INSTITUTE REPORT

MAX PLANCK INSTITUTE OF IMMUNOBIOLOGY AND EPIGENETICS

2016

2018
## Contents

### About us
- Preface ........................................ 04
- Highlights .................................... 08
- History of the Institute ......................... 10
- Previous Directors ............................ 12
- Organization of the MPI-IE ................. 14
- Administration and Service ................ 16
- Facts ........................................... 18
- The International Max Planck Research School .... 20
- Focus Immunobiology ......................... 22
- Focus Epigenetics ............................. 23

### Research Facilities
- Deep Sequencing Facility .................... 92
- Transgenic Mouse Facility .................. 94
- Fish Facility .................................. 96
- Fly Facility ..................................... 98
- Proteomics Facility ........................... 100
- Flow Cytometry and DNA Sequencing Facility ... 102
- Imaging Facility ............................... 104
- Laboratory Animal Facility ................ 106
- Metabolomics Facility ....................... 108
- Bioinformatics Facility ....................... 110

### Research Groups
- Senior Group Asifa Akhtar .................. 26
- Senior Group Thomas Boehm ............... 30
- Group Nina Cabezas-Wallscheid ........... 34
- Senior Group Rudolf Grosschedl .......... 38
- Group Dominic Grün ......................... 42
- Group Valérie Hilgers ......................... 46
- Group Nicola Iovino .......................... 50
- Senior Group Thomas Jenuwein .......... 54
- Group Tim Lämmermann ..................... 58
- Senior Group Edward Pearce ............... 62
- Senior Group Erika Pearce ................. 66
- Group Andrea Pichler ......................... 70
- Group J. Andrew Pospisilik ................. 74
- Group Angelika Rambold .................... 78
- Group Ritwick Sawarkar ..................... 82
- Group Eirini Trompouki ...................... 86

### Around the Institute
- Life at the Institute .......................... 114
- Life in and around Freiburg ............... 118
- Special Guest Seminar Series .............. 120
- Directions to the Institute ............... 122
- Imprint ...................................... 123
This report introduces the work of more than a dozen independent scientific research groups of the Max Planck Institute of Immunobiology and Epigenetics and reviews their progress over the last three years. As part of the Max Planck Society, Germany's most successful research organization, we are fully committed to Max Planck’s dictum “Insight must precede application.” We therefore wish to establish a culture, in which scientists are rewarded for originality and scope of their work as much as for the results. First and foremost, our continued success depends on the creativity of the minds that we can attract to this institute; this is the reason why we appreciate diversity and welcome people from all over the world. We put great emphasis on collaboration and camaraderie as essential ingredients of our success. Despite the intense competition in science, the spirit of the Musketeers of the Guard still holds: One for all and all for one. While we look back on the past three years in pride, we can look forward to even greater achievements in the future.

In the past three years, the institute attracted another two outstanding junior group leaders. In 2016 arriving from University of California at Berkeley, Valérie Hilgers started her laboratory to focus on gene regulatory mechanisms controlling the development and function of neurons. In 2017, Nina Cabezas-Wallscheid, who previously worked at the German Cancer Center in Heidelberg, established her group to continue work on the regulation of hematopoietic stem cell quiescence. Both have very quickly integrated into the institute and already become highly valued members of our dynamic faculty. Arrivals were accompanied by departures. In 2017, Rolf Kemler, who served as Director at the institute since 1992, closed his emeritus group. In 2018, Michael Reth, a senior research group leader at the institute and a professor at the University of Freiburg, left the institute and continues his successful research programme on lymphocyte signaling at the University. Ana Izcue, who was group leader from 2009-2015, accepted a position at the University Hospital Aachen to continue her work on in-
testinal immunity. Andrew Pospisilik, whose term as research group leader comes to an end in the fall of 2019, was appointed Professor and Director of Epigenetics at the Van Andel Research Institute (USA) in recognition of his groundbreaking work on mechanisms of epigenetic inheritance. We express our gratitude to their invaluable and outstanding contributions to the success of the institute and wish them well for the future.

Members of the institute continue to very actively contribute to the scientific life in Freiburg, mainly through Collaborative Research Centers (CRCs) and Research Clusters of the Excellence Initiative funded by the Deutsche Forschungsgemeinschaft (DFG). In a very welcome sign of ever increasing integration, more and more researchers from the University Hospital and the University of Freiburg have become principal investigators in our international Ph.D. programme. It was founded in 2006 as the International Max Planck Research School for Molecular and Cellular Biology (IMPRS-MCB) and, from 2019 onwards, will be continued as the Max Planck Research School for Immunobiology, Epigenetics and Metabolism (MPI-IEM). This School attracts many talented young scientists from all over the world and helps sustain scientific excellence at the institute. It is no surprise that our well-supported students excel in their research and are recognized for their outstanding achievements; for instance, in 2017, Aindrila Chatterjee was awarded the Otto Hahn Medal, which is awarded annually to the very best PhD students of the Max-Planck Society.

Our PhD students are organized in the PhDnet, which allows them speak with a forceful voice and makes sure that their opinions are heard. This level of cross-institutional organization was lacking for postdoctoral scientists, but they too have now formed an interest group, the PostdocNet, which represents a very diverse group of people whose achievements contribute much to the overall success of the institute.

Highlighting the ground-breaking discovery of genetic imprinting that laid one of the foundations of the modern field of epigenetics, Emeritus Director Davor Solter together with Cambridge University scientist Azim Surani received the Canada Gairdner International Award in 2018. In the same year, the German Research Foundation DFG awarded the Gottfried Wilhelm Leibniz Prize, one of the most distinguished German research awards, to Erika Pearce. Remarkable success also came for members of our junior faculty. No less than seven of our junior principal investigators won highly competitive grants from the European Research Council (ERC). Andrew Pospisilik was first awarded an ERC Starting grant, and subsequently an ERC Consolidator grant. Tim Lämmermann, Nina Cabezas-Wallscheid (who was named GSCN Young Investigator in 2018), and Valérie Hilgers are currently supported by ERC Starting grants, whereas Nicola Iovino (who became EMBO Young Investigator in 2017), Dominic Grü, and Ritwick Sawarkar enjoy the financial backing of ERC Consolidator grants. These achievements are particularly satisfying, since our junior principle investigators are under extreme pressure to prove themselves in a relatively short period of time. Congratulations to all of them.

Maintaining scientific excellence also requires efforts by everybody in the institute; this includes not only our scientists, but also our administrative and support staff members, without whom none of our efforts would come to fruition. I thank all members of the institute for their continued dedication to making this place one the premier institutions for life science research. The German tax-payer invests a lot of hard-earned money into our work and we therefore owe it to the public that we make the best of this support, and that we adhere to the highest standards of scientific and professional conduct.

I invite you to review this Annual Report, to find out about the extraordinary breadth of our scientific activities and to read more about some of the astounding achievements of the past three years. I trust that we can do even better in the future and look forward to sharing more of our scientific adventures with you in the years to come.

Thomas Boehm
Managing Director
Institute Highlights

2016

April

“Zukunftstag” 2016
The MPI-IE participates in the nationwide Girls’ & Boys’ Day initiative with a “Zukunftstag”. This annual event is held at the Institute premises. School children from Freiburg can learn more about the Institutes research and what it means to become a scientist. A variety of lectures were given as well as practical exercises & vocational events.

July

MaxDay 2016
The MaxDay is one of the central highlights of institutes life during the year. The one-day event generates interactive exchange between research groups, facilities and above all between the scientific and non-scientific staff of the institute by combined activities such as a science fair, a talk series, the IMPRS graduation ceremony and other activities.

October

Valérie Hilgers starts at the MPI-IE
Valérie Hilgers starts her research group on RNA biology at the MPI-IE.

2016-2017

November

Tim Lämmermann receives ERC Starting Grant 2016
Tim Lämmermann achieves one of the prestigious Starting Grants by the European Research Council in 2016, which will enable him to continue his work on Immune Cell Dynamics at the MPI-IE.

December

4th Epigenetics Meeting
The 4th Epigenetics Meeting takes place. This high-profile conference represents a broad range of topics in the field of chromatin and epigenetics research with more than 30 speakers. Approx. 120 scientists participate in the meeting to share exciting data and novel ideas.

May

Nina Cabezas-Wallscheid joins the MPI-IE
Nina Cabezas-Wallscheid starts her research group on hematopoetic stem cells in the department of Rudolf Grosschedl.

2017

July

Science Fair 2017
The MPI-IE participates in the Science Fair 2017 at the Freiburger Münstermarkt. The local event gives thousands of visitors the opportunity to get an insight in the research of the Institute. On these two days, visitors carried out different immunobiology and deep-sequencing games, observed fruit flies and looked at tissue sections under the microscope.

September

Nina Cabezas-Wallscheid receives ERC Starting Grant 2017
Nina Cabezas-Wallscheid has been awarded a Starting Grant by the European Research Council in 2017.

October

Nicola Iovino selected as EMBO Young Investigator
Nicola Iovino is honoured for his exceptional research and scientific potential with the Young Investigator Award by European Molecular Biology Organisation (EMBO).
Erika Pearce awarded Leibniz Prize
Erika Pearce receives one of the most distinguished German research prizes. The German Research Foundation (DFG) honours her work on Metabolism of Immune Cells with the Gottfried Wilhelm Leibniz Prize 2018.

Davor Solter receives Gardner International Award
Emeritus Director Davor Solter is awarded, together with British scientist Azim Surani, the Canada Gairdner International Award for pioneering the concept of genomic imprinting.

2018

January

Thomas Boehm new managing director of the MPI-IE
Thomas Boehm is elected the new managing director of the MPI-IE.

March

Erika Pearce awarded Leibniz Prize
Erika Pearce receives one of the most distinguished German research prizes. The German Research Foundation (DFG) honours her work on Metabolism of Immune Cells with the Gottfried Wilhelm Leibniz Prize 2018.

Davor Solter receives Gardner International Award
Emeritus Director Davor Solter is awarded, together with British scientist Azim Surani, the Canada Gairdner International Award for pioneering the concept of genomic imprinting.

July

Valérie Hilgers receives ERC Starting Grant 2018
Valérie Hilgers working in the field of RNA biology receives a Starting Grant by the European Research Council.

August

Nina Cabezas-Wallscheid receives GSCN Young Investigator Award
The German Stem Cell Network (GSCN) highlights the research of Nina Cabezas-Wallscheid with the GSCN 2018 Young Investigator Award.

September

Max Planck Day 2018
The Max Planck Society celebrates its 70th Anniversary with a Germany-wide Max Planck Day. At this large science festival, all institutes of the Max Planck Society simultaneously addressed the public to present their research and thus show how diverse and exciting all spectrums of the Max Planck Society are. The MPI-IE joined the central science fair in Munich and also created an exciting evening event in Freiburg to give the public insights into the past, present and especially the future of immunobiological and epigenetic research at the MPI-IE.

November

Three ERC Consolidator Grants for the Institute
Dominic Grün, Nicola Iovino and Ritwick Sawarkar are awarded individually one of the most prestigious Consolidator Grants of the European Research Council.

May

Pint of Science in Freiburg
Researchers of the MPI-IE bring perhaps the most unusual Science Festival to Freiburg. Pint of Science takes place in the city of Freiburg. On three nights, local scientists share their research stories with the public and bring ground-breaking discoveries from the lab to the local pub.

5th Epigenetics Meeting
For the 5th time the Max Planck Epigenetics Meeting, on the expanding field of epigenetics and chromatin, takes place at the MPI-IE in Freiburg. Renowned speakers, as well as excellent junior researchers used this opportunity to present their latest data and findings in over 40 lectures and poster presentations at this three-day meeting.
History of the Institute

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.
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.

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 Max Planck Institute of Immunobiology was renamed to Max Planck Institute of Immunobiology and Epigenetics.

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.

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

In beginning of 2019, the International Max Planck Research School for Immunobiology, Epigenetics, and Metabolism (IMPRS-IEM) became the successor of the successful IMPRS-MCB Ph.D. program.
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 cells 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.

