly specific inhibitor of the chaperone heat-shock protein 90 (Hsp90). **(A)** Changes in RNA-seq reads at one of the loci representing mouse endogenous retrovirus type K (ERV-K); **(B)** Changes in RNA-seq reads at one of the loci representing ERV-L.

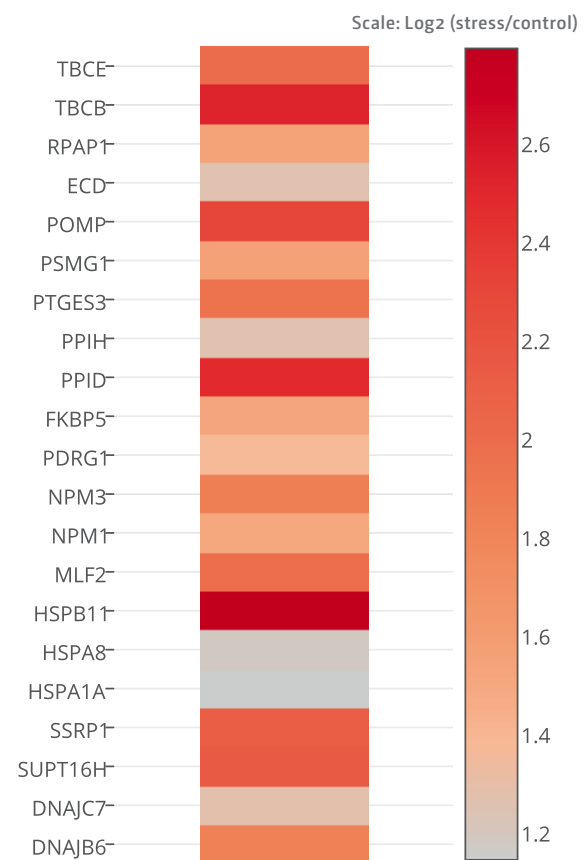
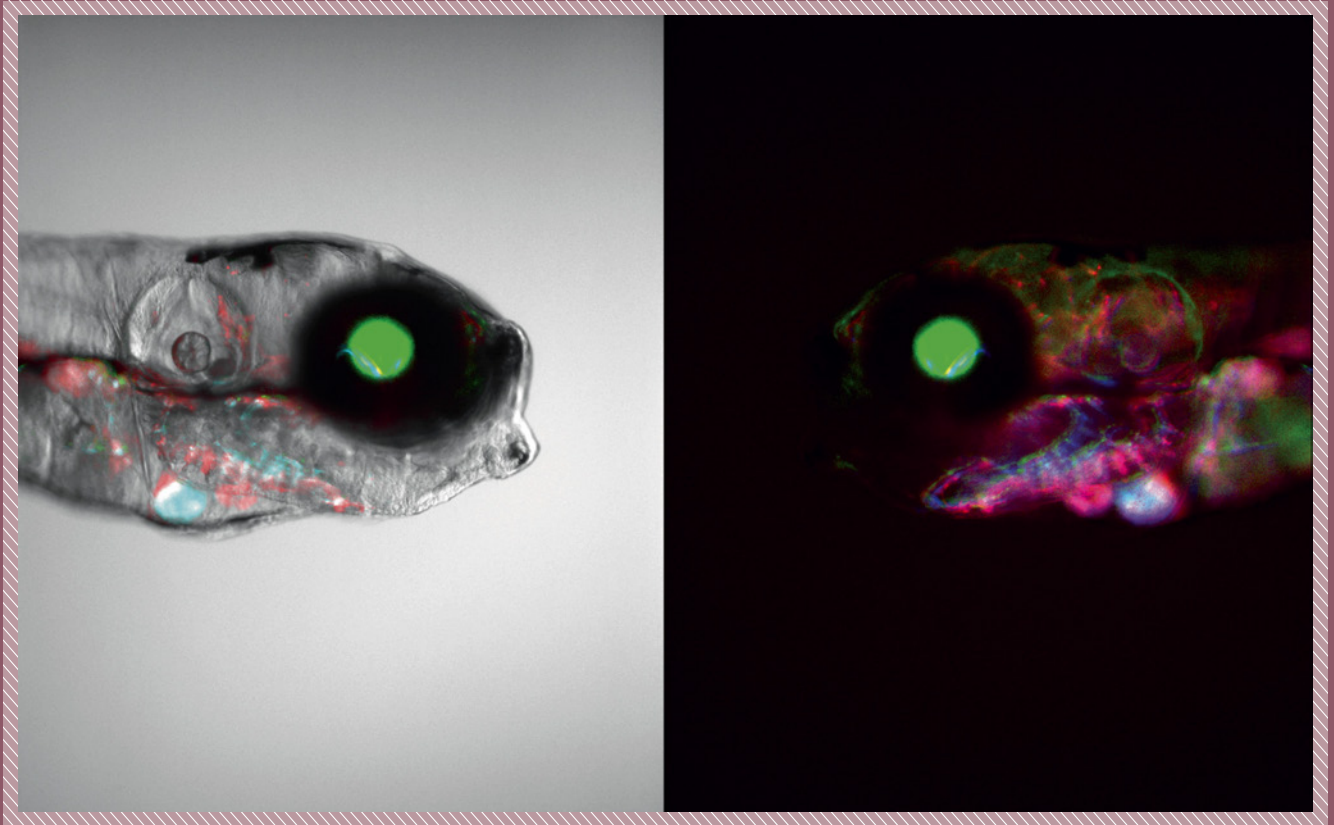


Figure 3 A heat map depicting quantitative changes in abundance of chromatin-associated proteins upon stress in human cells. SILAC-mass spectrometry done on chromatin of human cells subjected to thermal stress revealed an increase in the amount of several ubiquitin ligases, suggesting an increase in protein turnover.

abundance of chromatin-associated proteins on global scale, we demonstrated an increase in transcriptional repression- and histone modification machinery during stress. We aim to uncover the molecular pathways that sense stress and then cause relocalization of proteins to chromatin. Moreover, by globally analyzing the transcriptome and RNA polymerase II activity, along with histone modification profiles, we will functionally couple the proteome changes with transcriptional output.

Our studies of stress-induced chromatin changes extend to investigate if similar pathways play a role during cancer pathogenesis. In collaboration with colleagues at the Institute and at the University of Freiburg, we employ a mouse model of Acute Myeloid Leukemia (AML) driven by oncogenic fusion proteins of transcriptional regulators. We investigate if chromatin-associated proteostatic network is altered during oncogenesis, and if the activity of the network is causally associated with the disease. Finally, we are developing nuclear-specific inhibitors of chaperones and proteasomes in order to understand the precise contribution of proteostasis in this location to cellular health and disease.

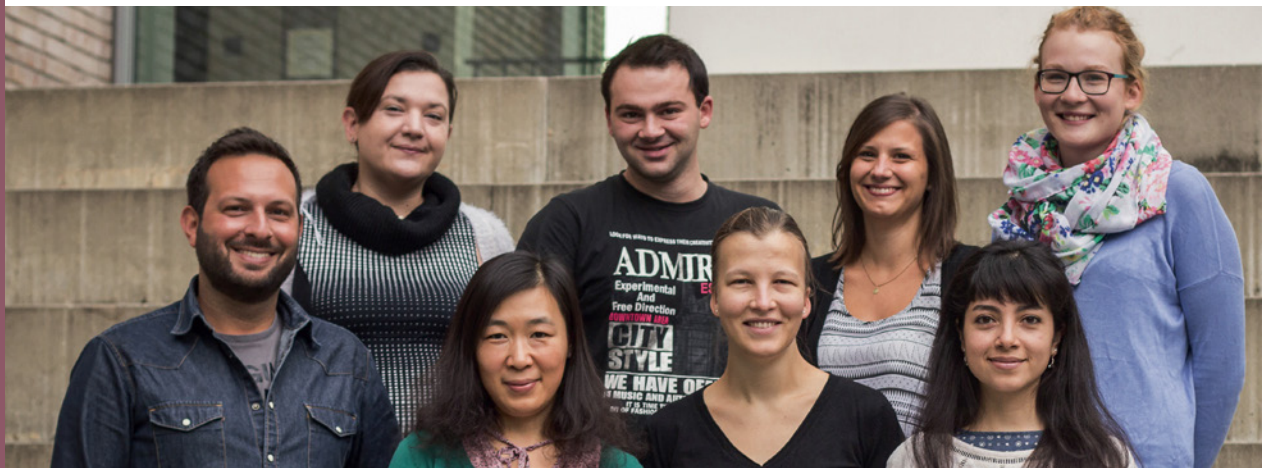


➤ LAB EIRINI TROMPOUKI

Fluorescent zebrafish, endothelial cells are marked with dsRED and CFP and hematopoietic cells with GFP.

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Bloody Signals



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➤ SELECTED PUBLICATIONS

Trompouki E (2015). Fish provide ID(H) eas on targeting leukemia. **Blood** **125**, 2880-2882.

Lin MI, Price EN, Boatman S, Hagedorn EJ, Trompouki E, Satishchandran S, Carspecken CW, Uong A, DiBiase A, Yang S, Canver MC, Dahlberg A, Lu Z, Zhang CC, Orkin SH, Bernstein ID, Aster JC, White RM and Zon LI (2015). Angiopoietin-like proteins stimulate HSPC development through interaction with notch receptor signaling. **eLife** **4**, 05544.

