Max Planck Institute of
Immunobiology and Epigenetics

INSTITUTE REPORT

MAX PLANCK INSTITUTE OF
IMMUNOBIOLOGY AND EPIGENETICS

2016
2018

MAX-PLANCK-GESELLSCHAFT

Contents

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Research Facilities

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Message from the Managing Director

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 Grün, 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

01

About us

Institute Highlights

Zukunftstag at the MPI-IE: First lab experiences for future scientists.

Science Fair and joint activities during the MaxDay in July 2016.

Poster session during the 4th Epigenetics Meeting 2016 at the MPI-IE

2016

April

"Zukunftstag" 2016

The MPI-IE participates in the nation-wide 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 activites 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).

01

Activities and visitors at the MPI-IE booth during the Science Fair 2017 in Freiburg.

More than 300 visitors listen to talks by MPI-IE scienstists at the Max Planck Day in September 2018.

A total of six ERC Grants went to the Junior Faculty of the MPI-IE in the last period.

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.

May

Pint of Science in Freiburg

Researchers of the MPI-IE bring perhaps the most unusal 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.

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.

December

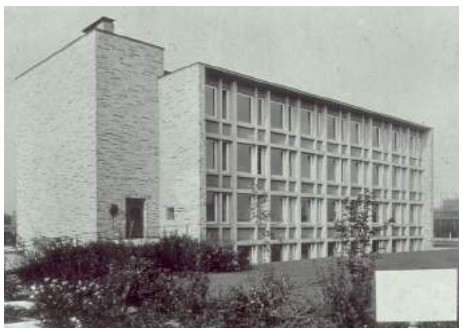
5th Epigenetics Meeting

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 theire latest data and findings in over 40 lectures and poster presentations at this three-day meeting.

History of the Institute

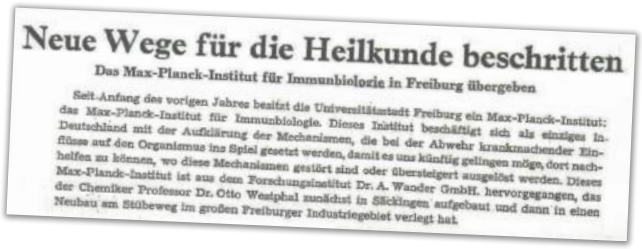
1961

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



The institute in 1962.

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



Neue Wege für die Heilkunde beschritten

Das Max-Planck-Institut für Immunbiologie in Freiburg übergeben

Seit Anfang des vorigen Jahres besitzt die Universitätsstadt Freiburg ein Max-Planck-Institut: das Max-Planck-Institut für Immunbiologie. Dieses Institut beschäftigt sich als einziges in Deutschland mit der Aufklärung der Mechanismen, die bei der Abwehr krankmachender Einflüsse auf den Organismus ins Spiel gesetzt werden, damit es uns künftig gelingen möge, dort nachhelfen zu können, wo diese Mechanismen gestört sind oder übersteigert ausgelöst werden. Dieses Max-Planck-Institut ist aus dem Forschungsinstitut Dr. A. Wander GmbH. hervorgegangen, das der Chemiker Professor Dr. Otto Westphal zunächst in Säckingen aufgebaut und dann in einen Neubau am Stübeweg im großen Freiburger Industriegebiet verlegt hat.

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

1984

Nobel Prize for director Georges Köhler

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

1961
The Max Planck Institute of Immunobiology (MPI-IB) was founded in 1961 on the premises of the former research institute of the pharmaceutical company Wander AG in Freiburg.

1970
Until the end of the 1970's, under the directorship of Otto Westphal, Herbert Fischer and Otto Lüderitz, the institute was primarily engaged in studying the interactions between infectious agents and the immune system, with special emphasis on the bacterial substance endotoxin.

1981
With the recruitment of Klaus Eichmann (1981) and Georges Köhler (1984), the thematic focus of the institute expanded to cellular and molecular mechanisms of B and T cells. Klaus Eichmann and colleagues were the first to describe the development of functional lymphoid tissue from embryonic stem cell lineages.