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

Management Board
Asifa Akhtar, Thomas Boehm, Rudolf Grosschedl, Thomas Jenuwein, Erika Pearce

represented by
Thomas Boehm,
Managing Director
(2018–2020)

Board of Trustees

Scientific Advisory Board
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. A director heads each department of the MPI-IE, also named ‘senior group leader’.

In addition, currently ten junior group leaders conduct research at the MPI-IE. They are either department-associated, department-independent or Max Planck research groups. All junior groups are considered equivalent. They have their own budget and pursue – within the framework of the MPI-IE – their research entirely independently. Junior group leader positions are established for five years (with the 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 head of administration. The managing directorship rotates every three years among the department heads of the MPI-IE. The management board establishes the general scientific and administrative policies and promotes long-term developments of the MPI-IE. Both the management board and the administration interact closely with the Max Planck Society in Munich regarding budgetary, personnel, and policy issues. In coordination with all group leaders and heads of infrastructure, the management board initiates the establishment of new scientific facilities and ensures a collaborative atmosphere at the institute. Regular 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 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 on 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.

**“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.
**Senior Executive Manager:** Christian Klatz
Assistant to Senior Executive Manager: Nathalie Schulz

**Controlling**
Saskia Moos

**Finance & Accounting**
Head: Florian Stiegeler
Susanne Demme
Jasmin Haas

**Reception**
Head: Daniela Moll
Sabrina Fögele
Gabriele Prosch

**Workshop**
Head: René Volz
Uwe Bachstein
Günther Barth
Thomas Fischer
Michael Herrn
Roland Kaiser
Steffen Pappenfuß
Volkers Wehrle

**Human Resources**
Head: Daniel Moll
Theresa Cimentepe
Veronica Klank
Nadine Laule
Dominique Schädler
Patricia Schätzle-Ott
Sabine Stallone

**Safety Department**
Head: Bernadette Lippok
Ute Lenz
Young-Min Kim
Marius Ruf
Andreas Rolke

**Basic Science Service**
Head: Bernadette Lippok
Anneka Hartl
Helga Herber
Doris Hoppe
Ute Lenz
Yan-Qing Li
Petra Lüderitz
Patricia Mayer
Roswitha Meller
Madalena Moreira
Andrea Reinecke

**Purchase Department**
Head: Michele Fieber
Christian Böhler
Regina Burger
Sven Mußmann
Sascha Wiencziers

**Public Relations**
Head: Marcus Rockoff

**Library**
Rose Black

**Cleaning**
Andrea Aukthun
Irmgard Bregenhorn
Michael Breithaupt
Filiz Kart
Floarea E. Zaharia

**IT-Service**
Head: Wolfgang Burger
Daniel Andris
Wolfgang Arndt
Ralf Gritzner
Christian Pagel
Wilhelm Rüsing
Luca Schuler-Köble

**Animal Welfare Department**
Inke Krupka-Dyaschneko
Stefanie Kunz

**Constructions**
Najeeha Amid

**Staff Restaurant & Lounge**
Martin Haberstroh (Tenant)
Facts

The 354 employees of the MPI-IE stem from all 5 continents and represent more than 40 countries of the world.

Internationality:

North & South America
1 Argentina
1 Brazil
3 Canada
3 Colombia
1 Cuba
3 Mexico
10 USA

Personnel

65 Postdocs
5 Directors
11 Group Leaders
16 Staff Scientists
53 Ph.D. Students
24 Trainees, student assistants
38 Administration
123 Technical Infrastructure
19 Guest Scientists

Gender

54% 46%
52.5% 47.5%
60.9% 39.1%

total scientific personnel non-scientific personnel

Publications

Over 700 publications since 2010

(Source: Scopus 2018)
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 Medical Center. One of the best examples of this fruitful cooperation is the University/MPI-IE Department of Molecular Immunology that had conducted research at the MPI-IE from 1996 until 2017. With the new joint University/MPI-IE Laboratory of Immunometabolism, established in 2015, the MPI-IE was able to continue this partnership. Furthermore, numerous research consortia build on the collaborations between the MPI-IE and the University. For example, MPI-IE research groups are integrated with the cluster of Excellence “BIOSS” and “CIBSS” as well as 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 Ph.D. 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

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 COORDINATOR

Dr. Kyle Austin
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. Our goal is 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 IMPRS Spokesperson and the Steering Committee. We currently have 54 students from 24 countries, and 28 laboratories (19 from MPI-IE and 9 from the University of Freiburg). We are also very proud of our growing Alumni group, which currently comprises 65 graduates. Most of our alumni continued their careers as postdoctoral fellows (67%) or moved into industry positions (22%).

Overview: Ph.D. at IMPRS-MCB

One distinctive aspect of our program is a rotation period. Students spend one month each in three different laboratories before beginning 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 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.

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 IMPRS-MCB structure. 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. representatives. 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.

“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 disregulation can significantly contribute to disease progression. Thus, epigenetic research promises far-reaching implications for new forms of therapy and diagnosis. Epigenetic changes also allow responses to environmental influences such as nutrition, stress and hormones. Intriguingly, there is growing evidence that epigenetic alterations might even be inheritable over a few generations.

The research groups of the Epigenetic Focus combine topics addressing dosage compensation, heterochromatin formation, and posttranslational modifications of histones. A variety of model organisms and experimental approaches (biochemistry, cell biology, Drosophila and mouse genetics, genome-wide profiling) are used to dissect the epigenome of distinct cell types.
Immuno-fluorescence 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, Janine Seyfferth, Thomas Stehle

**Postdoctoral Fellows**

Anastasios Alexiadis, Maria Felicia Basilicata, Aline Gaub, Shantanu Iyer, Amol Panhale, Gautier Richard, Tobias Bumpf, Bilal Nadeem Sheikh, Maria Shvedunova, Yidan Sun, Claudia Valsecchi, Meike Wiese, Yilong Zhou

**Ph.D. Students**

Hui-Ru (Elsa) Chen, Niyazi Umut Erdogdu, Sukanya Guhathakurta, Raed Hmadi, Adam Karoutas, Tanvi Sandeep Kulkarni, Cecilia Pessoa Rodrigues, Gina Vanessa Renschler, Maria Samata, Tsz Hong Tsang

**SELECTED PUBLICATIONS**


**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

1999-2001 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

2015-2017 Managing Director at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany
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 mediated by histone acetylation using X-chromosome specific gene regulation as a model system. More specifically, we are interested in addressing how the dosage compensation complex, composed of RNA and proteins [the MSL complex], gets targeted

Figure 1 Genetic information is encoded in our chromosomes (shown as different colored wool). Our lab is studying how this information is decoded (shown as a knitted work). We are particularly interested in how epigenetic regulation contribute to gene expression control.

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.
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 RNA helicases and 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. We have shown that roX RNAs harbor several binding sites for the MSL complex thus providing a platform for complex assembly. MLE, an RNA helicase, plays an important role in remodeling roX RNAs and for spreading of the MSL complex on the X chromosome. Our recent work has shown that MLE orthologue, DHX9 binds inverted repeats of Alu retrotansposable elements in humans which have tendency to form strong secondary structures. Thus, by resolving such structures DHX9 plays an essential role in posttranscriptional RNA homeostasis. In future we plan 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 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.

Figure 3 Schematic representation of the MOF’s dual role in the regulation of nuclear and mitochondria, thus connecting epigenetics and metabolism.

The role of the NSL complex in gene regulation

MOF histone acetyltransferase is part of an evolutionary conserved Non-Specific Lethal (NSL) complex in Drosophila and mammals. Interestingly, loss of MOF leads to early embryonic lethality indicating that this protein is essential during mouse development. Furthermore, MOF and H4K16ac are frequently misregulated in cancer suggesting that it is critical for cellular homeostasis of mammalian cells to maintain appropriate levels 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 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. 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 explore how interphase versus mitotic functions are coordinated by NSL complex members in mammals.

Connecting Epigenetics and Metabolism

We have recently uncovered a novel role of MOF beyond nuclear gene transcription. By confocal microscopy, we detect MOF, as well as members of the NSL complex, KANSL1 and KANSL3, in the mitochondria. Gene-expression analyses of MOF-depleted cells revealed that MOF regulates expression of genes involved in oxidative phosphorylation (OXPHOS) in aerobically respiring cells. Importantly, using a conditional knockout mouse model we demonstrated that mitochondria degenerate upon MOF loss in heart cardiomyocytes. This work provides a novel link between epigenetic and metabolism, which will be expanding on in future studies.

Figure 3 Schematic representation of the MOF’s dual role in the regulation of nuclear and mitochondria, thus connecting epigenetics and metabolism.
LAB THOMAS BOEHM
Transgenic mice expressing a nuclear YFP reporter in thymic epithelial cells reveal the different microenvironments in the outer cortex and the inner medulla of the mouse thymus.
Senior Group
Thomas Boehm
Design principles of adaptive immunity in vertebrates

LAB MEMBERS

Administration
Julia Rösner

Lab Manager
Annette Haas-Assenbaum

Technicians
Stefanie Birmelin, Dagmar Diekhoff, Ingrid Fidler, Christiane Happe, Brigitte Krauth

Project Leaders
Norimasa Iwanami (until 04/2018), Michael Schorpp, Jeremy Swann

Postdoctoral Fellows
Orlando Bruno Giorgetti, Divine Fondzemuy Lawir, Ryo Morimoto, Anja Nusser, Connor Patrick O’Meara, Inês Trancoso

Ph.D. Students
Mayumi Hirakawa, Iliana Siamishi

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


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. To this end, we are examining the thymopoietic activities of ancient forms of the master regulator of thymic epithelial cell differentiation, test the function of chimaeric versions of these factors, and also study the function of hypomorphic alleles. Our current data support the notion that the thymic environment evolves in a step-wise fashion, at each stage antedating the increased requirements associated with more complex patterns of T cell differentiation and selection.

In addition, we have embarked on a long-term project aimed at reconstituting the thymic niche in vivo, using...
a defined set of effector molecules. 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. Using a variety of novel reporter mouse strains marking different subsets of thymic epithelial cells, we are examining the molecular basis of thymus involution, a physiological process that leads to reduced output of naive T cells in ageing individuals.

Genetics of thymopoiesis and T cell development in vertebrates

A forward genetic screen in zebrafish was undertaken and the mutated genes in about 40 lines – all showing defects in thymopoiesis and T cell development – were identified by positional cloning. The results indicate an extraordinarily high degree of evolutionary conservation of key developmental pathways, such as those controlling T cell development, confirming that the zebrafish can serve as a versatile genetic model of vertebrate immune system development. On the other hand, we have identified a number of previously unappreciated genetic pathways important for T cell development, and are currently examining their relevance for the mammalian immune system.

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 development of the thymus equivalent, the T-like cell lineages, and the process of lamprey antigen receptor (VR) diversification by cytidine deaminases. We also explore the immunogenetics of sexual parasitism in anglerfishes, which represents an extraordinarily interesting example of natural parabiosis, with potential implications for our understanding of organ transplantation.
"Vitamin A-Retinoic Acid Signaling Regulates HSC Dormancy"

The cover art depicts dormant HSCs (HSCs in dormancy represented by night) which are regulated via vitamin A (symbolized by nutrients containing vitamin A such as carrot, broccoli, fish). The idea was developed by the artist Iris Joval and Nina Cabezas-Wallscheid.
LAB MEMBERS

Technician
Karin Jäcklein

Ph.D. Students
Polina Pavlovich, Katharina Schönberger, Yu Wei Zhang

Masters Student
Julian Meß

GROUP LEADER N. CABEZAS-WALLSCHEID

1982 born in Roses, Spain. Undergraduate Studies in Biotechnology at Autonomous University of Barcelona, Spain; Erasmus stage at University of Parma, Italy

2006-2010 PhD studies at the Laboratory of Molecular Mouse Genetics, Medical Center of the Johannes Gutenberg University of Mainz, Germany; Guest scientist at Harvard Stem Cell Institute, Boston, USA

2011-2017 Postdoctoral fellow at the Department of Stem Cells and Cancer, German Cancer Research Center, Heidelberg, Germany

Since 2017 Group Leader at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

SELECTED PUBLICATIONS


Group Nina Cabezas-Wallscheid

REGULATION OF HEMATOPOIETIC STEM CELL QUIESCENCE

How distinct factors influence hematopoietic stem cell self-renewal

Figure 1: The murine hematopoietic system: HSC = Hematopoietic Stem Cell; MPP = Multipotent Progenitor; CMP = Common Myeloid Progenitor; CLP = Common Lymphoid Progenitor; MEP = Megakaryocyte-Erythroid Progenitor; GMP = Granulocyte-Macrophage Progenitor; RBC = Red Blood Cell.