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BLOODY SIGNALS

Regulation of embryonic and adult hematopoiesis

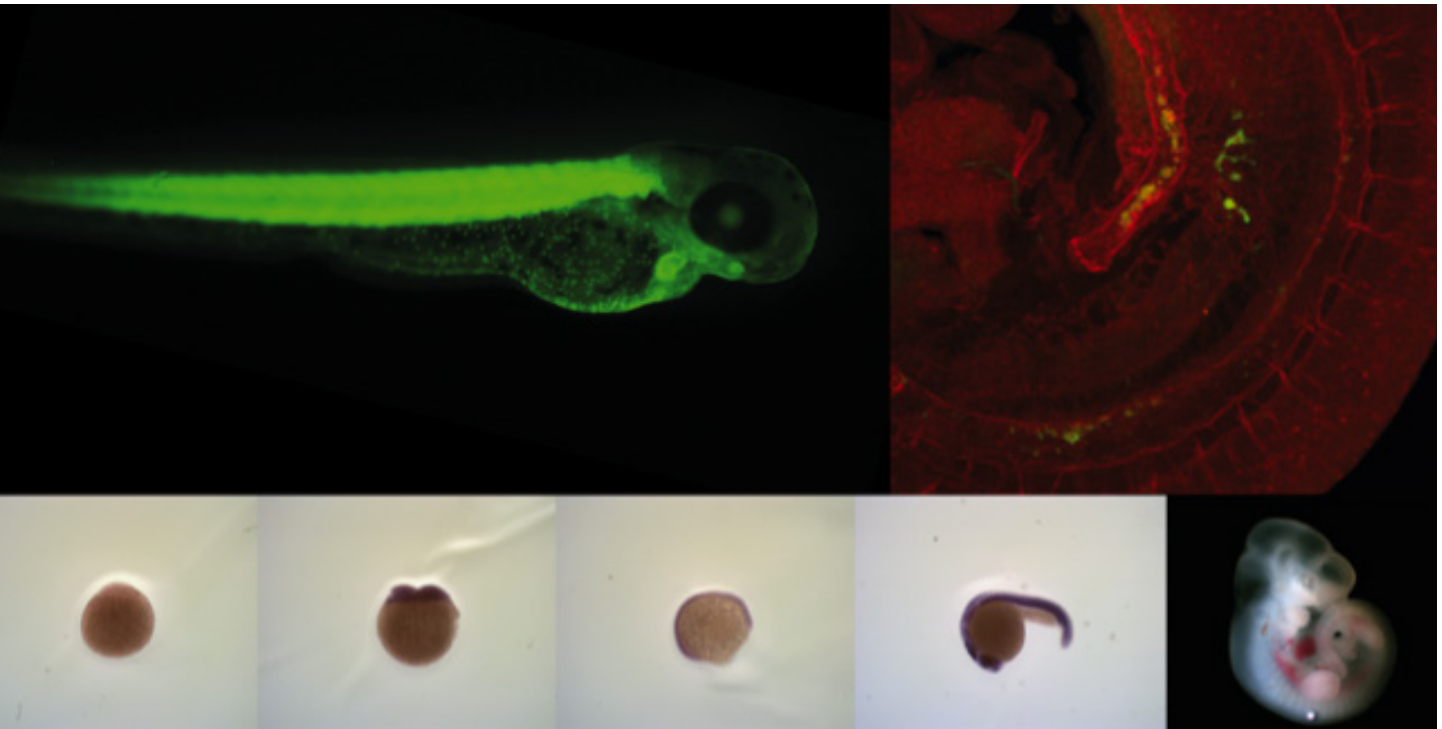


Figure 1 Different model organisms help to answer complex questions. In this figure zebrafish and mouse embryos are used to study hematopoiesis.

➤ Hematopoietic Stem Cells (HSCs) provide the foundation of the hematopoietic system in vertebrates. Being multipotent and capable of self-renewal, HSCs are responsible for constant production of all blood cell types throughout life. Because of their properties, HSCs are highly demanded in clinic daily. For example, HSCs are used for replenishing the hematopoietic system of acute myeloid leukemia (AML) patients after chemotherapy or for patients that need blood transfusions. However, due to their limited number and our inability to expand them sufficiently *in vitro* it is impossible, right now, to provide for these extensive needs. In addition, alterations in the properties of HSCs and their environment lead to disease. In order to be able to expand HSCs or cure hematopoietic malignancies arising from them, we need to understand the network of signals that govern their fate from the time they develop till maturity.

HSCs are initially generated during embryonic development, as proliferative cells that create the HSC pool of a vertebrate organism. During adulthood they reside in the bone marrow as quiescent HSCs and exit this state, almost exclusively, in case of stress or disease. In mice and zebrafish, HSCs are generated in the aorta-gonad-meso-

nephros (AGM) region from endothelial cells in a process termed endothelial-to-hematopoietic transition (EHT).

Multiple conserved signaling pathways governing EHT were identified in the recent years. For instance, Wnt, BMP, Vegf, and Notch signaling pathways are absolutely required for HSC emergence and have been utilized in attempts to generate functional HSCs from pluripotent stem cells *in vitro*. Due to the high complexity of HSC ontogeny, these approaches have thus far proven unsuccessful, indicating that missing key signals are yet to be discovered. Therefore, our first goal is to delineate precisely the mechanisms involved in HSC emergence *in vivo* and improve current strategies. In addition, many of these signaling networks that affect HSC ontogeny, play a role in HSC maintenance during adulthood. Our studies extend also to adult hematopoiesis, in an effort to create a temporal (from embryo till adult) signaling network imperative for hematopoiesis. Finally, we combine our results with published databases to study specific transcription factor networks that are deregulated in hematopoietic diseases. Our main goal is to identify combinations of mutated or deregulated transcription factors and unravel how they can lead to disease.

Signaling networks during hematopoietic ontogeny

Like all cells, HSCs are controlled by a complex cascade of signaling pathways that are either synergistic or antagonistic and ultimately determine cell decisions. Inflammatory signaling pathways like TLR, TNF, IFN were mostly associated with immune cells, but recently, it was shown that they can directly stimulate hematopoietic stem cells both under steady-state and stress conditions. Furthermore, inflammatory signaling was proven to be indispensable for HSCs emergence. We focus on understanding how inflammatory signaling affects HSC formation during hematopoietic development. Furthermore, our studies target on uncovering how inflammatory signaling can synergize with developmental pathways like Wnt, Notch and BMP to orchestrate HSCs development. We use zebrafish and mice to study HSC emergence and the epistatic interactions of different signaling pathways. We employ CRISPR-CAS9 technology, genetic and chemical screens, imaging and other methods in mice and zebrafish to unravel how HSCs are formed during EHT (Figure 1,2). Ultimately we follow the knowledge that we gain from the embryonic system in adult hematopoiesis. We examine how different inflammatory sensors are essential for the maintenance of the hematopoietic system in adult zebrafish and mice (Figure 2).

Transcription factor networks and disease modeling in zebrafish

Hematopoietic malignancies are usually complex, depending on many different mutations and diverse pathways. Zebrafish has emerged as an important model

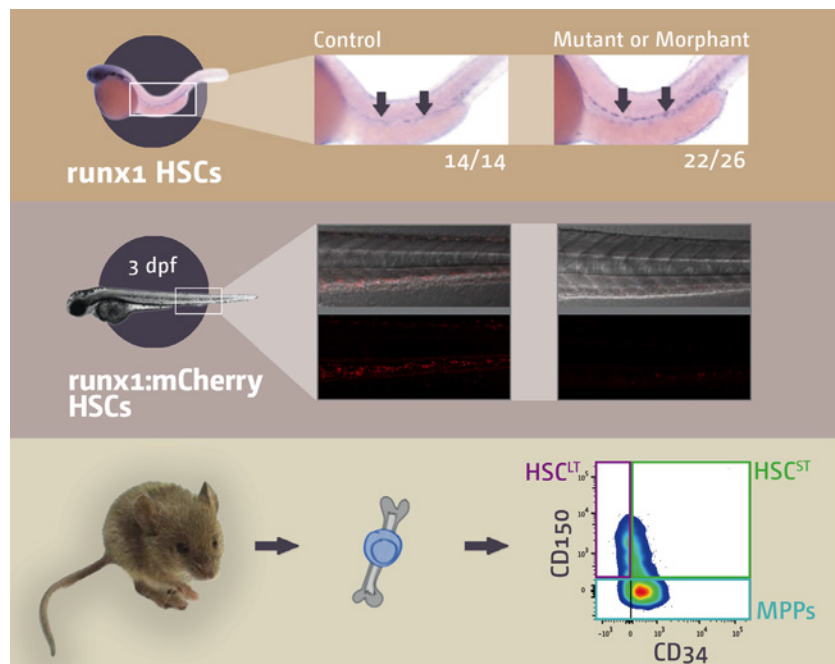


Figure 2 Identification of novel regulators of HSC emergence and maintenance in zebrafish and mice. HSCs are detected by whole mount in situ hybridization, live imaging or FACs sorting.

in biomedical sciences due to its genetic malleability and the possibility of performing large-scale chemical screens.

We focus on previously uncharacterized or poorly-characterized transcription factors that have been found deregulated or mutated in hematological malignancies. We use zebrafish to study the physiological role of these genes in vivo and determine epistatic relations between them. Moreover, we are trying to combine developmental biology with molecular biology techniques and high throughput sequencing to find upstream regulators and downstream targets of the respective transcription factors, thus creating a disease-related network (Figure 3). Our ultimate goal is to create disease models and use them to identify novel therapeutic substances by performing drug screens in zebrafish.

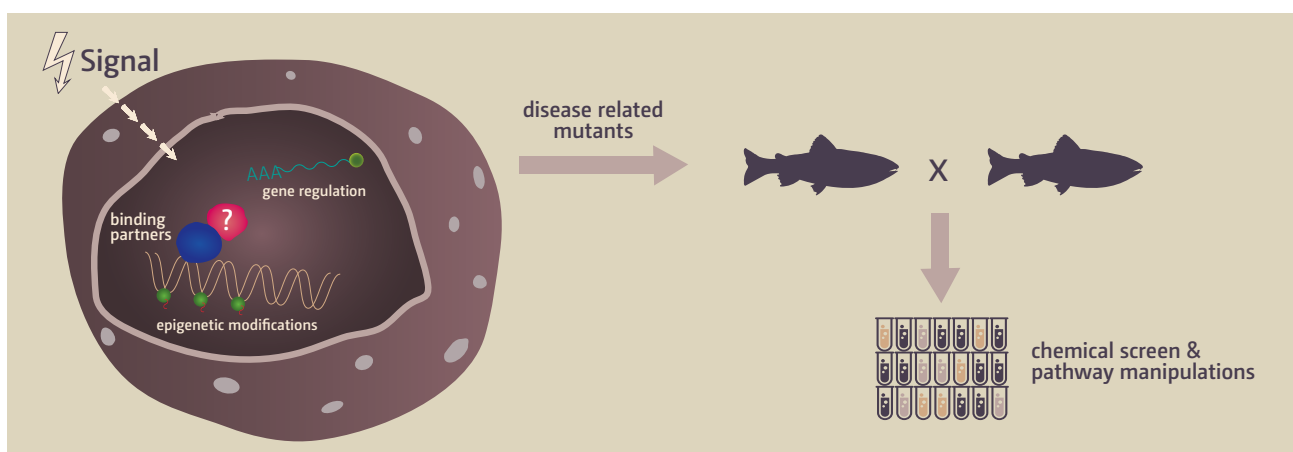


Figure 3 Transcription factor networks and disease modeling in zebrafish

Emeritus Group Rolf Kemler

CELL ADHESION AND WNT/ β -CATENIN SIGNALING IN MOUSE DEVELOPMENT

Key molecules in signal transduction and cell proliferation

➔ 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 cadherin-mediated adhesion and as the downstream effector of the canonical Wnt signaling pathway is addressed by combining *in vitro* and *in*

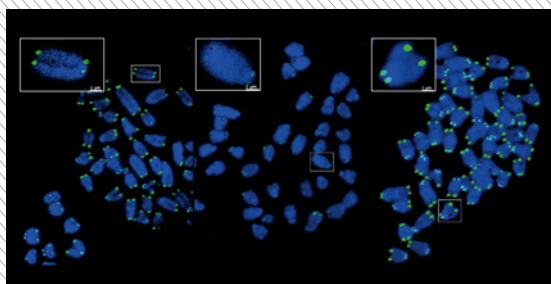


Figure 1 Wnt/ β -catenin signaling regulates Telomerase in stem cells and cancer cells. Telomere length determined by quantitative fluorescence *in situ* hybridization analysis.