1984
In 1984, Niels Jerne, Georges Köhler and César Milstein were awarded the Nobel Prize for their pioneering work on monoclonal antibodies using the hybridoma technique.

1990
Through a special funding by the State of Baden-Württemberg, developmental biology was added as another scientific focus, resulting in the recruitment of Davor Solter (1991) and Rolf Kemler (1992). Davor Solter was one of the first to identify genomic imprinting and his research focused on genetic and epigenetic mechanisms regulating mouse pre-implantation development. Rolf Kemler identified the first cell-cell adhesion molecule (E-cadherin) in mouse development and significantly advanced the understanding of mouse embryogenesis.

1998
With the appointment of Thomas Boehm (1998) as successor of George Köhler, developmental immunology was added as a new research focus. Efforts towards a stronger cooperation between MPI-IB and the Faculty of Biology at the University of Freiburg led to the establishment of the University department of molecular immunology at the MPI-IB and recruitment of Michael Reth as its head (1998). In addition, the Spemann Laboratory, consisting of three independent junior research groups, was established with the aim of promoting early independence of junior scientists.

01

2010 2011

Modern Lab in 2016 (Department of Thomas Boehm).

MPI-IB
1961-2010

MPI-IE
since 2010

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

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

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

The institute in 2016.

2004
With the appointment of Rudolf Grosschedl as successor of Klaus Eichmann (2004), the thematic connection between immunology and developmental biology was further strengthened and the molecular mechanisms of lymphoid cell differentiation and the regulation of genes via extracellular signals were added as new research areas.

2006
In 2006, the International Max Planck Research School for Molecular and Cellular Biology (IMPRS-MCB) was intitiated by Rudolf Grosschedl, in collaboration with colleagues of the University of Freiburg.

At the beginning of 2006, the President of the Max Planck Society launched a competition between all institutes of the Society to establish a new department with an innovative research theme. Among all proposals, "Epigenetics" was selected and Thomas Jenuwein (2008) accepted an offer of the Max Planck Society to direct the new department 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 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.

2015
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.

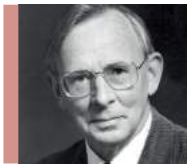
2019
In beginning of 2019, the International Max Planck Research School for Immunobiology, Epigenetics, and Metabolism (IMPRS-IEM) became the successor of the successful IMPRS-MCB Ph.D. program.

Previous Directors of the Institute

1961

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.

1970



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.

1980

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.

1990

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.

2000

Rolf Kemler (Director from 1992-2013) identified the first cell-cell adhesion molecule in mouse development, E-cadherin. He discovered catenins as cytoplasmic anchorage proteins. Particularly β-catenin is well-known because of its dual function in cell adhesion and Wnt signaling. Kemler was the first to establish mouse embryonic stem (ES) cells in Germany. He studied their differentiation potential and used gene targeting to investigate the function of cadherin and catenins in development, genomic maintenance and stem cell vs. oncogenic potential.

2010

01

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

- DEPARTMENT HEAD AND SENIOR GROUP LEADER
- GROUP LEADER
- SCIENTIFIC INFRASTRUCTURE
- JOINT UNIVERSITY/MPI-IE GROUP
- CENTRAL OFFICES
- SERVICE UNITS
- ADMINISTRATION