Adult hematopoiesis is responsible for the production of billions of mature blood cells every day (Figure 1). It is a hierarchically organized process that almost exclusively occurs in the red bone marrow. Hematopoiesis is tightly regulated, responding rapidly to stress stimuli, such as blood loss and inflammation, by modulating lineage commitment and terminal differentiation of progenitor cells.

Hematopoietic Stem Cells

Hematopoietic Stem Cells (HSCs) reside at the top of this hierarchy and represent an extremely rare cell population within the bone marrow. HSCs harbor long-term reconstitution capacities and have the ability to generate multipotent progenitors, which in turn differentiate into lineage-committed populations and subsequently into mature blood cells. Another fundamental feature of HSCs is their quiescent cellular status in terms of cell cycle activity and low biosynthetic activity. Quiescence or dormancy preserves and governs the life-long functionality of HSCs and protects them from accumulating genomic mutations potentially acquired during rapid cell divisions.

Regulation of Hematopoietic Stem Cells

Regulators of the dormant HSC state include cell-intrinsic signaling pathways as well as soluble components produced by the bone marrow niche. For instance, stress-signals such as interferons, lipopolysaccharide or stress-conditions including chemotherapy are known to cause HSC proliferation, thereby altering their homeostatic dormant status. In contrast to the factors that can activate and promote HSC exit from dormancy, little is known about the mechanism maintaining HSC quiescence. Importantly, dysregulation of this fine-tuned system may lead to aberrant hematopoiesis such as leukemia. Currently, the preservation of the dormant HSC status and the development towards a leukemic stem cell population is not well understood.

We have recently performed an extensive multi-layered OMICs analysis of HSCs and four multipotent progenitor populations (MPP1-MPP4) by combining DNA-methylome, whole-transcriptome and global proteome analyses (Figure 2). Our work identified exclusive gene expression clusters as potential gatekeepers of HSC self-renewal such as splicing variants, long non-coding RNAs...
Figure 2 Identification of regulatory networks in HSCs and their immediate progeny via integrated proteome, transcriptome, and DNA methylome analysis. Main findings summarizing Cabezas-Wallscheid et al. in Cell Stem Cell 2014.

and retinoic acid metabolism. In a follow-up study, we have shown by single-cell RNA-seq data that the molecular transition from the most inactive dHSCs cluster to the most active HSCs can be best described as a continuous stream-like process of steadily increasing metabolic activity. First protein synthesis and subsequently cell cycle-related components are continuously increasing in cells exiting the dormant state and moving towards an aHSC quiescence and cell cycle primed stage (Figure 3). We measured time for first division for 285 SICs by live cell imaging and found that aHSCs showed an average of 29.5 ± 0.7 hours to generate the first progeny, while dHSCs needed 40.8 ± 1.3 hours. This pronounced difference (11.3 hours) between two initially non-cycling populations suggests that dHSCs reside in a deeper level of quiescence, which is consistent with the molecular data. The association of delayed cell cycle entry with the extremely low biosynthetic activity defines the status of dormancy and distinguishes it from quiescence. Furthermore, we have observed that particular dietary habits, mainly vitamin A deficiency, is important for HSC maintenance, findings which pave the way to new fundamental and yet unsolved questions (Figure 4).

The goal of our laboratory is to cover novel ground on mechanisms that maintain HSC quiescence. We aim to investigate the impact of different nutritional regimes on HSC maintenance and to analyze the underlying regulatory mechanisms. Our ultimate goal is to translate these findings into human disease settings such as dietary deficiencies and leukemia. To address these biological questions, we are pursuing interdisciplinary projects which include the use of genetically modified mouse models, bone marrow imaging in combination with state-of-the-art population and single-cell OMIcs analysis.

Figure 3 Active HSCs are cell cycle primed. Left panel: heatmap representing cell cycle genes based on single-cell RNA-seq data of dormant and active HSCs. Middle and right panel: Microscopy images and quantification of CDK6 levels in dormant HSCs (dHSC), active HSCs (aHSCs) and MPP1.

Figure 4 Vitamin A/retinoic acid signaling regulates HSC maintenance versus differentiation. Left panel shows high levels of vitamin A (All-trans-retinoic acid = agonist of vitamin A) retains HSC quiescent upon stress. Right panel shows vitamin A–free diet triggers HSCs to differentiate.
mCherry-EBFs localizes to distinct domains in the nucleus of B cells. Intensity analysis of mCherry-EBFs domains inside B cell nuclei. Super-resolution images were processed via the combined Airyscan/Deconvolution module of Huygens. Domains of mCherry-EBFs were subsequently identified using the IMARIS spot function, and domain intensities plotted using the IMARIS Vantage module.
**LAB MEMBERS**

**Administration**
Marika Rott

**Technicians**
Ingrid Falk, Ganna Grosschedl, Dmitry Lupar, Hans-Jürgen Schwarz

**Postdoctoral Fellows**
Pierre Cauchy, Kyungjin Boo, Marta Derecka, Tanya Kapoor, Katharina Lupar, Yuthaphong Phongbunchoo, Hanibaskar Ramachandran, Nikolay Zolotarev

**Ph.D. Students**
Marc Bayer, Aurelie Lenaerts, Yuanting Wang

---

**SELECTED PUBLICATIONS**


---

**GROUP LEADER RUDOLF GROSSCHEDL**

1952 Born in Salzburg, Austria; Undergraduate studies in Biology in Freiburg, Germany

1978-1982 Ph.D. studies at University Zurich, Switzerland

1982-1986 Postdoctoral Fellow at MIT, Cambridge, MA, USA

1986-1999 Professor at the University of California, San Francisco and Investigator of Howard Hughes Medical Institute, Chevy Chase, Maryland, USA

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

**Senior Group Rudolf Grosschedl**

Regulatory Circuitries underlying B Lymphopoiesis
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) multi-lineage 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 extra-cellular 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 other transcription factors, in which positive feedback loops and cross-antagonism stabilize the establishment and maintenance of the B cell program. EBF1 is involved in activating genes associated with the B cell lineage and represses genes of alternative lineages. In addition, EBF1 poises genes for expression at later stages of differentiation. Genome-wide and kinetic analysis of EBF1 occupancy indicated that EBF1 binds naive progenitor chromatin prior to the generation of accessibility and activation of gene expression. Currently, we are studying the molecular mechanisms by which EBF can fulfill its diverse roles as a lineage-determining factor.

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. Recently, we found that SUMOylation of Satb2 during ectodermal differentiation is required for the down-regulation of pluripotency genes. Satb2 also plays a role in B lymphocytes by binding to AT-rich sequences that flank the intrinsic immunoglobulin heavy chain (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. We combine biochemical, imaging and genetic approaches, to elucidate how Satb proteins functionally organize chromatin.

Role of Mzb1 in peripheral B cell subsets

Peripheral B lymphocytes consist of multiple cell populations that differ in their phenotype, functional properties and anatomic locations. In addition to the vast majority of conventional B cells, also termed follicular B cells, which resides in lymph nodes and follicles of the spleen, marginal zone B cells occupy the marginal sinus of the spleen, and B1 cells are predominantly found in the peritoneal pleural cavities. B1 cells and marginal zone B cells have been termed ‘innate-like B cells’ because these cells quickly differentiate into antibody-secreting cells that produce ‘natural’, polyreactive antibodies. Therefore, these cells are considered to bridge the innate and adaptive immune responses. Previously, we identified and characterized 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. Current efforts focus on the mechanism by which Mzb1 regulates functions specific to innate-like B cells.

Figure 2 – A Dynamics of CpG DNA methylation around EBF1-occupied sites. Cloud maps presenting the levels of CpG methylation in ±100bp windows of persistent EBF1-occupied sites that are associated with low methylated regions. The levels at 24hrs and 72hrs after EBF1 induction and at the pro-B cell stage are compared with the levels before EBF1 induction (0 hrs). B Time-resolved analysis of transcript levels of up-regulated genes containing EBF1-occupied sites. Line plots show transcript levels that change >10-fold between 0 h of EBF1 induction and the pro-B stage.

Figure 3 Scheme of the regulatory circuitry of Satb2 in ES cells.
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 LEADER  DOMINIC GRÜN

- 1977 Born in Bergisch Gladbach, Germany
- 2004-2006 Ph.D. studies in theoretical physics and computational biology at the University of Cologne, Germany, and at New York University, New York, USA
- 2007-2010 Work in Industry
- 2010-2012 Postdoctoral fellow at the Max-Delbrück-Centrum, Berlin, Germany
- 2012-2015 Senior Researcher at the Hubrecht Institute, Utrecht, Netherlands
- Since 2015 Group Leader at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

LAB MEMBERS

Postdoctoral Fellows
Michael Bartoschek, Sagar

Ph.D. Students
Nadim Aizarani, Josip Hermann, Nina Pelto{kangas}, Patrice Zeis

SELECTED PUBLICATIONS


Group Dominic Grün

QUANTITATIVE SINGLE CELL BIOLOGY

Investigating cell fate decisions with high resolution

Figure 1 Model of stem cell differentiation. A multipotent cell is defined as an ensemble of metastable states. Transitions between these states are possible, until a cell commits to a terminal fate in response to a stochastic gene expression fluctuation or an external signal.

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, changes in the microenvironment 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 our lab we investigate how stem cells robustly maintain their pluripotent state and reliably execute differentiation programs with spatial and temporal precision in the presence of gene expression noise. We propose a model in which stem cells can exist in different metastable states, which are primed towards distinct lineages by subtle transcriptome modulations (Figure 1). While transitions between these stages are possible in the multipotent state, reinforcement of a primed state by random fluctuations or signalling events drives the cell into terminal differentiation. We explore the molecular mechanisms underpinning the transition from a multipotent state, exhibiting plasticity, to a lineage-restricted committed state. We have a particular focus on the role of gene expression noise in this process.

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. 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 chromatin accessibility,
DNA methylation, and genomic DNA. 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. Moreover, 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. We are also attempting to elucidate the impact of a cells’ microenvironment by high-resolution spatial gene expression analysis, e.g. by imaging utilizing single molecule fluorescence in situ hybridization.

**Differentiation of immune cells**

As a model of multi-lineage differentiation we are studying cells of the hematopoietic system with a focus on lymphocytes. Years of intense research have revealed the major hematopoietic cell types as well as multipotent progenitor populations with the help of flow cytometry on the basis of a relatively small number of cell surface markers. The development of sensitive high-throughput single-cell sequencing has revolutionized the identification of cell types and differentiation trajectories, and recent studies are changing our model of hematopoietic differentiation. We are studying differentiation of murine lymphoid cell populations in primary lymphoid organs, i.e. bone marrow and thymus, as well as differentiation of tissue-resident cells of the innate immune system. Our aim is to elucidate spatial heterogeneity, e.g. across tissues, and temporal heterogeneity of immune cell differentiation during life. In more detail, we are trying to identify regulatory mechanisms underpinning lineage choice and to understand the role of gene expression variability in this context. By means of single-cell RNA-seq analysis we are trying to create a high-resolution map of cell states and the differentiation trajectories connecting these states. To obtain temporal information, we couple snapshot single-cell transcriptome data to differentiation history by means of lineage tracing. Utilizing spatial transcriptomics and single molecule fluorescence in situ hybridization we investigate the role of the microenvironment in differentiation decisions. Our strategy implies the identification of novel cell type or state specific markers, enabling the purification of homogenous sub-populations for functional studies, e.g. by in vivo or in vitro differentiation assays.