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 mu-

tant 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 -Catenin-deficient 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.



➔ LAB MEMBERS

Administration
Rosemary Schneider

Technician
Katharina Maurer

Postdoctoral Fellow
Kathrin Hoffmeyer

➔ GROUP LEADER ROLF KEMLER

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

1971-1973 Ph.D. at the MPI of Immunobiology, Freiburg, Germany

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

1981-1987 Group Leader at Friedrich-Miescher-Laboratorium of the Max Planck Society, Tübingen, Germany

1987-1992 Group Leader at MPI of Immunobiology, Freiburg, Germany

1992-2013 Director at the MPI-IB/MPI-IE, Freiburg, Germany

Since 2013 Emeritus Group Leader at Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



➔ SELECTED PUBLICATIONS

Yakulov T, Raggioli A, Franz H, Kemler R. (2013) Wnt3a-dependent and -independent Protein Interaction Networks of Chromatin-bound β -catenin in Mouse Embryonic Stem Cells. **Molecular Cell Proteomics** **12**, 1980-1994.

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PAST RESEARCH GROUPS (2014-2016)

During 2014 and 2016 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.



PATRICK HEUN
(2005-2014)

Present affiliation: Wellcome Trust
Centre for Cell Biology, Edinburgh, UK

➔ 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. 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 segregation to the daughter cells. Using the fruit fly *Drosophila melanogaster* 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.



SIMONA SACCANI
(2005-2014)

Present affiliation: Institute for
Research on Cancer and Aging, Nice,
France

➔ Our research group studies the molecular mechanisms which regulate gene expression, using the NF- κ B 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- κ B family of transcription factors is crucial for the expression of multiple genes involved in cell survival, proliferation, differentiation and inflammation. The NF- κ B pathway is broadly active in multiple cell types, yet many NF- κ B target genes show a tightly-regulated pattern of expression, with stimulus-dependent activation restricted to a particular biological setting. To investigate the molecular mechanisms underlying this, we focus on differences in the chromatin structure of target promoters and enhancers, and on co-factors which can regulate the recruitment and activity of NF- κ B in specific cell-types.



HASSAN JUMAA
(2001-2013)

Present affiliation: Institute of Immunology,
University Medical Center Ulm,
Germany

➔ 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. Defects in the signal transduction of B cells result in immunodeficiency, autoimmunity or leukaemia. Studying signal transduction in B cell development allows multiple interactions and interdisciplinary collaborations with research groups in both basic science and in the clinic.



TARŌ FUKAO
(2008-2013)

Present affiliation: Tokyo, Japan

➔ The aim of our research group is to understand the biology of functional RNAs in the hematopoietic-immune system. We are focusing on the role of microRNAs in the mammalian hematopoietic-immune system. MicroRNAs are a class of non-coding RNAs binding to the complementary mRNAs and regulate their expression. They are found in animals, plants and viruses with their sequences conserved even among relatively distant species. Various reports have shown the involvement of microRNAs in a broad range of physiological events such as development, differentiation, proliferation, morphogenesis, apoptosis and metabolism. Studying the biology of microRNA in the immune system may directly contribute to understanding the molecular mechanism of immune system integrity. Furthermore, the knowledge of microRNA in immunology would provide clues to elucidate the molecular pathogenesis of infection and immune diseases such as autoimmune and inflammatory diseases.





03

Research Facilities

DEEP SEQUENCING FACILITY



High throughput data production with latest sequencing technology supporting a broad range of research applications. TOP: Illuminas' HiSeq2500
Bottom: NextSeq500 and MiSeq.

➤ SELECTED PUBLICATIONS

Arrigoni L, Richter AS, Betancourt E, Bruder K, Diehl S, Manke T and Bönisch U (2015). Standardizing chromatin research: a simple and universal method for CHIP-seq. **Nucleic Acids Research** DOI: [10.1093/nar/gkv1495](https://doi.org/10.1093/nar/gkv1495).

Mayer SC et al (2015). Adrenergic Repression of the Epigenetic Reader MeCP2 Facilitates Cardiac Adaptation in Chronic Heart Failure Novelty and Significance. **Circulation Research** **117**, 622-633.

Bönisch U, Arrigoni L, Betancourt E, Bruder K (2015). Automatisierte Herstellung von CHIP-Seq-Sequenzbibliotheken. **BIOspektrum** **5**, 526-527.

Roidl D, Hellbach N, Bovio PP, Villarreal A, Heidrich S, Nestel S, Grüning BA, Boenisch U, Vogel T (2015). DOT1L Activity Promotes Proliferation and Protects Cortical Neural Stem Cells from Activation of ATF4-DDIT3-Mediated ER Stress in Vitro. **Stem Cells** **34**, 233-245.

Gilsbach, Ralf et al. (2014). Dynamic DNA Methylation Orchestrates Cardiomyocyte Development, Maturation and Disease. **Nature Communications** **5**, 5288.

Öst A, Lempradl A, Casas E, Weigert M, Tiko T, Deniz M, Pantano L, Boenisch U, Itskov PM, Stoeckius M, Ruf M, Rajewsky N, Reuter G, Iovino N, Ribeiro C, Alenius M, Heyne S, Vavouri T, Pospisilik JA (2014). Paternal Diet Defines Offspring Chromatin State and Intergenerational Obesity. **Cell** **159**, 1352-1364.



MEMBERS



Technicians

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Laura Arrigoni

ULRIKE BÖNISCH

2002-2007 Undergraduate Studies in Nutritional Sciences, University of Hohenheim, Stuttgart Germany

2007-2012 Ph.D. 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



The Deep Sequencing Facility at the MPI-IE offers high-throughput sequencing services mainly for in-house research groups. The facility is equipped with modern state-of-the-art technology. Next to standardized quality controls, library preparation and deep-sequencing, the highly qualified staff constantly works towards optimization and automation of existing workflows according to the latest international standards. Apart from collaborating with all groups at the institute, the unit supports large consortia such as the German Epigenome Programme (DEEP) and CRC992 (Medical Epigenetics). In the context of these collaborations, we produce reference epigenomes from various tissues and human diseases.

Technology

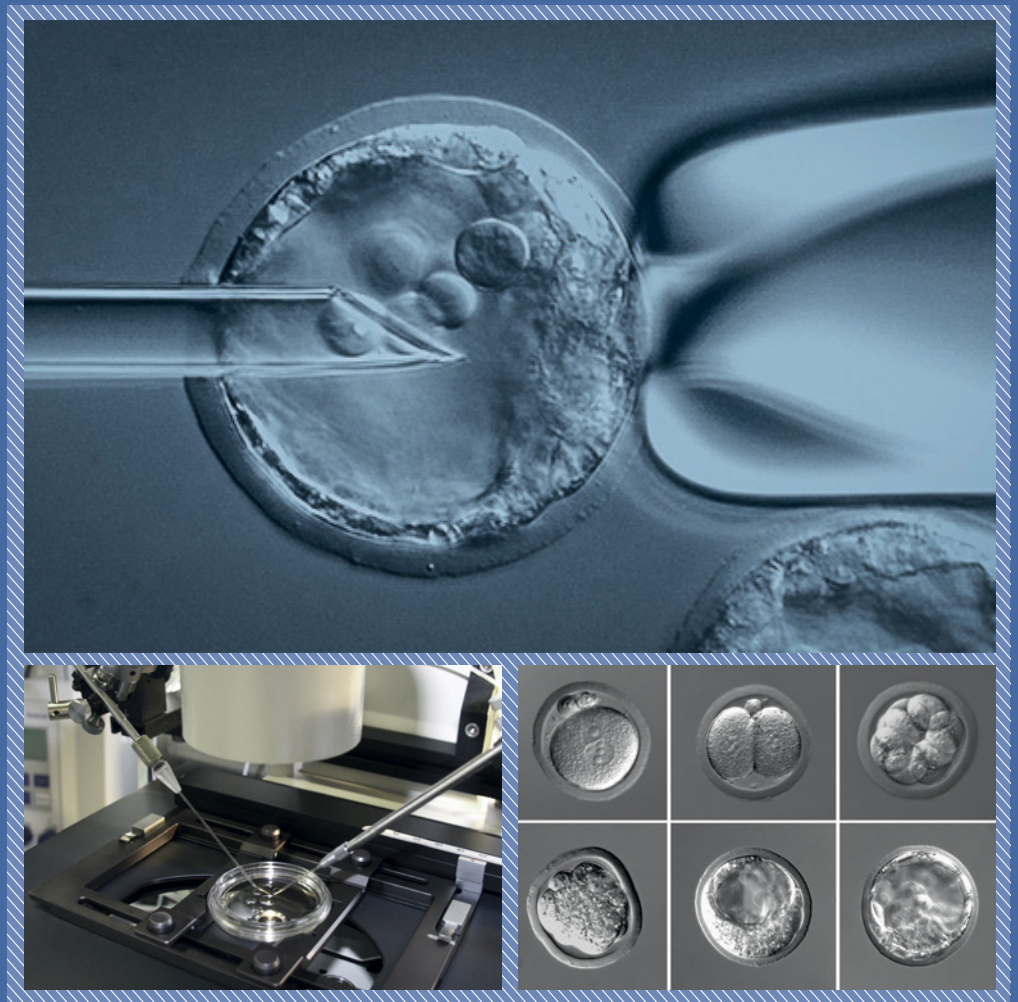
The unit employs state-of-the-art sequencing technology (Illumina HiSeq2500®, NextSeq500® and MiSeq®) and various protocols to infer the sequence content for a wide range of cellular samples submitted to the facility. We have implemented robust standard operation procedures, adequate instrumentation for parallelized sonication (Covaris E220™) and quality controls (Fragment Analyzer™, Qubit™ Fluorometer). To ensure standardized sample handling and fast

turnaround times, we have translated highly utilized protocols (ChIP-Seq, RNA-Seq) into automated workflows using liquid handling stations (epMotion 2075TMX, Eppendorf, IP-Star SX-8GCompact, Diagenode). This has significantly enhanced throughput and technical reproducibility.