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

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

- **P. Georgiev** Fly Facility
- **S. Avilov** Imaging
- **A. Würch** FACS
- **B. Kanzler** Trans-genesis
- **N. Iovino**
- **B. Lippok** Safety Dept.
- Animal Welfare Dept.
- **A. Rambold**
- **A. Akhtar** Chromatin Regulation
- **T. Boehm** Developmental Immunology
- **G. Mittler** Proteomics
- **N. Cabezas Wallscheid**
- **E. Trompouki**
- **R. Grosschedl** Cell. & Mol. Immunology
- **B. Lippok** Basic Science Service
- Animal House
- **R. Volz** Workshop
- **M. Rockoff** public relations
- **D. Moll** Human Resources
- **W. Burger** IT-Services
- **M. Fieber** Purchasing
- **C. Klatz** Senior Executive Manager
- **R. Black** Library
- **F. Stiegeler** Accounting
- **N. Amid** Constructions
- **A. Moos** Controlling
- **T. Jenuwein** Epigenetics
- **A. Pichler**
- **A. Pospisilik**
- **U. Bönisch** Deep Sequencing
- **T. Manke** Bio-informatics
- **K. Austin** IMPRS-MCB
- **E. Pearce** Immuno-metabolism
- **J. Büscher** Meta-bolomics
- **Department-independent Research Groups**
- **Max Planck Research Groups**
- **Ed. Pearce** Immuno-metabolism
- **D. Grün**
- **R. Sawarkar**
- **V. Hilgers**
- **T. Lämmer-mann**

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Prof. Dr. Sankar Ghosh
Columbia University, New York, USA


Prof. Dr. Anne Ferguson-Smith
University of Cambridge, Cambridge, UK

01

MANAGEMENT BOARD

Scientific Members of the Institute

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

Head of Administration

- Christian Klatz (since 2017)

EXTERNAL SCIENTIFIC MEMBERS

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

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

Christian Klatz
Senior Excecutive Manager

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.

Human Resources

Finance Department

Constructions

Safety Department

"Little Scientists" – child care facility

The institute offers child care premises located right next to the institute. The facility is run by Concept Maternel, a well-respected non-profit organization which runs several child care facilities and a primary school in Freiburg. Its pedagogical concept is based on the principles of Montessori, Freinet and Pikler and promotes the individual development of each child. Since June 2012, Concept Maternel provides education for children aged between 6 months to 3 years. Since spring 2014 a new and larger building in immediate vicinity of the institute enabled us to add a group for children aged 3 to 6 years to the facility. This way, MPI-IE employees will have the option of leaving their child in the same child care facility until primary school right next to the institute. The entire service is optimally adjusted to the international environment at the MPI-IE: German and English native speaking educators care for the children, opening hours are very flexible and very limited holiday closing days support the parents.

Workshop

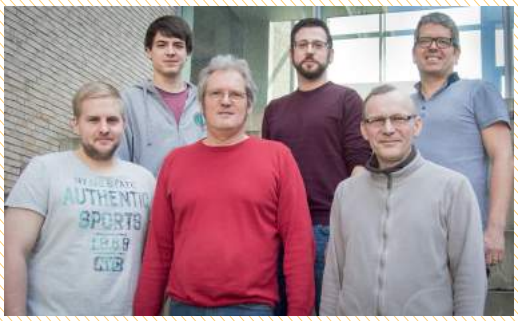
Basic Science Serivce

01

Library

Reception

Purchase Department



IT-Service

Senior Executive Manager: Christian Klatz

Assistant to Senior Executive Manager: Nathalie Schulz

Controlling

Saskia Moos

Finance & Accounting

Head: Florian Stiegeler

Susanne Demme
Jasmin Haaas

Human Resources

Head: Daniel Moll

Theresa Cimentepe
Veronika Klank
Nadine Laule
Dominique Schädler
Patricia Schätzle-Ott
Sabine Stallone

Purchase Department

Head: Michele Fieber

Christian Böhler
Regina Burger
Sven Mußmann
Sascha Wienczierz

Public Relations

Marcus Rockoff

Library

Rose Black

Reception

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Sabrina Fögele
Gabriele Prosch

Safety Department

Head: Bernadette Lippok

Ute Lenz
Young-Min Kim
Marius Ruf
Andreas Rolke

Animal Welfare Department

Inke Krupka-Dyaschneko
Stefanie Kunz

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

Workshop

Head: René Volz

Uwe Bachstein
Günther Barth
Thomas Fischer
Michael Herm
Roland Kaiser
Steffen Pappenfuß
Volker Wehrle