**The cellular composition of the liver.**

Another branch of the lab explores the cellular composition of the liver, with the goal of understanding differentiation pathways and plasticity of liver epithelial cells and their interaction with the liver resident and infiltrating immune cells. We are applying imaging-based high-resolution methods to reveal the spatial arrangement and interaction of these cell types in the normal liver and upon perturbations such as fatty liver disease and liver cancer.
LAB VALÉRIE HILGERS
Two 3’ UTR isoforms of the gene elav in the Drosophila embryonic nervous system. Yellow: cell nuclei; grey: ELAV protein that marks neurons; magenta: short elav 3’ UTR; cyan: long elav 3’ UTR.
LAB MEMBERS

Technician
Fernando Mateos

Postdoctoral Fellows
Sarah Holec, Barbara Hummel

Ph.D. Students
Judit Carrasco Sala, Dominika Grzejda, Carlos Alfonso Gonzalez, Stylianos Tsagkris

Bioinformatician
Michael Rauer

Masters Student
Marion Thomas

Trainee
Sophia Bares

GROUP LEADER VALÉRIE HILGERS

1982 Born in Saarbrücken, Germany. Undergraduate Studies in Biology at Universität des Saarlandes, Saarbrücken, Germany and Ecole Normale Supérieure, Paris, France

2006-2010 PhD studies at EMBL Heidelberg, Germany, and Temasek Life Sciences Institute, Singapore

2010-2016 Postdoctoral fellow at University of California, Berkeley, USA

Since 2016 Group Leader at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

SELECTED PUBLICATIONS


Using Drosophila as a model system, we study the molecular mechanisms underlying neuron-specific RNA processing. We also aim to understand how alternative RNA processing affects neuronal development and function.

Mechanism of RNA processing in neurons

One aim of our lab is to provide mechanistic insight into the unique co-transcriptional processes that give rise to ultra-long 3’ UTRs in neurons. The neuronal RNA-binding protein ELAV, which binds to newly transcribed polyadenylation sites and inhibits polyadenylation, mediates 3’ UTR extension. Our previous work uncovered an unexpected link between transcription initiation and alternative mRNA processing: ELAV binds to promoter regions of its target genes, and this association is facilitated by promoter-proximal RNA Polymerase II (Pol II) pausing. How ELAV at transcription initiation affects RNA processing many kilobases downstream remains mysterious. We are studying how epigenetic marks, promoter sequence, and Pol II pausing cooperate to promote neural-specific mRNA extension. Using functional genetics, RNA biochemistry, as well as whole-genome approaches and proteomics in fly tissues, we study the recruitment of ELAV at gene promoters and to nascent mRNA.
Differential regulation of extended mRNAs in neurons

Although a typical Drosophila 3′ UTR measures a few hundred base pairs, alternative 3′ UTRs can be up to 17kb long. This extreme addition of sequence creates substantial potential for posttranscriptional regulation. We believe that this extra layer of regulation is necessary for the mRNA to achieve a neuron-specific function. Ultra-long 3′ UTRs, through their unusually high number of targets sites for RNA-binding proteins, could represent a platform for regulated mRNA transport and translation, and may build a scaffold for mRNA granule assembly. Most extended mRNAs in turn encode crucial RNA regulators, which suggests that a network of cross-regulation between extended mRNAs and RNA-binding proteins supports neural function. Applying neuron biochemistry, whole-genome analyses and functional genetics, we will unravel this network and identify its components.

Function of alternative 3′ UTRs in neuronal processes

Quite notably, mRNAs with ultra-long 3′ UTRs are specifically expressed in neurons and encode crucial developmental regulators, in particular, RNA-binding proteins involved in neurodevelopmental and neurodegenerative disorders. The role of RNA-binding proteins and RNA granules in neurological pathologies, in particular neurodegeneration, has been well described. We propose that a balanced expression of ultra-long 3′ UTRs limits phenotypes of neurodegeneration in disease and in normal aging. Our findings indicate that deleting part of the alternative sequences can cause serious neurological phenotypes. We aim to establish the functional impact of ultra-long 3′ UTRs on neuronal function. Approaches include functional genetics and in vivo analysis, single-molecule live imaging, genomics and transcriptomics.

Figure 2 ELAV-mediated 3′ UTR extension is regulated at transcription initiation. ChIP-Seq metadata show that ELAV and paused Pol II are found at promoter regions of genes that undergo 3′ UTR extension. Model: In neurons, ELAV associates with the promoter region of its target genes, which is usually engaged by paused Pol II. During transcription, ELAV binds to the nascent transcript in the vicinity of each proximal polyadenylation site (PAS). The inhibition of cleavage and polyadenylation (CPA) at proximal sites causes transcriptional read-through and formation of an extended 3′ UTR.

Figure 3 Alternative mRNA processing linked to neuronal function. Transcripts with alternative, extended 3′ UTRs typically encode important effector proteins of various steps of mRNA metabolism. The represented example proteins play crucial roles in neuron development, morphogenesis and survival as well as synaptic transmission and memory. Many of these proteins are well-known players in neurodegenerative pathologies.

Figure 3 Alternative mRNA processing linked to neuronal function. Transcripts with alternative, extended 3′ UTRs typically encode important effector proteins of various steps of mRNA metabolism. The represented example proteins play crucial roles in neuron development, morphogenesis and survival as well as synaptic transmission and memory. Many of these proteins are well-known players in neurodegenerative pathologies.

Figure 3 Alternative mRNA processing linked to neuronal function. Transcripts with alternative, extended 3′ UTRs typically encode important effector proteins of various steps of mRNA metabolism. The represented example proteins play crucial roles in neuron development, morphogenesis and survival as well as synaptic transmission and memory. Many of these proteins are well-known players in neurodegenerative pathologies.
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

LAB MEMBERS

Technician
Eva Maria Löser, Melanie Schaechtle

Postdoctoral Fellow
Daniel Latreille, Michael Rauer

Ph.D. Students
Nazerke Atinbayeva, Dafne Ibarra Morales, Mariana Schulte-Sasse, Fides Zenk

GROUP LEADER NICOLA IOVINO

1977 Born in Italy, Undergraduate studies in Biology, University of Naples, Italy
2007 Ph.D. (Biology) at Rockefeller University, NY, USA & University of Rome, Italy
2007-2013 Postdoctoral fellow at the Rockefeller University, New York, USA and at IGH-CNRS, Montpellier, France
Since 2014 Group Leader at Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

SELECTED PUBLICATIONS


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.

**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).

3 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 germ line 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
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. We have now recently shown that that (i) the H3K27me3 mark in Drosophila is retained in the oocyte until late stages of oogenesis, that (ii) it is intergenerationally transmitted through the maternal germline to the early embryo, and that (iii) it is propagated throughout early embryogenesis restricting and defining cell identity at the time of the zygotic genome activation (Figure 1) (Zenk et al, 2017, Science)

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 maintained 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).
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 genome instability, chaotic chromatin information and impaired cell type identities.
Senior Group
Thomas Jenuwein
Epigenetic Control by Histone Lysine Methylation

LAB MEMBERS
Administration
Marcela Mare

Technicians
Bettina Engist, Lisa Jerabek, Lisa Kolodziejczyk, Birgit Koschorz

Staff Scientist
Nicholas Shukeir

Postdoctoral Fellows
Derek Atkinson, Reagan Ching, Ömer Copur, Kevin Daze (until Nov 2017), Thomas Montavon, Megumi Onishi-Seebacher (until March 2018), Deepika Puri (until June 2018), Oscar Velazquez Camacho (until April 2018)

Ph.D. Students
Katarzyna Duda, Thomas Fuhrmann, Hi-Hsuan Lo, Zoe Sawitzki (until Oct 2017), Kalina Swist-Rosowska

GROUP LEADER THOMAS JENUWEIN
1956 Born in Lohr am Main, undergraduate studies of Biology at University Erlangen and University Heidelberg, Germany
1983-1987 Ph.D. studies at EMBL, Heidelberg, Germany
1988-1993 Postdoctoral fellow at UCSF, San Francisco, USA
1993-2001 Group Leader at the Institute of Molecular Pathology (IMP), Vienna, Austria
2002-2008 Senior Scientist at the Institute of Molecular Pathology (IMP), Vienna, Austria
Since 2008 Director at Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany
2012-2014 Managing Director at Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

SELECTED PUBLICATIONS


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 (Figure 1).

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 safe-guarding 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.

De novo heterochromatin formation by DNA repeat sequences

Heterochromatin is nearly always nucleated by underlying DNA repeat elements. Although around half of the mammalian genome consists of repetitive elements, less than 10% of those retain transcriptional activity. Only transcriptionally competent long intergenic nuclear elements (LINE) and endogenous retroviruses (ERV) and the major satellite repeat (MSR) sequences are decorated with Suv39h-dependent H3K9me3 (Bulut-Karslioglu et al. 2014). Major satellite repeats (MSR) and intact MSR elements are the primary chromatin regions to be silenced by the Suv39h enzymes in mouse ES cells and permutated or truncated MSR elements fail to accumulate Suv39h-dependent H3K9me3. These data allow the dissection of genomic repeat sequences that would direct the de novo formation of heterochromatin.

Based on our genome-wide epigenetic profiling, we identified genomic regions in mouse ES cells that lack the main histone modifications, display little or no DNA methylation and have no RNA output. We have started to insert one or three copies of the basic unit (234 bp) of the MSR and of permutated MSR derivatives into these inert regions of the mouse genome by CRISPR/Cas9-mediated integration of DNA sequences. The initial data indicate that 1 intact copy of the MSR is insufficient,
but that 3 reiterated and intact MSR copies induce a local enrichment of H3K9me3 over the integration site. These analyses will allow the definition of genomic repeat sequences that can direct the de novo formation of mouse heterochromatin.

**MSR 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 by constituting a structural component of a distinct heterochromatin configuration. MSR transcripts largely lack poly(A) signals and remain chromatin associated. The data reveal RNA-DNA hybrids and single-stranded RNA 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 heterochromatin.

Figure 2. 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. In addition, MSR transcripts also facilitate recruitment and secure retention of the Suv39h enzymes to heterochromatin.

**Novel SU(VAR) factors and signaling to heterochromatin**

Genetic screens for suppressors of position effect variegation (PEV) in *Drosophila* have been instrumental to identify crucial chromatin regulators that dictate heterochromatin formation. The laboratory of Gunter Reuter (University of Halle/Wittenberg) has pioneered the isolation and characterization of PEV modifier genes, such as, for example, *Su(var)3-7* (encoding a Zn-finger protein), *Su(var)3-9* (enabling the discovery of the mammalian Suv39h enzymes as the first KMT), *Su(var)3-3* (encoding a H3K4 histone demethylase) and several other important *Su(var)* genes. While to date around 25 *Su(var)* genes have been molecularly defined, genetic analyses in *Drosophila* predict the presence of around 60 novel *Su(var)* loci (Figure 3). Conserved SU(VAR) factors between *Drosophila* and mouse will be selected for functional analyses in heterochromatin formation and repeat-dependent gene silencing. Intriguingly, several out of the novel *Su(var)* genes in *Drosophila* encode components for Ecdyson signaling and suggest that heterochromatin formation is not only developmentally regulated but could respond to hormone signaling. We treated mouse ES cells and differentiated MEFs with a variety of steroid-related compounds and found that Genistein (a plant phytoestrogen) can massively derepress MSR transcription in MEFs. This example highlights the significant insight that can be gained by the functional analysis of novel SU(VAR) factors and reveals that MSR derived heterochromatin is particularly sensitive to stress signaling pathways.

**Image:**

- Figure 2: Major satellite repeat transcripts organize an RNA-nucleosome scaffold at mouse heterochromatin. Model depicting the classic system of position effect variegation (PEV) in *Drosophila*, in which the white gene (which codes for a red eye pigment) is translocated into a block of heterochromatin and stochastically silenced, resulting in variegated eye pigmentation. Suppressors of variegation, so-called *Su(var)* genes, encode chromatin factors and enzymes that stabilize a heterochromatin state. *Su(var)* gene mutants therefore weaken heterochromatin and lead to transcriptional activation of the translocated *In(s)wm4* white gene that is detected by a full red eye colouring.