Methodological development

We support a broad range of next generation sequencing applications over various sample types and input amounts including ChIP-Sequencing, Whole-Genome Sequencing, Methylation Sequencing as well as several RNA-Sequencing techniques such as mRNA-, Total-RNA and small-RNA Sequencing. To enable research with the latest genomic tools and tailor protocols to the specific needs of the research groups the unit supports methodological developments, such as the optimization, automation and standardization of new protocols. In recent developments and collaborations we have successfully established protocols for single-cell transcriptome analysis and standardized ChIP-seq processing for small cell numbers.

TRANSGENIC MOUSE FACILITY



Top: Embryonic stem cells injection of a mouse blastocyst; Left: Microinjection chamber on an inverted microscope; Right: Preimplantation stages of mouse embryonic development.

➤ SELECTED PUBLICATIONS

Balbach ST, Esteves TC, Houghton FD, Siatkowski M, Pfeiffer MJ, Tsurumi C, Kanzler B, Fuellen G, Boiani M (2012). Nuclear reprogramming: kinetics of cell cycle and metabolic progression as determinants of success. *PLoS One* 7(4), e35322.

Bedzhov I, Liszewska E, Kanzler B, Stemmler MP (2012). Igf1r signaling is indispensable for preimplantation development and is activated via a novel function of E-cadherin. *PLoS Genet* 8(3), e1002609.

Corbeaux T, Hess I, Swann JB, Kanzler B, Haas-Assenbaum A, Boehm T (2010). Thymopoiesis in mice depends on a Foxn1-positive thymic epithelial cell lineage. *Proceedings of the National Academy of Sciences USA* 107, 16613-16618.



MEMBERS



Technician
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Student Assistant
Ana Paula Sanchez Carranza

BENOÎT KANZLER

1996 Ph.D. thesis in Cellular and Molecular Biology, 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

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 gene-targeting technology for generating genetically altered mouse models 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. The centralization of such services has proven to provide enormous benefits for cost effective research and saving resources.

Our barrier facility maintains a specific pathogen free (SPF) health status, housing gene modified mice in sterile and ventilated cage systems (IVCs) that are serviced by intensively trained and skilled animal care staff. Microinjected embryos are reimplanted under strict sterile conditions and SOPs 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 analyzing their mouse lines in compliance with the German law.

In parallel, we strive to implement and develop new technologies, e.g. establishment of new methods for ES cell derivation or genome edition using the CRISPR/Cas system. As an example, we recently succeeded in deriving several new C57Bl/6N feeder-dependant and feeder-independent ES cell lines with high germline transmission potential.

In order to keep track of all experimental parameters, conduct data analysis and edit statistics and reports, a TG-Unit 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.

FISH FACILITY



The zebrafish, *Danio rerio*, is the most used animal model in the fish facility of the MPI-IE.

➤ SELECTED PUBLICATIONS

- Hess I, Boehm T (2012). Intra-vital imaging of thymopoiesis reveals dynamic lympho-epithelial interactions. **Immunity** **36**, 298-309.
- Iwanami, N, Mateos, F, Hess, I, Riffel, 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. **Journal of Immunology** **15**, 7060-7066.
- Soza-Ried, C, Hess, I, Netuschil, N, Schorpp, M and Boehm, T (2010). Essential role of c-myb in definitive hematopoiesis is evolutionarily conserved. **Proceedings of the National Academy of Science USA** **107**, 17304-17308.



MEMBERS

Technicians

Donatus Bönsch
Uwe Heinzmann
Oleksandr Kuzmenko

Mirco Schultis
Dominik Zota

MICHAEL SCHORPP

1993-1996 Ph.D. 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 at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

➤ 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 extracorporeal 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.

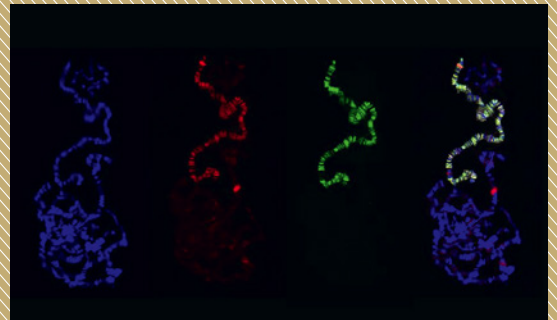
Service
The fish facility at the Max Planck Institute of Immunobiology and Epigenetics was opened in 2007. It houses zebrafish (*Danio rerio*) and Medaka (*Oryzias latipes*) fish. Each room is equipped with an independent water treatment unit. Ac-

cess 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 ENU-induced 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, conventional transgenesis
- Cell transplantations in embryos and adult fish
- ENU mutagenesis, CRISPR/Cas9 mutagenesis
- Homozygosity mapping/positional cloning
- Pressure-driven microinjection of mRNA, DNA, or anti-sense morpholino oligonucleotides into fertilized eggs
- Sperm cryoconservation and in vitro fertilisation

FLY FACILITY



Top: Plamen Georgiev and a Ph.D. student exploring *Drosophila* in the Fly room. Left: Fruit flies under the microscope. Right: Polytene chromosome squash preparation from male third instar *Drosophila* larva labeled with antibodies against MSL1 (red) and MLE (green) decorating the X chromosome.

➤ SELECTED PUBLICATIONS

Ramirez F, Lingg T, Toscano S, Lam KC, Georgiev P, Chung HR, Lajoie B, de Wit E, Zhan Y, de Laat W, Dekker J, Manke T, Akhtar A (2015). High-affinity sites form an interaction network to facilitate spreading of the MSL complex across the X chromosome in *Drosophila*. **Molecular Cell** **60**, 146-62.

Quinn JJ, Ilik IA, Kun Qu, Georgiev P, Chu C., Akhtar A., Chang HY (2014). Revealing long noncoding RNA architecture and functions using domain-specific chromatin isolation by RNA purification. **Nature Biotechnology** **32**, 933-940.

Dias J, Van Nguyen N, Georgiev P, Gaub A, Brettschneider J, Cusack S, Kadlec J, Akhtar A (2014). Structural analysis of the KANSL1/WDR5/KANSL2 complex reveals that WDR5 is required for efficient assembly and chromatin targeting of the NSL complex. **Genes Development** **28**, 929-942.

MEMBERS



Technicians

Ute Lenz
Noel Gutierrez

PLAMEN GEORGIEV

2004–2007 Ph.D. studies in Molecular biology & Genetics at Cambridge University, Cambridge, UK

2007–2010 Postdoctoral fellow at the Babraham Institute, Cambridge, UK

Since 2010 Head of the Fly Facility at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



➤ 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.

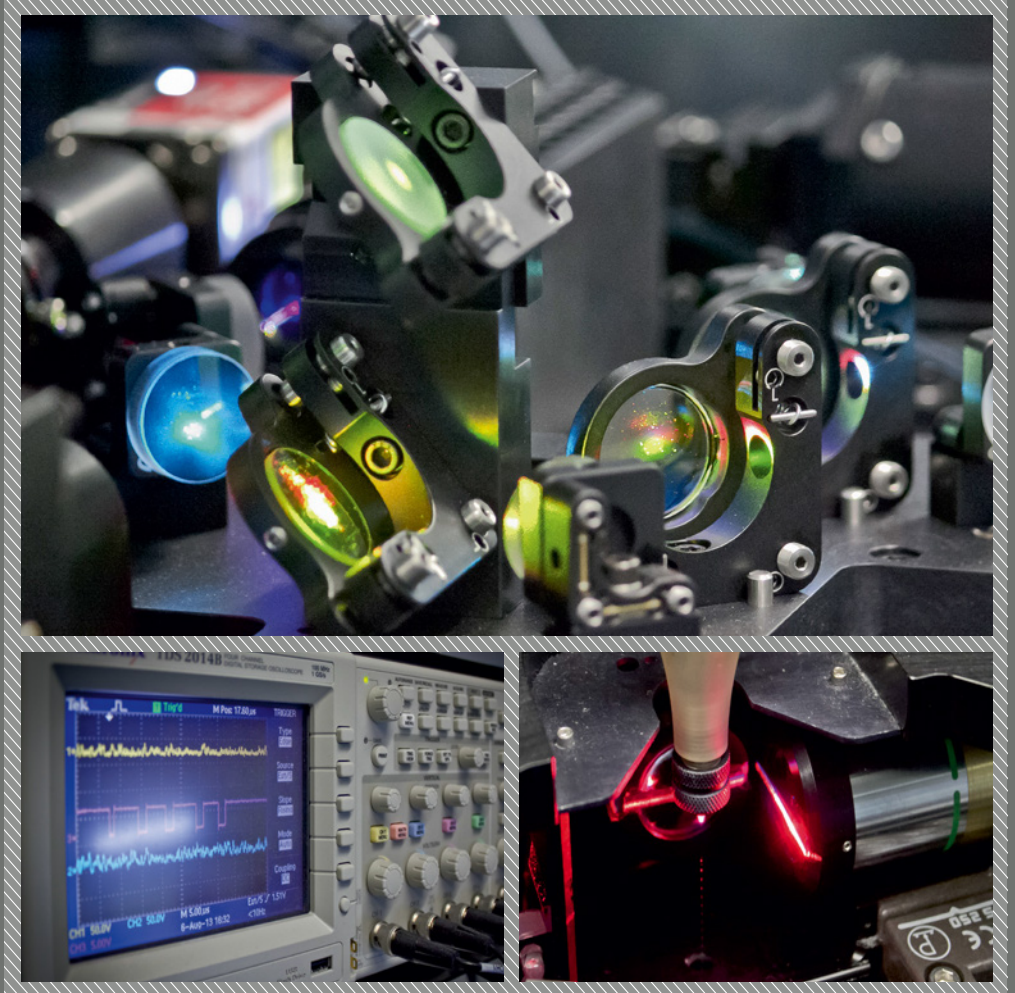
Importantly, many aspects of development and behaviour in *Drosophila* parallel those in humans. The completion of both the human and *Drosophila* genome sequencing projects revealed that more than 75% of human genetic disease genes have clear homologues in the fruit fly. Taking advantage of the significantly 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 CO₂ 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 as 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 infrastructure, consult and support in applying advanced genetic techniques (such as gene targeting by homologous recombination or TALENS, ZFNs, CRISPR/Cas9-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.