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

IT-Service

Head: Wolfgang Burger

Daniel Andris
Wolfgang Arndt
Ralf Gritzner
Christian Pagel
Wilhelm Rüsing
Luca Schuler-Köble

Constructions

Najeeha Amid

Staff Restaurant & Lounge

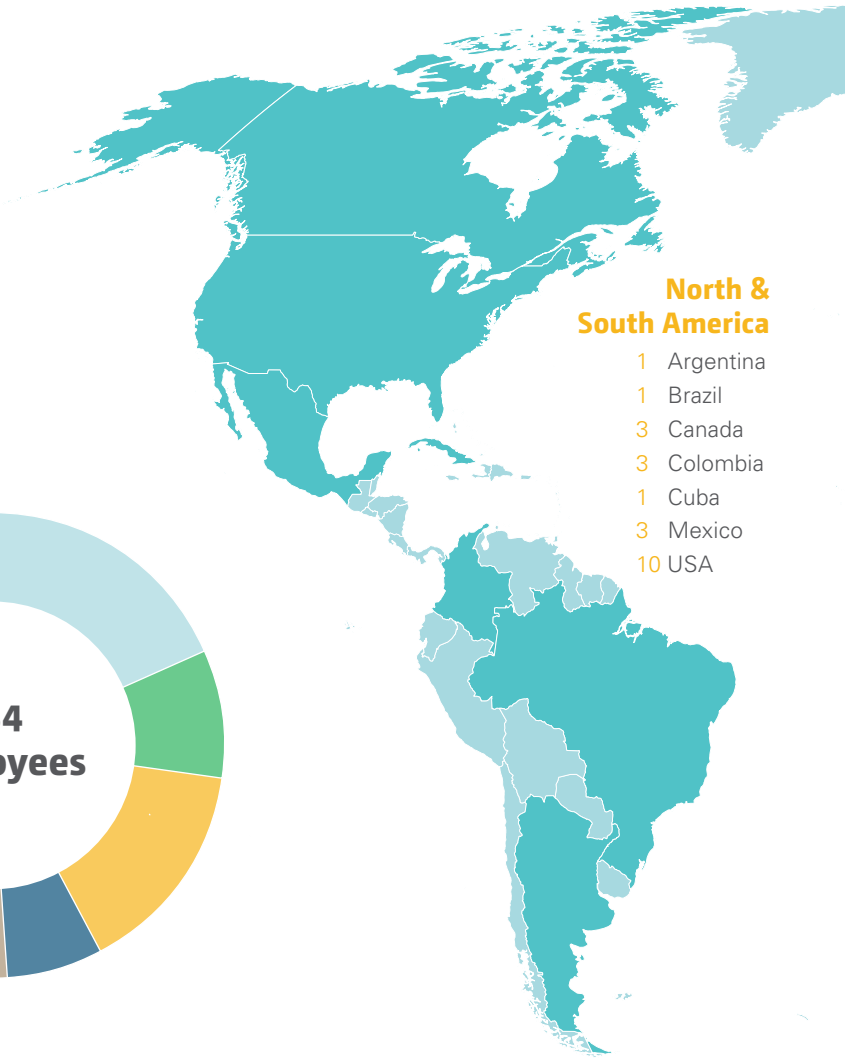
Martin Haberstroh (Tenant)

Facts

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

data as of April 2018

Internationality:

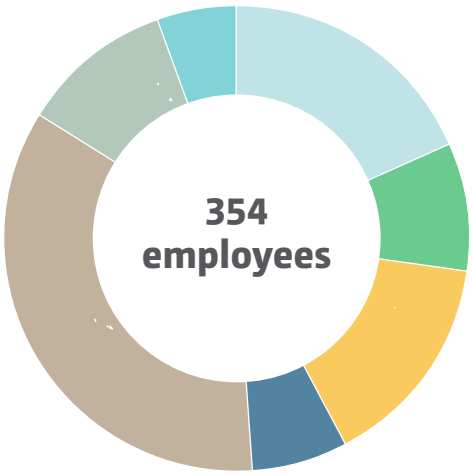


North & South America

1 Argentina
1 Brazil
3 Canada
3 Colombia
1 Cuba
3 Mexico
10 USA

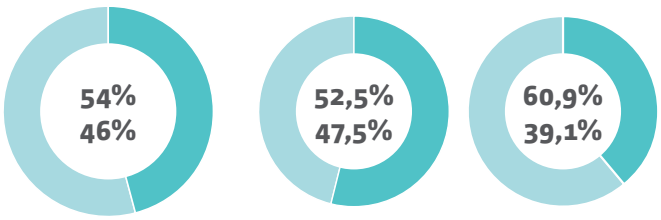
Personnel

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



Gender

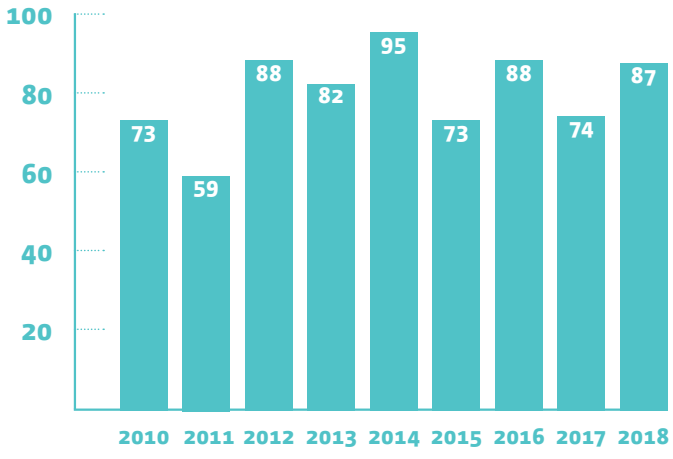
♀ ♂



total | scientific personnel | non-scientific personnel

Publications

Over 700 publications since 2010



(Source: Scopus 2018)

Europe

3 Austria	1 Cyprus	11 France	8 Greece	3 Netherlands	2 Romania	3 Spain	2 Ukraine
1 Beligum	1 Czech Rep.	196 Germany	10 Italy	10 Poland	3 Russia	5 Switzerland	
4 Bulgaria	1 Finland	8 Great Britain	2 Irisch	2 Portugese	1 Serbia	3 Turkey	