- Figure 3: A new *Su(var)* screen in *Drosophila* identifies novel *Su(var)* factors. The Figure depicts the classic system of position effect variegation (PEV) in *Drosophila*, in which the white gene (which codes for a red eye pigment) is translocated into a block of heterochromatin and stochastically silenced, resulting in variegated eye pigmentation. Suppressors of variegation, so-called *Su(var)* genes, encode chromatin factors and enzymes that stabilize a heterochromatin state. *Su(var)* gene mutants therefore weaken heterochromatin and lead to transcriptional activation of the translocated *In(s)wm4* white gene that is detected by a full red eye colouring.
LAB TIM LÄMMERMANN
Innate immune cells as sentinels of our body periphery
LAB MEMBERS

Technician
Alina Gavrilov, Petra Wirth

Postdoctoral Fellow
Michael Mihlan, Elisa Nent

Ph.D. Students
Sarah Bambach, Katharina Glaser, Korbinian Kienle, Neil Paterson, Manuel Stecher

GROUP LEADER TIM LÄMMERMANN

1999-2004 Undergraduate Studies in Molecular Medicine at University Erlangen-Nürnberg, Germany

2004-2009 Ph.D. studies at the Max Planck Institute of Biochemistry, Martinsried, Germany

2009-2014 Postdoctoral fellow at the National Institutes of Health, National Institute of Allergy and Infectious Diseases, LSB, Bethesda, USA

Since 2015 Group Leader at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

SELECTED PUBLICATIONS


Lämmermann T (2016). In the eye of the neutrophil swarm – navigation signals that bring neutrophils together in inflamed and infected tissues. Journal of Leukocyte Biology 100, 55-63.

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. While we have learnt from 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 (Figure 1).

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 accu-
mulation 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. By using intravital two-photon microscopy, we could recently define a multistep attraction cascade that guides neutrophils at sites of local sterile injury 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 (Figure 2). While our previous work provided an initial molecular map for neutrophil swarm formation, we are currently investigating how the swarming response is terminated during the resolution phase of inflammation.

Positioning & migration patterns of other innate immune cells

For an optimal innate immune response, a variety of leukocytes with diverse effector functions exhibit coordinated cell migration, tissue positioning, and intercellular interactions (Figure 1, 3). This includes immune cells that are recruited from the bloodstream, which coordinate their function with sessile immune cell types that already seeded the tissue during embryonic development. How chemotactants and adhesion receptors influence the motility patterns of leukocytes in vivo has long been unexplored. For migration in the inflamed tissue interstitium, it is now generally considered that leukocytes move in an adhesion-independent fashion and follow sources of soluble chemokines.

Our current work systematically addresses if this paradigm holds true for all immune cells. Furthermore, we are investigating how immune cells influence the dynamics of each other and how this depends on the specific architecture of healthy and inflamed tissue compartments. To address 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 Neutrophil swarming upon local sterile injury. Upon local tissue injury, neutrophils (red) close to the damage site sense short-range attractant factors. Cell death occurring at the injury site initiates amplified neutrophil recruitment from distant tissue regions. Hereby, single neutrophils release the lipid leukotriene B4 (LTB4), which further amplifies local cell death signals. LTB4 acts as a signal relay molecule between neutrophils, which enhances the radius of neutrophil tissue recruitment. 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.

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.
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.

LAB MEMBERS

Technicians
Ali Hackl, Annette Elizabeth Patterson

Postdoctoral Fellows
Alanna Cameron, George Caputa, Angela Castoldi, Katarzyna Grzes, Aga Kabat, Mai Matsushita, David Esteban Sanin Pena

Ph.D. Student
Nikki van Teylingen Bakker

Masters Students
Lea Flachsmann, Helen Kleinfelder

GROUP LEADER EDWARD PEARCE

1958 Born in Cardiff, UK; 1983 Ph.D. at the NIMR, Mill Hill, UK
1983-1990 PostDoc Training at National Institutes of Health, Bethesda, USA
1990-2001 Assistant/Associate Professor at Cornell University, Ithaca, NY, USA
2001-2009 Associate/Full Professor, University of Pennsylvania, Philadelphia, PA, USA
2009-2011 Member and Chief Scientific Officer, Trudeau Institute, Saranac Lake, NY, USA.
2011-2015 Professor, Department of Pathology and Immunology, Washington University School of Medicine St. Louis, St. Louis, MO, USA
Since 2015 Senior Group Leader MPI-IE and Professor at Faculty of Biology, University of Freiburg, Germany

SELECTED PUBLICATIONS


Macrophages adopt different activation states depending on the overall immunologic context. For example, in settings where TLR agonists are present, especially in combination with a local source of the cytokine interferon-γ, macrophages become pro-inflammatory and assume microbicidal properties. In contrast, the cytokines IL-4 and IL-13 promote alternative macrophage activation, which is important for wound healing and tissue homeostasis as well as for immunity to helminths and for cancer metastasis. Differential functions in inflammatory and alternatively activated macrophages are supported by distinct metabolic processes, with the former using Warburg metabolism, and the latter emphasizing fatty acid oxidation. We have active research programs addressing the regulation and function of these metabolic processes and since the last report we have published on the importance of mTORC2 in macrophage alternative activation, and on the importance of autocrine type 1 interferon signaling in metabolic reprogramming in pDCs (Fig. 1).

Our long term goal is to be able to manipulate metabolic processes in immune cells in order to promote or inhibit their activation and/or longevity, and in so doing develop novel approaches for improving or limiting immune responses as required.

**Immun cell-intrinsic metabolic reprogramming**

We are interested in how the transition from resting to activation is regulated in cells of the innate immune system. Innate immune cells are a diverse group of cell types, such as macrophages and plasmacytoid dendritic cells (pDCs) that express receptors (e.g. Toll-like receptors, TLRs) which recognize molecular motifs characteristic of particular pathogens or cellular damage, as well as receptors for cytokines and other intercellular signaling molecules. Receptor ligation leads to changes in gene expression and cellular biology, and cells activated in these ways can orchestrate responses to infection or damage and play a role in promoting and shaping adaptive immunity. Unregulated activation of immune cells can lead to their participation in disease processes such as autoimmunity and allergy. It is clear that immune cell activation is underpinned by cellular metabolic reprogramming that is tailored towards the bioenergetic and biosynthetic needs of activated rather than resting cells.

Macrophages adopt different activation states depending on the overall immunologic context. For example, in settings where TLR agonists are present, especially in combination with a local source of the cytokine interferon-γ, macrophages become pro-inflammatory and assume microbicidal properties. In contrast, the cytokines IL-4 and IL-13 promote alternative macrophage activation, which is important for wound healing and tissue homeostasis as well as for immunity to helminths and for cancer metastasis. Differential functions in inflammatory and alternatively activated macrophages are supported by distinct metabolic processes, with the former using Warburg metabolism, and the latter emphasizing fatty acid oxidation. We have active research programs addressing the regulation and function of these metabolic processes and since the last report we have published on the importance of mTORC2 in macrophage alternative activation, and on the importance of autocrine type 1 interferon signaling in metabolic reprogramming in pDCs (Fig. 1).

The biology of inflammatory and alternatively activated macrophages can be further modified by additional extrinsic signals such as prostaglandin E2 (PGE2), which itself has been reported to induce a type of alternative activation. We found that PGE2 causes the dissipation of macrophage mitochondrial membrane potential and in al-
alternatively activated macrophages a significant change in expression of a set of genes that includes RELMα. Our data indicate that this reflects effects of PGE2-induced signaling on the expression of genes encoding components of mitochondrial shuttles, systems which exist to balance NAD+/NADH ratios between mitochondria and cytoplasm. The indication from our data that mitochondrial membrane potential can control nuclear gene expression has led us to an ongoing exploration of mechanisms of mitochondria/nuclear communication.

Warburg metabolism, a process by which ATP is produced from glucose independently of mitochondrial oxidative phosphorylation, is dependent on NAD+, which is a required cofactor for GAPDH, a central enzyme in glycolysis. NAD+ is produced by the oxidation of NADH, and the interconversion of NADH to NAD+ is regulated to maintain redox balance. However, NAD+ can be consumed by various enzymatic reactions, and when this happens de novo synthesis of new NAD+ or NAD+ salvage from other molecules, is required to replenish NAD+ pools. It is recognized that expression of the NAD+ salvage pathway enzyme NAMPT is increased in inflammatory macrophages, and we have been working to understand the underlying reasons for this response. Our work has shown that in macrophages stimulated with LPS, NAD+ is depleted due to ROS production from Complex III of the electron transport chain, ensuing DNA damage, and accompanying activation of PARPs which consume NAD+.

Under these circumstances, NAMPT expression is required to regenerate NAD+ to maintain GAPDH activity for Warburg metabolism (Fig. 2) and cellular activation, since loss of function of NAMPT has marked inhibitory effects on these processes.

**Figure 2** Adoption of Warburg metabolism is critical for macrophage activation in response to lipopolysaccharide (LPS). Macrophages stimulated with LPS increase expression of nicotinamide phosphoribosyltransferase (NAMPT), a key enzyme in NAD+ salvage, and loss of NAMPT activity alters their inflammatory potential. We have found that: a) NAD+ depletion and increased NAMPT expression occur rapidly after inflammatory activation and coincide with DNA damage caused by reactive oxygen species (ROS), b) ROS are produced by Complex III of the mitochondrial electron transport chain, and c) ROS are required for macrophage activation. We found that DNA damage is associated with PARP activation, which results in NAD+ consumption; in this setting increased NAMPT expression allows the maintenance of NAD+ pools sufficient for GAPDH activity and Warburg metabolism. Our findings have provided an integrated explanation for dependency on the NAD+ salvage pathway in inflammatory macrophages.

---

**Immune cell/adipose tissue interactions**

Adipose tissues provide central triacylglycerol stores which can be broken down to provide fatty acids as fuels for distal tissues. The cellular components of adipose tissue include adipocytes, preadipocytes, and various immune cells. Recent work has emphasized the importance of immune cells in adipose tissue homeostasis and focused on the role of alternatively activated macrophages in healthy adiposity. There is also growing interest in the role of adipose tissue in wound healing, a process in which alternatively activated macrophages are also implicated.

The key initiating cytokines for alternative activation are IL-4 and IL-13, which are defining cytokines made by Th2 cells, ILC2s, and other cellular components of type 2 immunity. Type 2 immunity is critical for resistance to parasitic helminths, but also plays a dominant role in allergy/asthma. We have been exploring the connection between adipose tissue, and immune cells resident within it, in the response to intestinal helminth infections. Using single cell RNAseq we have discovered a subset of IL-33-receptor expressing Th2 cells, resident within mesenteric fat. This population expands significantly in response to infection, and based on RNAseq data is distinct from Th2 cells within the mesenteric LN or intestine. We hypothesize that this distinct population of Th2 cells resides within adipose tissue because it can either influence adipose tissue biology or is itself influenced by adipose cells in some way that is important for immunity to helminth parasites, and we are engaged in research to address these possibilities.

In a project in collaboration with Dr. Erika Pearce, we are examining the role of adipose tissue in lymph node biology. It is recognized that lymphoid organs are embedded in adipose tissue, but the significance of this for immunity is unknown. We are pursuing this by examining adipocyte biology within perinodal fat deposits, compared to distal adipose tissues, before and during the initiation of an immune response, and asking whether perinodal fat is necessary for immune response induction and resolution. 