FLOW CYTOMETRY & DNA SEQUENCING FACILITY



Top: A complex mirror system leads the laser beams to the interrogation point where the laser intersects with the samples.

➤ SELECTED PUBLICATIONS

Gilsbach R, Preissl S, Grüning BA, Schnick T, Burger L, Benes V, Würch A, Bönisch U, Günther S, Backofen R, Fleischmann BK, Schübeler D and Hein L (2014). Dynamic DNA methylation orchestrates cardiomyocyte development, maturation and disease. **Nature Communications** 5, 5288.

Engelke R, Riede J, Hegermann J, Würch A, Eimer S, Dengjel J and Mittler G (2014). The Quantitative Nuclear Matrix Proteome as a Biochemical Snapshot of Nuclear Organization. **Journal of Proteome Research** 13, 3940-3956.



MEMBERS



Technicians

Sebastian Hobitz
Maïke Knoblauch

Konrad Schuldes
Doris Wild

ANDREAS WÜRCH

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

1993-1999 Research Assistant at the Department of Cellular and Molecular Immunology, 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



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 many advanced flow cytometry applications.

Sequencing Facility

The DNA Sequencing Core Facility provides access to automated DNA sequencing. The sequencing service processes samples on a 48-capillary ABI 3730 DNA Analyzer. We had a total of 50.000 sequences last year.

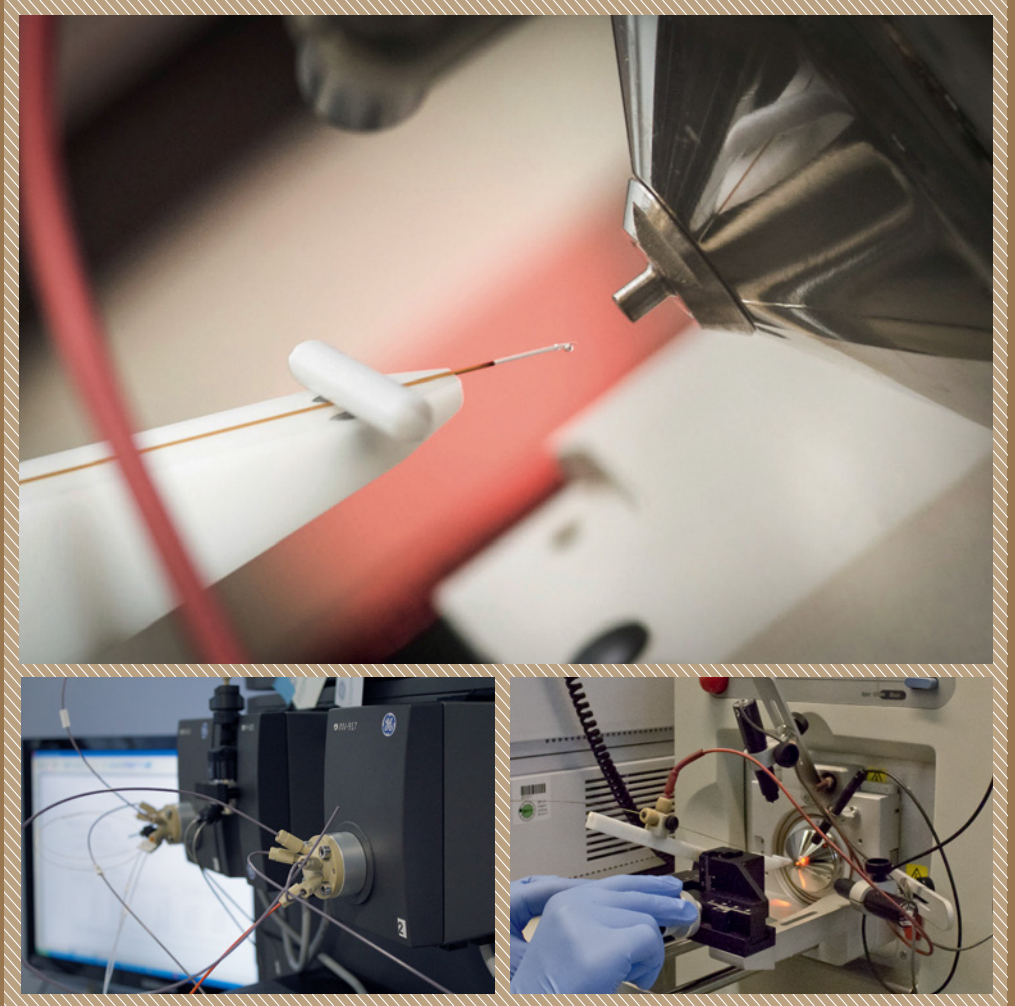
The facility is equipped with the most advanced, state-of-the-art flow cytometers. For sorting, we have a Beckman Coulter MoFlo XDP sorter, two Becton Dickinson (BD) FACSAria sorters, a (BD) Influx sorter, a (BD) AriaFusion and a Miltenyi autoMACS magnetic sorter. For analysis of cell samples our facility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

teen fluorescent parameters (Influx, AriaFusion and Aria III), respectively. 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 96-well 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 2014 we sorted more than 6000 samples.

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)

PROTEOMICS FACILITY



Top: Nano capillary chromatography column (tip diameter ≈ 0.008 mm) interfaced with electrospray ionization source of mass spectrometer. Left: High Performance Liquid Chromatography (HPLC) system for separation of peptides and proteins. Right: Setting up peptide analysis on a LTQ-Orbitrap mass spectrometer.

➤ SELECTED PUBLICATIONS

Hein K, Mittler G, Cizelsky W, Kühl M, Ferrante F, Liefke R, Berger IM, Just S, Sträng JE, Kestler HA, Oswald F, Borggreffe T (2015). Site-specific methylation of Notch1 controls the amplitude and duration of the Notch1 response. *Science Signaling* **8**, ra30.

Engelke R, Riede J, Hegermann J, Wuerch A, Eimer S, Dengjel J, Mittler G (2014). The Quantitative Nuclear Matrix Proteome as a Biochemical Snapshot of Nuclear Organization. *Journal of Proteome Research* **13**, 3940-3956.

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 Research* **19**, 284-293.



MEMBERS



Technician
Gabriele Nerz

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Witold Szymanski

Ph.D. Student
Monika Puchalska

GERHARD MITTLER

1998-2002 Undergraduate Studies in Biochemistry, University of Bayreuth, Germany

1998-2002 Ph.D. studies (Protein Biochemistry and Molecular Cell Biology), Genecenter LMU and Helmholtz Center Munich

2002-2005 Postdoctoral fellow, Center of Experimental Bioinformatics, University of Southern Denmark, Odense, Denmark

Since 2005 Head of Proteomics Facility, Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



The unit is offering a state-of-the-art biomolecular mass spectrometry (MS) analysis service and is collaborating with other research groups at the institute in order to develop custom-tailored protein-protein and protein-nucleic acid complex purification and MS analysis strategies to address well-defined biological questions.

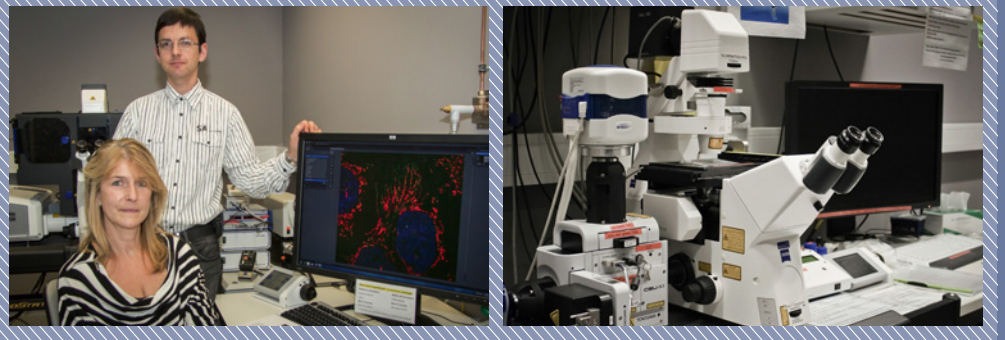
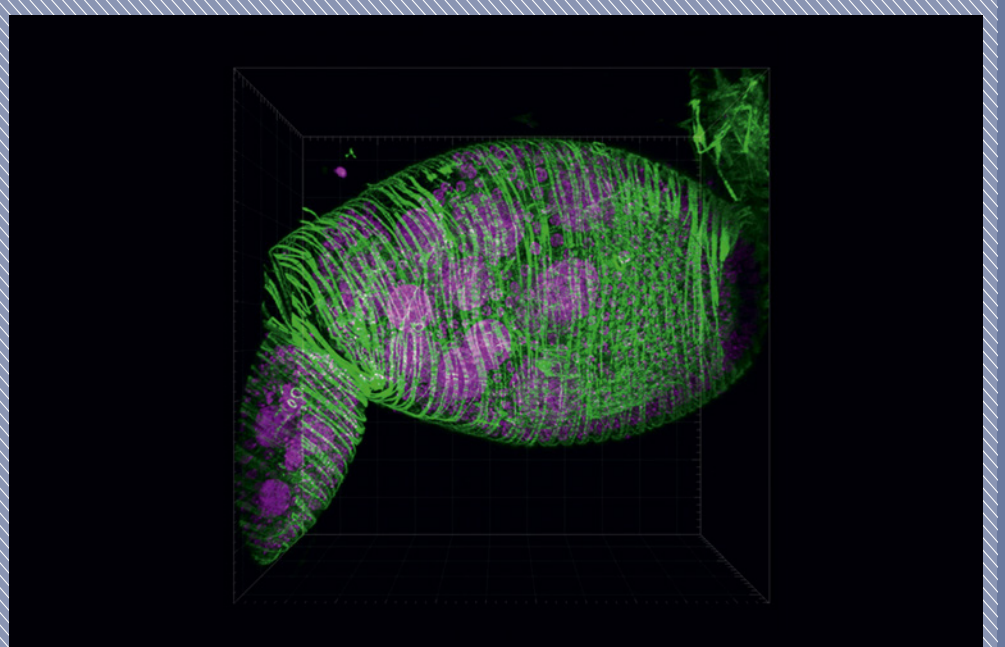
The facility is exclusively employing HPLC ESI-MS techniques because of its sub-femtomol sensitivity and high resolving power. Accordingly, the unit is running two quadrupole orbitrap instruments (QExactive series) and one hybrid linear ion trap (LIT) FT-MS instrument (Orbitrap XL+ETD), that are coupled online via electrospray ionization (ESI) source interfaces to nanoLC systems. We use both nanoHPLC as well as nanoUHPLC for upfront reversed-phase (RP) separation of complex peptide mixtures generated by tryptic digestion of proteomes. NanoUHPLC enables the use of columns with a length of up to 50 cm that in conjunction with sub-2 μ m bead capillary columns offer superior separation power that is best combined with very fast scanning QExactive MS. Our equipment allows us to employ the most commonly used fragmentation technologies (CID, HCD, ETD, MSA) for MS/MS identification and PTM analysis of proteins, enabling the in depth characterization of proteins and protein complexes.

Depending on the sample proteolytic digestions are performed either in-gel, in solution or by FASP (filter-aided sample preparation). Sample preparation by SPE (solid phase extraction) is performed offline (semi automated) in a microcolumn-in-a-tip (STAGE tips) format. The STAGE tip set-up is very flexible and can

accommodate reversed phase (standard), strong anion and cation exchange (SAX, SCX), HILIC (hydrophilic interaction chromatography) as well as affinity chromatography (e.g. Titania-MOC for phosphopeptides) beads. Pre-fractionation of very complex samples is done either at the protein level by 1D SDS-PAGE or at the peptide level via peptide-SAX/SCX or IEF. For offline protein and peptide chromatography microbore HPLC systems are used. Isoelectric focusing (IEF) is performed on an Agilent 3000 off-gel fractionator.

The unit is very experienced in metabolic labelling of cell lines by the SILAC methodology and relative quantification of SILAC-MS data is currently achieved with the help of the MaxQuant and Perseus software environment. For conducting label-free quantitative proteomics experiments, we make use of the MaxQuant LFI algorithm. The in-house bioinformatics pipeline additionally consists of a PEAKS™ workstation connected to a Mascot™ Server for automated multi-engine ("in chorus") database searching. Standard service includes protein ID from silver- or colloidal coomassie-stained gel bands. Peptide mapping for protein characterization, investigation of bead-associated and other medium-complexity proteomes (e.g. protein complexes), studies of PTMs and quantitative analysis are much more time consuming and are therefore considered as collaborations. Special and proteome-wide analysis identifying and quantifying proteins in the range of four to six thousands are evaluated and ranked by an in-house committee.

IMAGING FACILITY



Top: 3D reconstruction of a *Drosophila melanogaster* ovary. Images acquired on LSM780NLO. Sample prepared by Iovino lab.

➤ SELECTED PUBLICATIONS

Meunier S, Shvedunova M, Van Nguyen N, Avila L, Vernos I, Akhtar A (2015). An epigenetic regulator emerges a microtubule minus-end binding and stabilizing factor in mitosis. **Nature Communications** **6**, 7889.

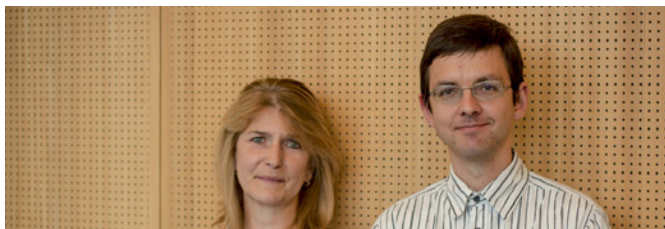
Maity PC, Blount A, Jumaa H, Ronneberger O, Lillemeier BF and Reth M (2015). B cell antigen receptors of the IgM and IgD classes are clustered in different protein islands that are altered during B cell activation. **Science Signaling** **8**, ra93.

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Öst A, Lempradl A, Casas E, Weigert M, Tiko T, Deniz M, Pantano L, Boenisch U, Itskov PM, Stoeckius M, Ruf M, Rajewsky N, Reuter G, Iovino N, Ribeiro C, Alenius M, Heyne S, Vavouri T and Pospisilik JA (2014). Paternal Diet Defines Offspring Chromatin State and Intergenerational Obesity. **Cell** **159**, 1352-1364.



MEMBERS



Technicians
Visnja Jevtic
Petra Kindle

SERGIY AVILOV

2000-2004 Ph.D. studies at Palladin Institute for Biochemistry, Kiev, Ukraine and Institute for Biophysics and Radiation Biology, Budapest, Hungary

2005-2007 Postdoctoral fellow at Strasbourg University, Strasbourg, France

2008-2013 Scientist at European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany and Grenoble, France

Since 2014 Head of the Imaging Facility at the MPI-IE, Freiburg, Germany



Understanding the roles gene products play in biological systems is a key challenge in the post-genomic era. Thanks to tremendous progress of instrumentation, software and molecular “tools” (genetically encoded fluorophores, ion sensors etc.), fluorescence microscopy evolved from an “illustrative” method to a stand-alone technology in modern life sciences. Fluorescence microscopy is in principle capable to visualize almost every structure and every event of interest. Details smaller than optical resolution limits can be visualized by recently developed “super-resolution” techniques. In many cases, dynamic non-invasive observations of live objects are possible, and quantitative information can be extracted, including those on concentration, specific interactions and mobility of a molecule of interest.

A range of state-of-the-art light microscopy instruments and image processing tools are available at the Imaging Facility of MPI-IE. The Facility maintains the equipment and assists with image acquisition and analysis. The Facility usage is growing: over 5000 total usage hours were registered in 2015, in comparison to ≈2850 hours in 2014; there are nearly 80 active users. The Facility renews its equipment and widens the arsenal of available techniques. In 2014-2015, Imaging Facility acquired new high-end “mobile” microscope stage incubation unit for live microscopy, an image analysis workstation and advanced software. Most important, the Institute won a “Replacement Investment” grant of MPG which enabled the Imaging Facility to acquire a brand-new multi-photon and confocal imaging system (Zeiss LSM780 NLO), which is

suitable for intra-vital imaging. Even more opportunities in experiment design and model organism range appeared since this system is installed in the Biosafety level 2 containment.

Overview of equipment and software

- **Zeiss LSM 780 NLO** high-end system for two-photon and confocal imaging
- **Zeiss LSM 780**, a versatile confocal laser scanning microscope with spectral detectors
- **Zeiss Elyra PS1**, a cutting-edge multi-modal system combining PALM&STORM super-resolution, SIM super-resolution, total internal reflection (TIRF) and confocal modes
- **Zeiss Cell Observer SD**, a Nipkow spinning disc microscope enables very fast and long-term observations of live samples with minimized photodamage
- **Zeiss Axiovert 200M**, an epifluorescence microscope equipped with microinjection system
- **Zeiss Imager Z1 ApoTome**, an upright system for imaging in fluorescence and bright-field illumination modes
- **Zeiss Imager Z1 Apotome 2**, upright system for optical sectioning fluorescence imaging
- **Two Tokai Hit**, “mobile” microscope incubation units for gas and temperature control of the samples under observation, compatible with all inverted microscopes of the Facility
- **Imaris**, a “flagship” software for multidimensional image presentation and analysis, including object tracking, surface rendering, particle analysis
- **Huygens**, dedicated software for image restoration based on different deconvolution algorithms, that permit the recovery of objects from images that are degraded by blurring and noise
- **Image J/Fiji**, the most popular open source software, for which hundreds of plug-ins exist, for great variety of image analysis procedures
- **Zen Offline**, proprietary software of Carl Zeiss for viewing and basic analysis of raw image datasets acquired on Zeiss microscopes

LABORATORY ANIMAL FACILITY



Top: The building of the animal facility guarantees the highest level of maintenance standards and research opportunities. Left: Cryopreservations straws, in which cryoprotected embryos were individually stored to put them in special freezers. Right: Housing of our mice.



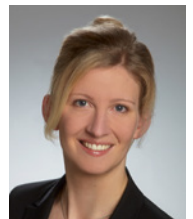
⇒ INKE KRUPKA DYACHENKO

1999-2006 Studies in veterinary medicine at the University of Leipzig, Germany; License to practice veterinary medicine

2006-2008 Ph.D. studies at the Institute of Immunology, University of Leipzig, Germany

2009-2015 Scientific Associate, Institute of Infectious Diseases and Zoonoses, Ludwig Maximilian University of Munich, Germany

since 2015 Head of the Laboratory Animal Facility at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



⇒ 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*), maintenance of the latter two being the responsibility of departmental scientists.

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, immunodeficient and germfree mice are kept in small isolator units that are serviced by intensively trained and skilled animal care staff. 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.

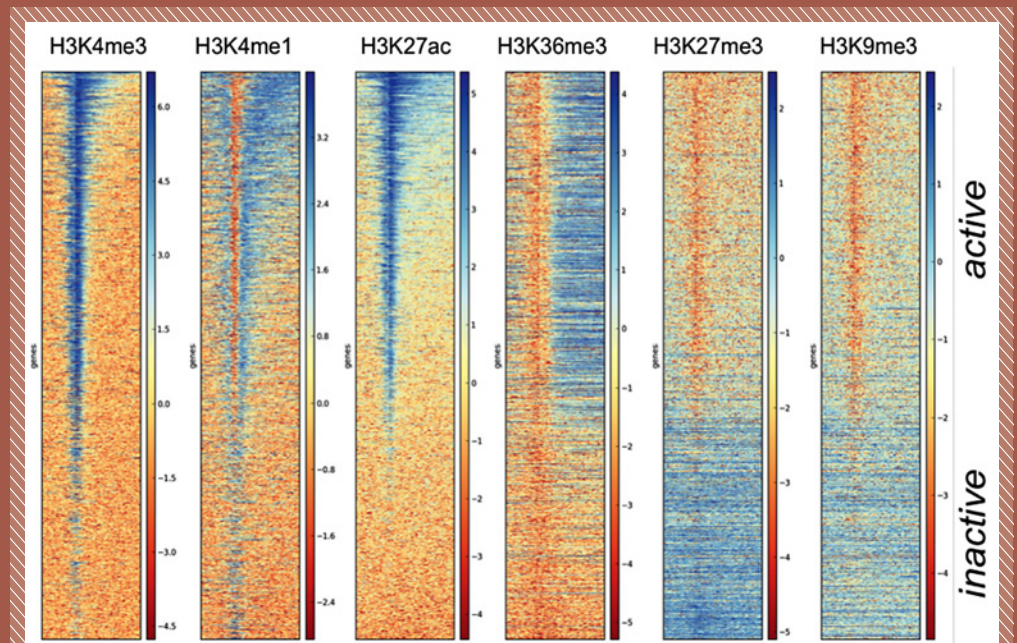
During 2016, the construction of the new building for animal holding and breeding of mice exceeding the new European animal welfare regulations and standards will start.