---

**Diagram**

- Glucose → NAD+ → Acetyl CoA → CIII
- NADH → G6P → F6P → G3P → 2PG → PEP → Pyr → PARP activation → DNA repair
- ROS → NADH → NAD+ → NMN → NAMPT
- NAD+ salvage
- LDH → Lactate
- GAPDH
- G6P → F6P → F1,6BP → 3PG → 1,3BPG
- Acetyl CoA → TCA cycle

---

**Legend**
- PARP activation
- DNA damage
- Repair
- Cytokine production
Mitochondrial activity is dynamically regulated in immune cells. Recent work from the lab shows that polyamines are required for the efficient translation of the mitochondrial targeting sequences of many metabolic enzymes.
Assistant Members
Kimberly Mueller

Technicians
Jonathan Curtis, Andrea Quintana de Blas

Lab Assistants
M. Ibrahim Alsakani, Lea Flachsmann, Fabian Hässler, Raima Kyle, John Sutherland

Postdoctoral Fellows
Petya Apostolova, Francesc Baixauli Celda, George Caputa, Marco Cavallari, Mauro Corrado, Cameron Field, Joy Edwards-Hicks, Beth Kelly, Ramon Klein Geltink, Ryan Kyle, Michael Stanczak, David O’Sullivan, Daniel Puleston, Matteo Villa

Bioinformatician
Nisha Rana

Masters Student
Hauke Weiß

Scientific Communication

Johan Fridén

Interns
Gustavo Carrizo, Nina Ligthart

Ph.D. Students
Michael Buck, Jing Qiu

SELECTED PUBLICATIONS


GROUP LEADER ERIKA PEARCE

1972 Born in New York (NY), undergraduate studies of biology at Cornell University, Ithaca, USA

2001-2005 Ph.D. studies in cellular and molecular biology at the University of Pennsylvania, Philadelphia, USA

2006-2009 Postdoctoral Fellow at the University of Pennsylvania, Philadelphia, USA

2009-2011 Assistant Member at Trudeau Institute, Saranac Lake (NY), USA

2011-2013 Assistant Professor of pathology and immunology at the Washington University School of Medicine, Saint Louis (MO), USA

2014-2015 Associate Professor of pathology and immunology at the Washington University School of Medicine, Saint Louis (MO), USA

Since 2015 Director at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany
Senior Group Erika Pearce

METABOLIC REGULATION OF T CELL FUNCTION AND FATE

Understanding metabolic reprogramming in T cells during infection and cancer

T cell response to acute infection

What are the metabolic requirements during these phases?

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 reinfection. 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.

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 1). 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 enforce either the effector or memory fate of a T cell.

Immune cell metabolism in the tumor microenvironment

During a productive immune response to cancer, naïve tumor antigen-specific T cells become activated and produce a variety of effector molecules that mediate tumor clearance. However, T cells often experience a progres-
ly regulated process that occurs in the cytoplasm of all cells. In eukaryotes, amongst other wide-ranging functions the polyamine spermidine is needed to hypusinate a conserved lysine residue in the translation elongation factor eukaryotic initiation factor 5A (eIF5A). We recently investigated how this process impacts cell metabolism and found that hypusination of eIF5A is critical for TCA cycle integrity and mitochondrial respiration. Hypusinated eIF5A (eIF5AH) enables efficient translation of a subset of TCA cycle and mitochondrial electron transport chain (ETC) enzymes that mediate stable TCA cycle flux and oxidative phosphorylation (OXPHOS). Our results suggest that eIF5AH facilitates efficient production of proteins with difficult to translate mitochondrial targeting sequences (MTS), an activity that is consistent with eIF5A in overcoming ribosome stalling. We found that eIF5AH is required for the differential activation of bone marrow-derived macrophages (BMMs), a cell type whose divergent functional fates are defined by a metabolic switch between respiration and glycolysis. Our findings have implications for controlling metabolism in immune cells to therapeutically modulate inflammation. We hope that our work will allow us to develop new ways to target immune cell function through metabolism, with a long-term goal of mitigating human disease.

Ancillary Metabolic Pathways

The contribution of core metabolic pathways to immune cell bioenergetics has been vigorously investigated in recent years. However, precisely how other peripheral metabolic pathways support immune cells beyond energy generation is less well understood. Recent work in the laboratory focuses on so-called ancillary metabolic pathways, and how they affect the function of immune cells to support processes beyond ATP production and ultimately contribute to protective immunity. One such pathway, the polyamine biosynthesis pathway, is a high-

Figure 2 Metabolic tug-of-war within the tumor microenvironment. The balance of nutrients and oxygen within the tumor microenvironment controls immune cell function. Glucose and amino acid consumption by tumor cells can outpace that of infiltrating immune cells, specifically depriving them of nutrients to fuel their effector function.

Figure 3 Ancillary Metabolic Pathways Are Intimately Intertwined with Core Metabolism Core metabolic pathways (gray shaded) use most of the carbon equivalents derived from nutrients for the production of energy, to control redox balance, and to generate biomass. “Peripheral” pathways are also intertwined with core metabolism. In this figure, we focus our attention on some of the documented interactions between “peripheral” and core metabolic pathways.
SUMO chain assembly. Workman (red) represents the conjugating SUMO enzymes.
LAB MEMBERS

Group Leader Andrea Pichler
SUMO & Ubiquitin control

Group
Andrea Pichler
SUMO & Ubiquitin control

Postdoctoral Fellows
Nathalie Eisenhardt, Chronis Fatouros, Easa Nagamalleswari

Ph.D. Student
Dragana Ilic

GROUP LEADER ANDREA PICHLER

1966 Born in St. Pölten, Austria; Undergraduate studies in Biology at University of Vienna, Austria
1998 Ph.D. studies at University of Vienna, Austria
1998-2005 Postdoctoral fellow at the Novartis Research Institute, Vienna, Austria, at the Max Planck Institute of Biochemistry, Munich and at the University of Göttingen, Germany
2006-2009 Independent Project Leader at the Max F. Perutz Laboratories, Vienna, Austria
2010 Habilitation at the University of Vienna, Austria
Since 2010 Group Leader at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

SELECTED PUBLICATIONS

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). 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.
The ZNF451 family, a novel class of SUMO enzymes

Very recently, we discovered a novel family of SUMO conjugating enzymes with E3 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 sumoylation 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).
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.
**LAB MEMBERS**

**Administrative Assistant**
Michal Dror

**Technicians**
Vanessa Wegert, Till Wörpel

**Project Leader**
Steffen Heyne

**Postdoctoral Fellows**
Erez Dror, Anne Drougard, Mary Iconomou, Heidi Lempradl, Iliaria Panzeri

**Ph.D. Student**
Chih-Hsiang Yang

---

**SELECTED PUBLICATIONS**


---

**GROUP LEADER J. ANDREW POSPISILIK**

1976 Born in Vancouver, Canada, Undergraduate studies in Physiology at the University of British Columbia, Vancouver, Canada

1998–2003 Ph.D. studies at the University of British Columbia, Vancouver, Canada

2004–2009 Postdoctoral fellow at the Institute of Molecular Biotechnology, Vienna, Austria

2010–2019 Group Leader at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

Since 2019 Director of the Center of Epigenetics and Professor at Van Andel Institute, Grand Rapids, USA
Mapping (epi)genome variation as a mode of disease etiology

The first comprehensive attempts to provide an epigenetic framework on a genome wide scale are now being realized en masse (e.g. NIH Roadmap). These efforts, many of which were initially performed 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.

We aim to answer the question, “Who could I have been?”. Our lab aims to challenge dogmas in understanding the mechanisms underpinning disease and evolution. Current estimates place the prevalence of diabetes, neurodegeneration, cancer and obesity beyond 1 billion individuals worldwide. These represent the world’s chief economic and health care challenges of the day. 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 phenotypic variation and thus to disease susceptibility. These paradigms include, among others, metabolic- and 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. Our efforts are split into two avenues:

**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.

**Mapping (epi)genome variation as a mode of disease etiology**

The first comprehensive attempts to provide an epigenetic framework on a genome wide scale are now being realized en masse (e.g. NIH Roadmap). These efforts, many of which were initially performed 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 rigor of offspring. These projects are funded by the Max Planck Society, the ERC, as well as individual and consortia grants from the DFG (Neuromac / eCLASH); and BMBF (DEEP / EpiTriO) 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, and phenotypic variation as an underpinning of disease.

Stochastic and intergenerational determinants of phenotypic variation

Another 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 evidence 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. The demonstration of polyphenism in mice and humans will transform modern medicine, as well as our current understandings of population genetics and evolution.
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.
Technical Assistant
Kerstin Lucht

Ph.D. Students
Ev-Marie Schuster, Julia Zimmermann

Masters Student
Paulina Staus (until 02/2019)

GROUP LEADER ANGELIKA RAMBOLD

1978 born in Munich, Germany; Undergraduate studies in Cell Biology at the Max Planck Institute of Biochemistry, Martinsried, Germany

2004-2008 Ph.D. studies at the Max Planck Institute of Biochemistry, Martinsried, Germany, and the Ludwig Maximilian University, Munich, Germany

2009-2014 Postdoctoral studies at the National Institutes of Health, National Institute of Child Health and Development, Cell Biology and Metabolism Branch, Bethesda, MD, USA

2014-2015 Project Leader at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

Since 2015 Group Leader at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

LAB MEMBERS

SELECTED PUBLICATIONS


Group Angelika Rambold

ORGANELLE NETWORKS IN CELLULAR IMMUNITY

Communication between cell organelles shape the function of immune cells

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 contributed 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 intra-cellular 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 & 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 organelar 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.

Figure 2 During an immune response different organelles (differentially colored) adapt their morphology and inter-organelle interaction position and functional interaction.

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

![Image of metabolic pathway]

**Figure 4** 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.

![Image of aberrant lysosomal size and positioning]

**Figure 5** Aberrant lysosomal size and positioning in macrophages generated from Wildtype (top) or a murine Chediak Higashi Syndrome model (bottom).
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.

© Dr Fernando Aprile-Garcia
LAB MEMBERS

Postdoctoral Fellows
Fernando Aprile-Garcia, Barbara Hummel, Sergio Leone

Ph.D. Students
Prashant Rawat, Aneliya Yoveva

Masters Students
Lukas Mann, Lena Tittel

Medical Student
Ashkan Kharavan

GROUP LEADER RITWICK SAWARKAR

1980 Born in Mumbai, India; Undergraduate studies in Biology at Mumbai, India
2010 Ph.D. (Biology) at Indian Institute of Science, Bangalore, India
2009-2014 Postdoctoral Research at ETH-Zurich, Basel, Switzerland
Since July 2014 Group Leader at Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

SELECTED PUBLICATIONS


Group Ritwick Sawarkar

REGULATION OF TRANSCRIPTION BY PROTEIN HOMEOSTASIS

The role of molecular chaperones and proteasomes in gene expression

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 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.

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.
LAB EIRINI TROMPOUKI

Fluorescent zebrafish, endothelial cells are marked with dsRED and CFP and hematopoietic cells with GFP.
Group Leader Eirini Trompouki
Bloody Signals

LAB MEMBERS

Technician
Lhéarna Klaeylé

Postdoctoral Fellow
Clapes Thomas

Ph.D. Students
Stylianos Lefkopoulos, Aikaterini Polyzou, Pia Prater

Masters Student
Sofoklis Trigkas-Chatziandreou

SELECTED PUBLICATIONS


GROUP LEADER EIRINI TROMPOUKI

1977 Born in Athens, Greece; Undergraduate studies in Biology at the National and Kapodistrian University of Athens, School of Biology, Athens, Greece

2001-2006 Ph.D. studies in Molecular Biology at the BSRC Alexander Fleming and the National and Kapodistrian University of Athens, Medical School, Athens, Greece

2007-2013 Postdoctoral fellow at Children’s Hospital Boston, Harvard Medical School, Boston, USA

Since 2013 Group Leader at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany
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-mesonephros (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 HSC 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 HSC 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 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.
DEEP SEQUENCING FACILITY

High throughput data production with the latest sequencing technology supporting a broad range of research applications. **Top:** high throughput sample preparation for transcriptome analysis of 96 samples using an automated platform and customized protocols. **Bottom:** course for sample preparation for deep sequencing.

### SELECTED PUBLICATIONS


Next-generation sequencing (NGS) technology and applications are extremely fast evolving. As core laboratory at MPI-IE the Deep Sequencing Unit supports technologically advanced projects and 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 workflows to ensure highest quality at reduced turnaround time. Advising and teaching is a central part of our work. In hands-on sample preparation courses, participants are learning latest methodologies and best laboratory practices. The unit is collaborating with all groups at the institute and supports large consortia such as the German Epigenome Programme (DEEP) and CRC992 (Medical Epigenetics).