The Mouse House offers high standard services

- 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

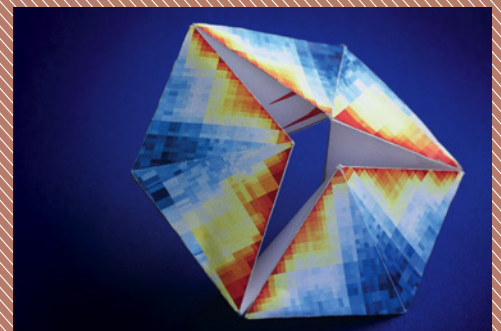
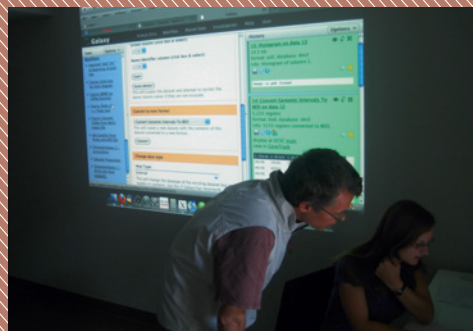
For mouse strain management and coordination of the services offered, a software program (TBase) developed by Dr. Peter Nielsen, has become indispensable. TBase provides users with 24-hour access to their data, facilitates communication between animal care staff and researchers, and provides an ideal 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 authorities.

BIOINFORMATICS FACILITY



Top: Genome-wide data integration reveals clusters of active and inactive genes. In this figure each panel corresponds to a map of one specific histone modification across the transcription start site of more than 20,000 annotated genes. Together these maps amount to an epigenetic fingerprint (in this case of a healthy liver cell). Left:

The facility provides regular workshops and hands-on training courses in bioinformatics. Right: A self made kaleidocycle whose sides are decorated with chromosome conformation capture data summarizing 1 billion sequencing reads.



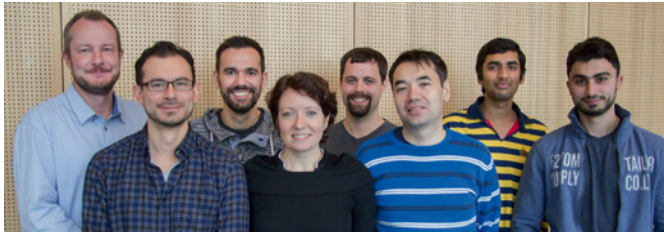
➤ SELECTED PUBLICATIONS

Ramirez F, Lingg T, Toscano S, Lam KC, Georgiev P, Chung HR, Lajoie B, de Wit E, Zhan Y, de Laat W, Dekker J, Manke T, Akhtar A (2015). High-affinity sites form an interaction network to facilitate spreading of the MSL complex across the X chromosome in *Drosophila*. **Molecular Cell** **60**, 146-62.

Ramírez F, Dündar F, Diehl S, Grüning BA, Manke T (2014). deepTools: a flexible platform for exploring deep-sequencing data. **Nucleic Acids Research** **42**, W187-191 (Web Server issue).

Bulut-Karslioglu A, De La Rosa-Velázquez IA, Ramirez F, Barenboim M, Onishi-Seebacher M, Arand J, Galán C, Winter GE, Engst B, Gerle B, O'Sullivan RJ, Martens JH, Walter J, Manke T, Lachner M, Jenuwein T (2014). Suv39h-dependent H3K9me3 marks intact retrotransposons and silences LINE elements in mouse embryonic stem cells. **Molecular Cell** **55**, 277-290.

MEMBERS



Bioinformaticians

Fabian Kilpert
Fidel Ramirez
Andreas Richter

Devon Ryan
Katarzyna Sikora
Abdullah Sahyoun

Ph.D. student

Vivek Bhardwaj

THOMAS MANKE

1994-1998 Ph.D. studies (Theoretical Physics) at Cambridge University, Cambridge, UK

1998-2010 Postdoc at Tsukuba University, Japan, at Columbia University, New York, USA, and at the Max Planck Institute for Molecular Genetics, Berlin, Germany

Since 2010 Head of Bioinformatics/Deep-Sequencing Facility, Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany



➤ 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 large-scale experiments and the capacity to analyze genome-wide data. Apart from supporting many collaborative projects at the MPI-IE, the group interacts very closely with the Deep-Sequencing Unit that generates data at an unprecedented scope, resolution and rate. The Bioinformatics Group is operating a powerful Data Center to process, analyze and visualize this information. We also provide regular training and help our colleagues to interpret genome-wide data.

Data Center

We operate a state-of-the-art data center with a dedicated cooling system, more than 800 high-speed cores, 200TB 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 Unit and other internal users. We provide access to hundreds of standardized tools, protocols and Linux software for data management and analysis.

Web Services

Apart from offering direct access to our Linux servers, we also host a number of different web-

services (Galaxy, deepTools, Rstudio) 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.

International Cooperations

As central component of two large consortia – the CRC 992 “Medical Epigenetics” and the German Epigenome Program (DEEP) – the Bioinformatics Group supports international efforts to understand human diseases in the context of epigenetic alterations. Together with the Deep Sequencing Facility, we specialize in the generation of histone modification maps that serve as important standard and reference for genome-wide analyses and mechanistic studies.

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.



Around the Institute



The regulation of size and time in gene regulation
Keep Me...

Pushing
the limits
of the future

LIFE AT THE INSTITUTE

An integral part of creating an inspiring place for excellent research is a vibrant life at the institute. Throughout the entire year various events both for employees as well as the public take place.

➤ Marathon Women & Men

It is a great event every year in April: after winter the runner's scene meets in Southern Baden to start the new season. For this event or various corporate runs in and around Freiburg, members of the institute stick together to form a runners team. It accommodates all types of abilities from newcomers till experts, and thus everybody at the institute is welcome to join the MPI-IE running team.





➔ MAXDAY

During the summer time the MPI-IE invites all staff members to the MaxDay. This internal event aims to bring all employees of the institute together and wants to promote the exchange between research groups, facilities and above all between the scientific and non-scientific employees of the institute. Throughout the day different scientific talks, performances by employees and games take place to show the multifaceted and vibrant life at the institute. A joint poster session in

which all research groups, scientific facilities as well as administrative departments present their work in the most creative manner introduces all aspects of the institute: from the activities in the labs to the work of the teams of personnel, finance or purchase. Another highlight of the Max-Day is the official IMPRS graduation ceremony at the MPI-IE. Doctoral students who successfully completed the IMPRS program are honored for their achievements by the entire institute.

LIFE AT 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? Regularly in summer, all employees of the MPI of Immunobiology and Epigenetics (MPI-IE) and the MPI of Foreign and international Law (MPIICC) are invited to join Max Planck Health Day. With this initiative, the two Max Planck institutes set a sign for a healthier working environment. The employees receive advice for ergonomic work places, participate in back coachings and learn about strategies of stress prevention.



➤ OUTREACH EVENTS

Engagement with the public is one of the core tasks of the MPI-IE communication goals. Thus this different outreach events with the participation of researchers, facilities as well as administration take place. For instance, in cooperation with the local newspaper Badische Zeitung (BZ) the MPI-IE invited readers to visit the institute. Visitors got the opportunity to join a guided tour through the institute and gain insights into core scientific infrastructure units of the MPI-IE: flow cytometry, proteomics, deep sequencing and modern microscopy.

Regularly the MPI-IE also participates in the Science Fair at the Freiburger Münstermarkt. At this local event thousands of visitors have the chance to gain insight into the ongoing research at the 58 participating scientific institutions and companies. With the help of various posters and information material presented at the stand scientists of the MPI-IE explained the relevance of immunobiology and epigenetics. Visitors could play different immunobiology and deep-sequencing games, observe fruit flies and look at tissue sections under the microscope.

LIFE IN AND AROUND FREIBURG

Freiburg im Breisgau is a city, where Black Forest idyll meets cutting-edge research. But the city has a lot more to offer: the warm climate, a historical city center and a diverse and vibrant cultural life.

Life in Freiburg

➤ 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 more than 220.000 inhabitants is characterized by 25.000 students at the University of Freiburg. 16 percent among them are from abroad, half European and non-European, which adds to the international flair of the town.

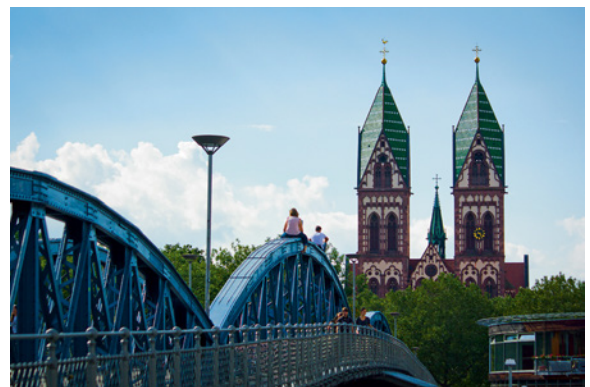
The calm climate and the local cuisine of Baden, crowned with numerous first class restaurants in and around Freiburg, make Freiburg a town highly attractive to visitors from all over the world. In the historical city center

with the famous Freiburg Münster cathedral and square as well as the Augustinerplatz with the Augustiner Museum it is possible to linger in one of the small and cozy cafes and bars or enjoy a shopping trip.

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.



“Venice for your feet”: Freiburg has an unique system of small canals (called Bächle) that run throughout the centre. During the summer, the running water provides natural cooling of the air and offers a pleasant gurgling sound. © Rob Faulkner



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



FREIBURG IM BREISGAU

	Freiburg	Germany
☀	Ø 1.811h	Ø 1.480h
🌡	Ø 11°C	Ø 8,7°C

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 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. Further lakes and a wonderful countryside with more than 23.000 kilometers of hiking trails is an ideal terrain not only for nature lovers for hiking, hillwalking, skiing, mountain biking, and paragliding.

With innumerable museums, exhibitions, open-air theatres, castles, churches and monasteries the entire region offers entertainment and pleasure to discover a rich history and heritage.

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 headquarters of various pharmaceutical companies are located here. From there, some of the most prominent Swiss mountains like the Eiger can be reached easily.

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.

2014

27. January 2014

Edith Heard

Genetics and Develop. Biology Unit, Institute Curie, Paris, France

Nuclear dynamics and chromatin changes during X-chromosome inactivation

30. January 2014

A. Neil Barclay

Sir William Dunn School of Pathology, University of Oxford, UK

The leukocyte surface – complexity, structure, interactions, evolution, and disulfide bonds

13. February 2014

Adam Antebi

Max Planck Institute for Biology of Ageing, Cologne, Germany

Hexosamine pathway metabolites enhance protein quality control and prolong life

27. February 2014

Sarah Teichmann

EMBL-EBI & Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK

Gene expression genomics in T cells

06. March 2014

Garnett H. Kelsoe

Department of Immunology, Duke University, Durham, NC, USA

A population genetics for B lymphocytes: The shapes of tolerance and antigen-driven selection

13. March 2014

Anne Ephrussi

Developmental Biology Unit, EMBL, Heidelberg, Germany

Assembly and transport of oskar mRNPs in the *Drosophila* oocyte

21. March 2014

Frederick Alt

Harvard Medical School, Boston, USA

Antibody Diversity, Genomic Instability, and Cancer

01. April 2014

Felix Randow

Medical Research Council, Cambridge, UK

Autophagy in host-pathogen interactions

02. April 2014

Burkhard Becher

Institute of Experimental Immunology, University of Zürich, Switzerland

Cytokines: how T cells instruct myeloid cells in chronic inflammatory disease

03. April 2014

Ton Schumacher

Netherlands Cancer Institute, Amsterdam, The Netherlands

Dissecting T cell immunity in mice and men

10. April 2014

Erika Pearce

Washington University School of Medicine, St. Louis, USA

Metabolic Regulation of T Cell Function and Fate

11. April 2014

Margaret Goodell

Baylor College of Medicine, Houston, USA

Regulation of normal and malignant hematopoiesis by DNA methylation

24. April 2014

Josef Penninger

Institute of Molecular Biotechnology, Vienna, Austria

How to license innate immunity to kill cancer metastases

22. May 2014

Xiaowei Zhuang

Harvard University, Cambridge, MA, USA

Bioimaging at the nanoscale: single-molecule and super-resolution fluorescence microscopy

05. June 2014

Ben Black

University of Pennsylvania, Philadelphia, USA

Centromere identity and the nature of the chromatin containing the variant histone, CENP-A

10. June 2014

Emmanuelle Passegue

University of California, San Francisco, USA

The Many Faces of Hematopoietic Stem Cells



Discussion in the lecture hall and in the lounge after the seminar.



12. June 2014

Thorsten Hoppe

University of Cologne, Germany

Ubiquitin sets the timer: Impacts on Stress Response and Aging

26. June 2014

Phillip Zamore

HHMI, University of Massachusetts Medical School, Worcester, USA

Mechanisms and Functions of RNA Silencing Pathways

11. July 2014

Edward J. Pearce

Washington University School of Medicine, St. Louis, USA

Metabolic reprogramming in the activation of mononuclear phagocytes

17. July 2014

Wolfgang Zachariae

Max Planck Institute of Biochemistry, Martinsried, Germany

Masters of Reduction: Control of Chromosome Segregation in Meiosis

24. July 2014

Helle Ulrich

Institute of Molecular Biology, Mainz, Germany

Coping with DNA damage during replication

2. October 2014

Brigitta Stockinger

MRC National Institute for Medical Research, London, UK

Environmental influences on inflammatory immune response

23. October 2014

Ajay Chawla

Cardiovascular Research Institute, University of California, San Francisco, USA

Innate Immunity and Metabolic Homeostasis

3. November 2014

John Lis

Cornell University, Ithaca, USA

Targeted and genome-wide views into the mechanisms of transcription and its regulation in living cells

5. November 2014

Alexander Tarakhovsky

Rockefeller University, New York, USA

Control of gene expression by histone mimics

17. November 2014

Jörn Walter

Universität des Saarlandes, Fachrichtung 8.3 Genetik & Epigenetik, Saarbrücken, Germany

From high resolution epigenomics to deep insights in cell programs

2015

13. March 2015

Matthias Peter

Institute of Biochemistry, ETH Zürich, Switzerland

Regulation of DNA replication and repair by ubiquitin-dependent mechanisms

20. March 2015

Elaine Fuchs

The Rockefeller University, New York, USA

Stem Cells in Silence, Action and Cancer

9. April 2016

Maria-Elena Torres Padilla

IMBC, Strasbourg, France

Epigenetic mechanisms in early mammalian development

16. April 2015

Timm Schroeder

Department of Biosystems Science and Engineering, ETH Zürich Switzerland

Long-term single cell quantification: New tools for old questions

17. September 2015

Ineke Braakman

University of Utrecht, The Netherlands

Chaperone-assisted protein folding in the secretory pathway

24. September 2015

Jim Hagman

National Jewish Health Center, University of Colorado, Denver, USA

Requirements for Chd4-NuRD in early B cell development

08. October 2015

Ulrich Hartl

Max Planck Institute of Biochemistry, Martinsried, Germany

Long-Chaperone functions in protein folding and proteostasis

27. October 2015

Andrew Jackson

MRC Human Genetics Unit, University of Edinburgh, UK

How do Ribonucleotides embedded in DNA cause neuroinflammation

12. November 2015

Katel Patel

MRC Laboratory of Molecular Biology, Cambridge, UK

The genetic basis for protection against genotoxic metabolites in mammals

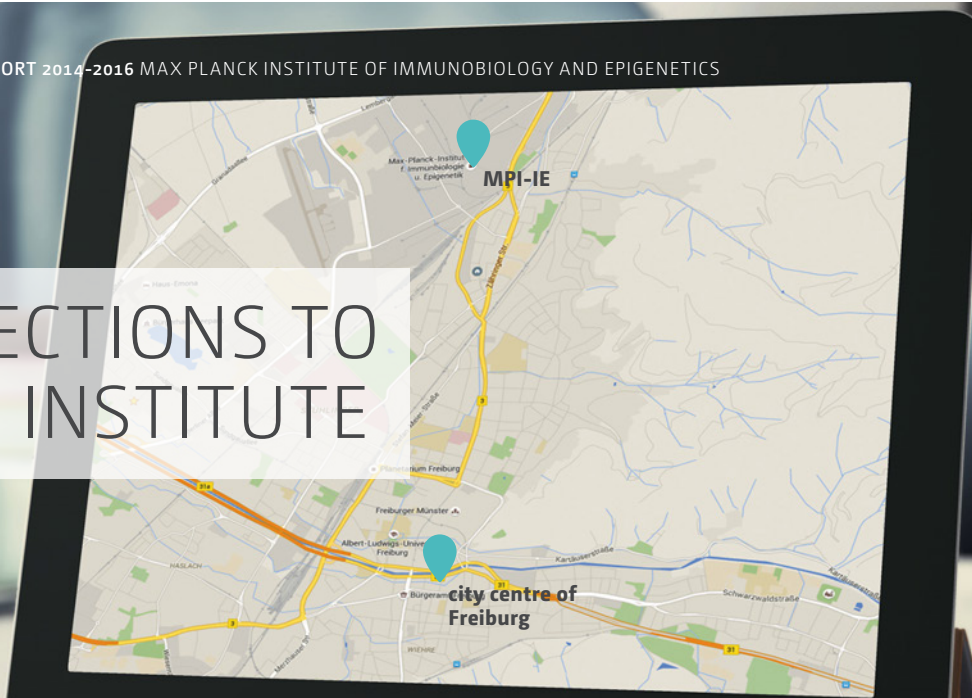
10. December 2015

Kim Nasmyth

Department of Biochemistry, University of Oxford, UK

It's not just in our DNA: how are our chromosomes held together?

DIRECTIONS TO THE INSTITUTE



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Max Planck Institute of
Immunobiology and Epigenetics
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Germany

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Fax: +49 761 5108-221
Mail: presse@ie-freiburg.mpg.de
Web: www.ie-freiburg.mpg.de

By public transport (from main train station)

You find the tramway station at the south end of the main train station (The way is signposted). To get to the MPI-IE take **tram no. 5** to "Zähringen". Get off at stop "Tullastraße" (8 stops). Walk along the "Tullastraße" until "Zinkmattenstraße" (first exit at the roundabout) and follow this street until "Stübeweg" on your left. You find the MPI-IE on the left side (overall a 15-20 min walk).

Alternatively, you can use busses from the ZOB (central bus station) next to the main train station:

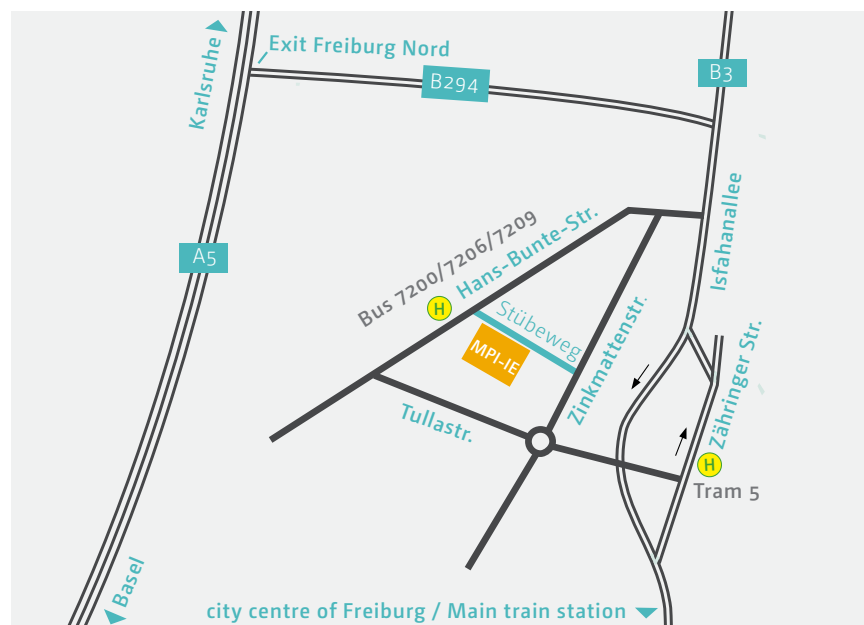
- **7200** to „Emmendingen Bahnhof“
- **7209** to „Denzlingen Bahnhof“

Get off at stop "Stübeweg" (approximately a 15 min ride). The bus stop is located at the corner of Hans-Bunte-Straße and Stübeweg on the left. The MPI-IE is on the right hand side of Stübeweg (overall a 2 min walk).

By car

If you arrive via freeway A5, Karlsruhe to Basel, take the exit for "Freiburg Nord". Keep in the left lane and take highway B294 towards Freiburg. After approximately 3.5km highway B294 runs into highway B3 towards Freiburg. Take the exit for "Industriegebiet Nord" and keep in the left lane. Turn left at the traffic light into "Hans-Bunte-Straße". Finally turn left again into "Stübeweg". The MPI-IE is on your right.

Map



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 (approximately a 1h bus ride). It will stop at Freiburg main train station.