Technology
The unit employs state-of-the-art sequencing technology (Illumina HiSeq3000®, HiSeq2500®, NextSeq500®, MiSeq® and Oxford Nanopore) and various protocols to infer the sequence content for a wide range of cellular samples submitted to the facility. Standard operation procedures and robust instrumentation are in place to ensure sample preparation according to latest international standards. To handle thousands of highly diverse samples per year, the unit applies automation and software supported process structuring. For high-throughput library preparation a liquid handling station (Biomek i7, Beckman Coulter) is installed that enables end-to-end automation for RNA- and DNA-library preparation. Up to 96 RNA-Seq or ChiP-Seq samples can be prepared in parallel at highest quality without any user intervention. Continuously we are bringing protocols onto the robotic platform; massive testing ensures comparability and reproducibility. A laboratory management software (Parkour) was developed that functions as central platform for sample tracking and quality management. Parkour is a convenient tool for facility users to submit and track their samples. The unit profits from the software as central laboratory notebook and team planning tool.

Methodological development
We support a broad range of next-generation sequencing methods including ChiP-Sequencing, Whole-Genome Sequencing, Methylation Sequencing as well as several RNA-Sequencing techniques such as mRNA-, total-RNA, small-RNA, single cell RNA-sequencing and long-reads sequencing (Oxford Nanopore). All developments are focusing on method applicability to a wide range of sample types and input amounts without further adjustments. In this regard, we have developed NEXSON (nuclei extraction by sonication) to extract nuclei from any formaldehyde fixed material (Arrigoni et al. 2016). Pure nuclei preparations can be used for standardized chromatin preparation and ChiP-Seq. The method was recently expanded to allow intranuclear chromatin cutting and insertion of chromatin barcodes for high-throughput ChiP (Arrigoni et al. 2018). The procedure is called RELACS (Restriction Enzyme-based Labeling of Chromatin in Situ) and can generate ChiP-seq libraries from hundreds of samples within three days and with less than 1000 cells per sample.
TRANSGENIC MOUSE CORE FACILITY


Transgenic technology has widely been recognized as a powerful tool for analysis of in vivo gene function and generation of animal mouse models for the advance of science and human health. The Transgenic Mouse Core Facility (TMCF) strives to provide high-quality, cutting edge transgenic and assisted reproduction technologies, in both a time-effective and cost-efficient manner. We provide investigators with novel mouse lines generated either by transgenesis (via pronuclear/cytoplasmic 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 various CRISPR/Cas9 approaches), e.g. “knockouts”, conditional mutants or gene-edited animals. In collaboration with several groups of the institute we use and further develop the CRISPR/Cas9 technology for editing the mouse genome via direct microinjection into zygotes. As an alternative to microinjection we are currently establishing a new in vivo electroporation method, with a NEPA21 Super Electroporator, for CRISPR reagents delivery into mouse embryos.

Standard assisted reproduction technology services include IVF, embryo transfer, import/export of mouse lines and cryopreservation of embryos and sperm. Our barrier transgenic facility maintains a specific pathogen free (SPF) health status, housing gene modified mice in individually ventilated cage (IVC) systems that are serviced by intensively trained and skilled animal care staff. Embryos (e.g. micromanipulated, to revitalise or from imported mouse lines) are transferred under strict sterile conditions into pseudopregnant foster mothers inside this barrier. Serology testing of selected animals is systematically completed to confirm their health status, thus allowing the transfer of the transgenic founders or positive gene targeted offspring to other areas of the Animal House. After germline transmitters have been identified the investigators will only then become responsible for breeding and analysing their mouse lines under strict compliance to the German Law. Researchers are responsible for keeping accurate and regularly updated mating/offspring/genotyping records for each individual genetically modified mouse line in our internal mouse colony management database, PyRat.

We are supporting and promoting the 3Rs principles of human experimental techniques by striving to develop and implement new technologies, as for example: establishing new ways to make precise and targeted changes to the genome using the CRISPR/Cas editing system, increasing the efficiency of various assisted reproduction technology methods or minimizing pain by replacing injection anaesthesia with inhalational anaesthesia. We welcome any opportunity to promote design, knowledge and experience to help to attain the research goals of the investigators. As active members of the International Society for Transgenic Technologies (ISTT) we participate in increasing education, communication and technology sharing to enhance scientific research and advance the field of animal transgenesis.
FISH FACILITY

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

SELECTED PUBLICATIONS


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. Access to the facility is restricted and automatically recorded; technical parameters of the entire facility are remotely controlled. A separate quarantine room provides opportunity for short-term experiments without compromising the high hygienic standards of the main facility. Currently, about 50 different lines carrying ENU- and CRISPR/Cas9-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 antisense morpholino oligonucleotides into fertilized eggs
- Sperm cryoconservation and in vitro fertilisation

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

MEMBERS
Technicians
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

MEMBERS
Technicians
Uwe Heinzmann
Oleksandr Kuzmenko
Mirco Schultis
Dominik Zota
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


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.
PROTEOMICS FACILITY

SELECTED PUBLICATIONS


Trung, NT, Kremmer, E, Mittler, G (2016). Biochemical and cellular characterization of transcription factors binding to the hyperconserved core promoter-associated M4 motif. BMC Genomics 17, 693.


The unit offers a state-of-the-art biomolecular mass spectrometry (MS) analysis service and collaborates with other research groups at the institute in order to develop custom-tailored protein-protein and protein-nucleic acid complex purification and MS analyses strategies to address biological questions. Accordingly, the unit is running two quadrupole orbitrap instruments (QExactive series) and one linear ion trap (LIT) FT-MS instrument (OrbitrapXL+ETD), that are coupled online via electrospray ionization (ESI) source interfaces to nanoUHPLC (LC-MS). NanoUHPLC reversed phase (RP) separation enables the use of columns with a length of up to 50 cm that in conjunction with sub-2µm bead capillary columns offer superior separation power for complex tryptic peptide mixtures. 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. Recently, we added targeted MS (PRM: Parallel Reaction Monitoring) offering routine sub-femtomol sensitivity as well as next-generation proteomics via DIA/HRM (Data Independent Acquisition/Hyper Reaction Monitoring) to our portfolio. Depending on the sample, proteolytic digestions are performed either in-gel, in solution/suspension (iST or SP3 technology) 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. This 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 beads (e.g. titania-MOC for phosphopeptides). Pre-fractionation of high complexity samples is done either at the protein level by SDS-PAGE or at the peptide level via peptide-SAX, SCX, RP or RP-SCX (iST). The unit is very experienced in metabolic labeling of cells (by SILAC) and quantification of SILAC-MS data is achieved with the MaxQuant and Perseus software environment. Similarly, for conducting label-free quantitative proteomics, we make use of the MaxQuant LFQ algorithm. The bioinformatics pipeline additionally consists of a PEAKS Studio workstation connected to a Mascot Server for automated peptide sequence tag assisted multi-engine database searching. Likewise, DIA/HRM data are processed on a Spectronaut Pulsar workstation. Standard service includes protein ID from silver or colloidal coomassie stained gels. Peptide mapping (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. Proteome-wide analysis identifying and quantifying protein in the range of five to seven thousand protein groups is available (SILAC-MS, LFQ-MS, DIA/HRM-MS). Recently, in a close collaboration with the Akhtar department we have co-developed CAPRI (Crosslinked and Adjacent Peptides based RNA binding domain Identification), which extends our portfolio towards the MS-based identification of RNA-binding protein domains.
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


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.

The facility is equipped with the most advanced, state-of-the-art flow cytometers. For sorting, we have four Becton Dickinson (BD) FACSAria Fusions, one BD FACSAria III, one BD Influx sorter, one Beckman Coulter MoFlo XDP sorter and a Miltenyi autoMACS-Pro magnetic sorter. For analysis of cell samples our facility provides two LSR II and three 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 up to twenty fluorescent parameters (Influx, Aria III and Aria Fusions), 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 96-well or 384-well plates. 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 2018 we sorted more than 10,000 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)

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.
IMAGING FACILITY

Top: Structured illumination super-resolution image of a live mammalian cell. In green mitochondrial DNA, in orange microtubules and in magenta mitochondria. Elyra PS1 microscope. Scale bar, 10 microns. Sample prepared by Imaging Facility.

SELECTED PUBLICATIONS


Facilities Imaging

Thanks to tremendous progress of instrumentation, software and molecular “tools” (such as genetically encoded fluorescent tags and ion sensors), light microscopy evolved from an “illustrative” method to a stand-alone technology in modern life sciences, which is capable to visualize nearly every structure and every event of interest in a biological sample. In many cases, dynamic non-invasive observations on live samples are possible, and quantitative information on concentrations and specific interactions of a molecule of interest can be extracted.

At the Imaging Facility, a large arsenal of light microscopy techniques and image analysis tools are available. The facility currently manages 8 advanced fluorescence microscopes of various types, including 5 systems suitable for live samples, a biosafety level S2 containment, high-end workstations and advanced programs for bioimage analysis. The Facility staff assists about 80 users from MPI-IE in all microscopy-related subjects, including choosing the optimal technique, microscope operation, image processing and data interpretation.

Usage of the Facility increased from total 5000 hrs in 2015 to over 11000 hrs in 2018. To further improve the service and user experience, Facility staff continuously communicates with the research groups, informally and through regular Users Committee meetings. Staff members teach microscopy and image processing in various formats, from one-to-one hands-on sessions to seminars and EMBO courses. Further, Imaging Facility is involved in method development. The Facility constantly renews its equipment and expands the arsenal of available techniques.

In October 2017, LSM880 Airyscan FAST, a high-end confocal microscope with super-resolution capacity, was installed. In 2018, a light sheet microscope has been acquired by Akhtar Department and installed at the Imaging Facility. Thanks to unique light path configuration, the microscope permits fast 3D imaging of very large (over 1 cm) samples, such as live embryos or whole organs of small animals. The Imaging Facility is a member of the German Society for Microscopy and Image Analysis and Microscopy and Image Analysis Platform Freiburg (MIAP).

Featured instruments
- LSM880 Airyscan FAST, high-end confocal system equipped with recently invented “Airyscan” array detector which enables one to significantly improve speed, sensitivity and/or resolution (up to 1.7 fold), in comparison to any conventional confocal system.
- Elyra PS1, a multi-modal system which combines PALM&dSTORM super-resolution, SIM super-resolution, total internal reflection (TIRF) and confocal imaging modes.
- Cell Observer SD, a Nipkow spinning disc microscope, enables high-frame-rate and long-term observations of live samples with little photodamage, as well as fast acquisition of 3D datasets.
- LSM780 NLO, an inverted combined multi-photon and confocal system enabling one to acquire high-quality images deep in intact live samples.

MEMBERS
Technicians
Visnja Jevtic
Petra Kindle
Roland Pohlmeyer

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

FEATURED INSTRUMENTS
- LSM880 Airyscan FAST, high-end confocal system equipped with recently invented “Airyscan” array detector which enables one to significantly improve speed, sensitivity and/or resolution (up to 1.7 fold), in comparison to any conventional confocal system.
- Elyra PS1, a multi-modal system which combines PALM&dSTORM super-resolution, SIM super-resolution, total internal reflection (TIRF) and confocal imaging modes.
- Cell Observer SD, a Nipkow spinning disc microscope, enables high-frame-rate and long-term observations of live samples with little photodamage, as well as fast acquisition of 3D datasets.
- LSM780 NLO, an inverted combined multi-photon and confocal system enabling one to acquire high-quality images deep in intact live samples.
LABORATORY ANIMAL FACILITY

Top: Mouse (Mus musculus) of our facility with polycarbonat enrichment showing typical explorative behaviour. Bottom: IVC-Housing of our mice.
The Animal Facility at the Max Planck Institute of Immunobiology and Epigenetics has supported research for more than 50 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 wellbeing 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 September 2017, the construction of the new building for animal holding and breeding of mice exceeding the new European animal welfare regulations and standards has started.

The MPI-IE Mouse House offers high standard services

- Animal colony maintenance
- Hygiene monitoring and veterinary service
- Training for scientists, caretakers and trainees
- Assistance in experimental design and techniques
- Tissue biopsies, blood and organ collection
- Import and export of animals

For mouse strain management and coordination of the services offered, a software program (PyRAT) is introduced in 2019 and will replace the 4D based TBase software developed by Dr. Peter Nielsen. PyRAT 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. PyRAT also enables the users to monitor procedures and animals that have been licensed by the local authorities.
INSTITUTE REPORT 2016–2018  MAX PLANCK INSTITUTE OF IMMUNOBIOLOGY AND EPIGENETICS

M E T A B O L O M I C S  F A C I L I T Y

Top: Metabolites were measured by GC-MS. Then peaks were automatically detected and integrated by our in-house data processing. Left: $^{13}$C tracing samples in autosampler awaiting measurement on GC-MS. Right: Sample prep for quantification of polar metabolites on LC-MS.

SELECTED PUBLICATIONS


All living cells are metabolically active, i.e. they take up substrates and use them to fuel a network of biochemical reactions. The intermediates of this network are called metabolites. Their entirety is the metabolome. Metabolomics is the study of these chemical fingerprints. Like the genetic code, the metabolic network is highly conserved across species; therefore metabolomics can work for any biological model.

Metabolites directly reflect a cell’s physiological state, and thus integrate all levels of regulation from gene expression to enzyme kinetics. Moreover, metabolomics is the ideal technology to dynamically observe the response to environmental perturbations such as the availability of nutrients or changes in temperature, because metabolites can respond within seconds. To capture the metabolic state of cells, the metabolomics core facility currently offers targeted and non-targeted quantification of metabolites by GC-MS (gas chromatography coupled to mass spectrometry) and LC-MS (ultra high pressure liquid chromatography coupled to mass spectrometry).

The flow of metabolites through the metabolic network can be traced by feeding cells with substrates such as glucose or glutamine that are labeled with stable heavy isotopes, typically $^{13}$C. We then measure how many labeled atoms were incorporated into key metabolites. From this data, we can infer how much the cell used different metabolic pathways.

We currently operate 3 mass spectrometers: A GC-MS (Agilent 7890 GC coupled to 5977 MS) which we mainly use for label tracing and fatty acid analysis, a LC-QQQ-MS (Agilent 1290 Infinity II UHPLC coupled to 6495 QQQ-MS) for targeted quantification of polar metabolites and lipids and a LC-QTOF-MS (Agilent 1290 Infinity II UHPLC coupled to Bruker Impact II QTOF-MS) for discovery metabolomics and label tracing. All sample data is securely stored and organized in our database and is conveniently accessible through our intranet page. To generate results in a fast and reproducible manner, we have developed a highly automated pipeline for the processing of GC-MS and LC-MS data that provides users with results in a spreadsheet and graphical formats.

The metabolomics core facility is more than an analytics lab. We assist users at every step of their metabolomics experiments including experimental design, sample prep, data processing, and data interpretation.
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


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 1000 high-speed cores, a petabyte of 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 a 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 multiple times per year and are open to all members of the MPI-IE and Ph.D. students.
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.

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 (MPICC) 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 workplaces, participate in back coachings and learn about strategies for stress prevention.
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 MaxDay 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

Engagement with the public is one of the core tasks of the MPI-IE communication goals. On public events such as the nation-wide Max Planck Day, the Freiburg Science Fair, Pint of Science or the “Zukunftstag”, scientists of the MPI-IE regularly explained the relevance of immunobiology and epigenetics to the public.
OUTREACH EVENTS

Making science compelling and accessible to the public is done by a range of formats for different audiences. Regularly the MPI-IE participates in the Science Fair at the Freiburg minster market. At this local event thousands of visitors have the chance to gain insight into the ongoing research at 55 participating scientific institutions and companies. With the help of posters, exhibits and information material presented at a booth, scientists of the MPI-IE explained the relevance of immunobiology and epigenetics. Occupational orientation is in focus at the MPI-IE “Zukunfts- tag” that takes place yearly on the occasion of the German-wide Girls’ and Boys’ Day initiative. School children from Freiburg get the chance to have their first lab experiences and learn what it means to become a scientist. In 2018, the Max Planck Society had its 70th Anniversary and celebrated the so-called Max Planck Day. To this event, the MPI-IE invited the interested public into the auditorium of the University to follow an exciting program giving insights into past, present and especially the future of immunobiological and epigenetic research at the institute.
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.

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 mild 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 historic 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 summertime, various open-air concerts and festivals take place, the Zeltmusikfestival ZMF being one of the biggest.

“Venice for your feet”: Freiburg has a unique system of small canals (called Bächle) that run throughout the center. 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.
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 famous 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 beautiful 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 countless 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 LECTURE SERIES

The Special Guest Lecture 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.

2016

03. March 2016
Gerard Karsenty
Columbia University Medical Center, New York, NY, USA
The impact of bone on whole-organism physiology

07. April 2016
Reina Mebius
VU University Medical Center, Amsterdam, The Netherlands
Lymph node stromal cells in control of immune cells

Sebastian Amigorena
Immunity and Cancer Dept, Curie Institute, Paris, France
Antigen cross presentation and T cell activation by dendritic cells

17. October 2016
Mina Bissel
Lawrence Berkeley National Laboratory, Berkeley, CA, USA
Closing the loop: How a linear DNA sequence becomes a three dimensional tissue and how it may become malignant and metastatic

13. October 2016
Meinrad Busslinger
Research Institute of Molecular Pathology, Vienna, Austria
Control of B cell immunity and leukemia by the transcription factor Pax5

Marc Schmidt-Supprian
Ludwig Maximilians University, Munich, Germany
Investigating lymphomagenesis, NF-kB activation and beyond

09. January 2017
Ben Lehner
Centre for Genomic Regulation, Barcelona, Spain
Trans-generational epigenetic inheritance of environmental information in C. elegans

03. March 2016
Harinder Singh
Cincinnati Children’s Research Hospital, Memphis, USA
Using single cell transcriptomics to analyze immune cell fate choice and dynamics

07. April 2017
Monsef Benkirane
Human Genetics Institute, Montpellier, France
Paving the way towards elimination of HIV persistent CD4+ T cell in vivo

2017

17. October 2016
Meinrad Busslinger
Research Institute of Molecular Pathology, Vienna, Austria
Control of B cell immunity and leukemia by the transcription factor Pax5

27. April 2017
Didier Trono
School of Life Sciences Ecole Polytechnique Fédérale de Lausanne, Switzerland
Mobile genetic elements, polydactyl proteins and the species-specificity of human biology

11. May 2017
Nicolas Gompel
Ludwig Maximilians University, Martinsried, Germany
Regulatory evolution and the diversification of pigmentation patterns in Drosophila

22. May 2017
Bart Lambrecht
VIB UGent Center for Inflammation Research, Gent, Belgium
Dendritic cell – Epithelial cell interactions at the heart of the allergy epidemic
### 2018

<table>
<thead>
<tr>
<th>Date</th>
<th>Speaker</th>
<th>Institution/Location</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>06. July 2017</td>
<td>Danny Reinberg</td>
<td>Howard Hughes Medical Institute, New York, USA</td>
<td>Epigenetics: One genome, multiple phenotypes</td>
</tr>
<tr>
<td>12. September 2018</td>
<td>Julius Brennecke</td>
<td>Institute of Molecular Biotechnology, Vienna, Austria</td>
<td>Ubiquitin sets the timer: Impacts on stress response and aging</td>
</tr>
<tr>
<td>12. September 2018</td>
<td>Jens Brüning</td>
<td>MPI for Metabolism Research, Cologne, Germany</td>
<td>Inflammatory signaling in obesity and metabolic disease</td>
</tr>
<tr>
<td>12. October 2017</td>
<td>Emmanouil Dermitzakis</td>
<td>University of Geneva, Switzerland</td>
<td>Contribution of non-coding DNA to complex disease and cancer</td>
</tr>
<tr>
<td>23. November 2017</td>
<td>Paul Martin</td>
<td>University of Bristol, UK</td>
<td>Studying inflammation in wound healing and cancer</td>
</tr>
<tr>
<td>05. December 2017</td>
<td>John O’Shea</td>
<td>NIH, Bethesda, USA</td>
<td>Genomic views of lymphocyte differentiation and activation</td>
</tr>
<tr>
<td>15. February 2018</td>
<td>Ivan Dikic</td>
<td>Goethe University, Frankfurt am Main, Germany</td>
<td>Ubiquitin and autophagy networks in health and disease</td>
</tr>
<tr>
<td>02. March 2018</td>
<td>Alexander Stark</td>
<td>Research Institute of Molecular Pathology, Vienna, Austria</td>
<td>Decoding transcriptional regulation</td>
</tr>
<tr>
<td>15. March 2018</td>
<td>Luca Scorrano</td>
<td>Venetian Institute of Molecular Medicine, Padova, Italy</td>
<td>Keeping mitochondria in shape: a matter of life and death</td>
</tr>
<tr>
<td>22. March 2018</td>
<td>Richard Morimoto</td>
<td>Northwestern University, Illinois, USA</td>
<td>The biology of proteostasis in aging and neurodegenerative disease</td>
</tr>
<tr>
<td>05. April 2018</td>
<td>Nikolaus Rajewsky</td>
<td>Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, Berlin, Germany</td>
<td>Single cell sequencing in space and time</td>
</tr>
<tr>
<td>26. April 2018</td>
<td>Ralf Sommer</td>
<td>MPI for Developmental Biology, Tübingen, Germany</td>
<td>Nature vs. Nurture: Genetics and epigenetics of phenotypic plasticity and the first self-recognition system in nematodes</td>
</tr>
<tr>
<td>17. May 2018</td>
<td>José Enriquez</td>
<td>National Center for Cardiovascular Research, Madrid, Spain</td>
<td>Genomic views of lymphocyte differentiation and activation</td>
</tr>
<tr>
<td>06. June 2018</td>
<td>Andrea Musacchio</td>
<td>MPI of Molecular Physiology, Dortmund, Germany</td>
<td>Feedback control of mitosis: down to mechanisms</td>
</tr>
<tr>
<td>12. July 2018</td>
<td>Bertie Gottgens</td>
<td>University of Cambridge, UK</td>
<td>Regulatory Networks and Cellular States of Normal and Malignant Blood Development</td>
</tr>
<tr>
<td>13. September 2018</td>
<td>Andreas Trumpp</td>
<td>DKFZ, Heidelberg, Germany</td>
<td>Stem Cells during Normal Physiology and Cancer</td>
</tr>
<tr>
<td>25. September 2018</td>
<td>Alex Schier</td>
<td>University of Basel, Switzerland</td>
<td>Chromatin roles for SUMO in innate immunity and cell identity</td>
</tr>
<tr>
<td>27. September 2018</td>
<td>Anne DeJean</td>
<td>Institut Pasteur, Paris, France</td>
<td>Genetic conflicts shape meiosis, centromeres and species</td>
</tr>
<tr>
<td>15. November 2018</td>
<td>Harmit Malik</td>
<td>Fred Hutchinson Cancer Research Center, Seattle, USA</td>
<td>Resident and Recruited Macrophages in Health and Disease</td>
</tr>
<tr>
<td>23. July 2018</td>
<td>Martin Guilliams &amp; Charlotte Scott</td>
<td>VIB Center for Inflammation Research (IRC), Ghent University, Belgium</td>
<td></td>
</tr>
</tbody>
</table>
DIRECTIONS TO
THE INSTITUTE

Max Planck Institute of Immunobiology and Epigenetics
Stübeweg 51
D-79108 Freiburg
Germany

Phone: +49 761 5108-0
Fax: +49 761 5108-221
Mail: presse@ie-freiburg.mpg.de
Web: www.ie-freiburg.mpg.de

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).

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.

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.

© pikcha (shutterstock)