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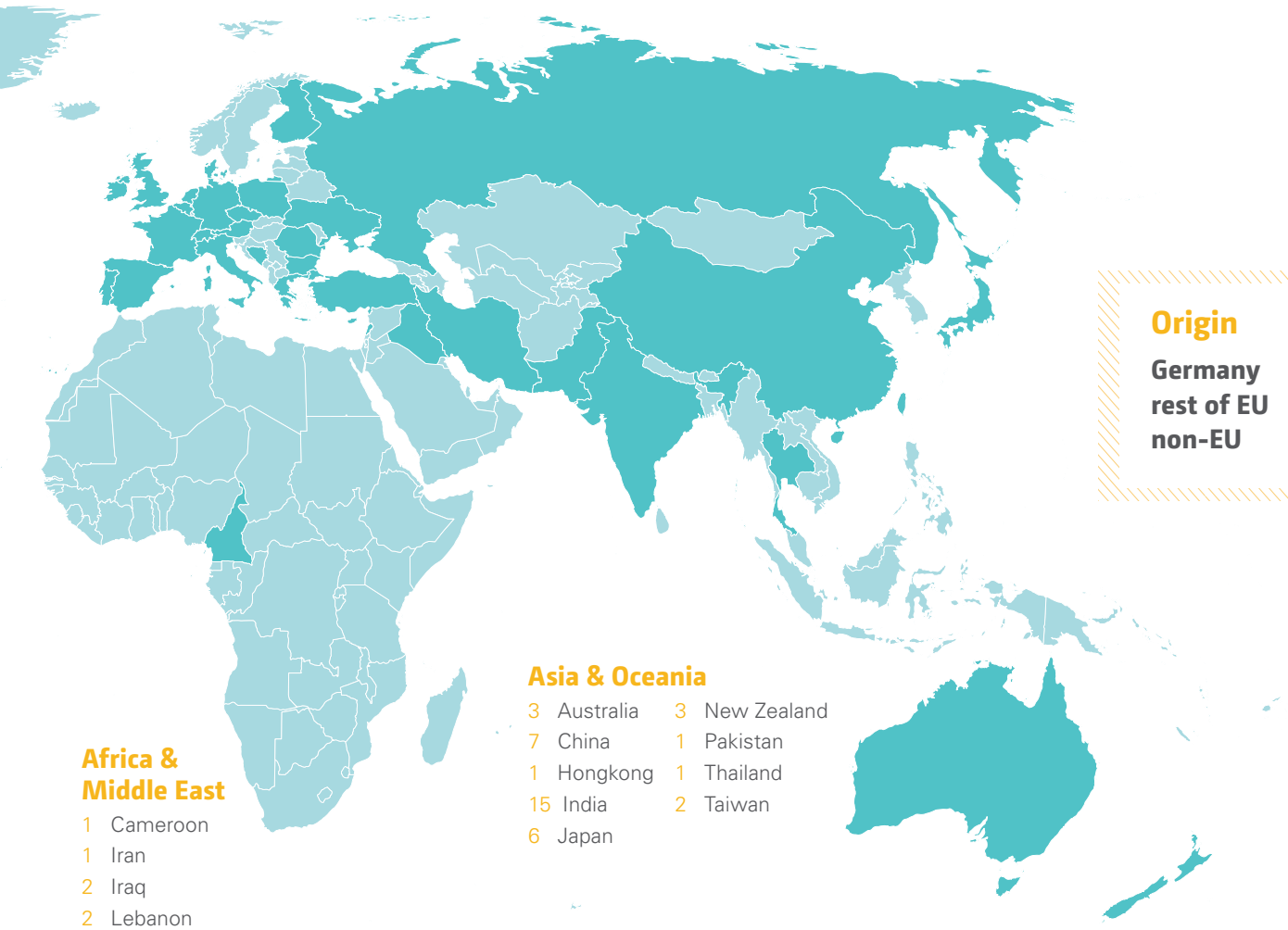


Origin

Germany	**56,5%**
rest of EU	**19,9%**
non-EU	**23,6%**

Asia & Oceania

3 Australia	3 New Zealand
7 China	1 Pakistan
1 Hongkong	1 Thailand
15 India	2 Taiwan
6 Japan	

Africa & Middle East

1 Cameroon
1 Iran
2 Iraq
2 Lebanon

Collaborations

Traditionally, the Max Planck Institute of Immunobiology and Epigenetics (MPI-IE) has long-standing interactions and collaborations with the University of Freiburg and its associated 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.

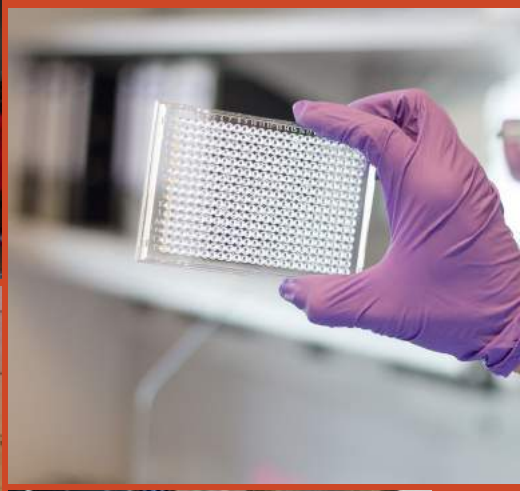
Furthermore, numerous research consortia build on the collaborations between the MPI-IE and the University. For example, MPI-IE research groups are integrated with the cluster of Excellence "BIOSS" and "CIBSS" as well as the Center of Chronic Immunodeficiency "CCI". Also, several SFBs (collaborative 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







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

Transferable skills
- Scientific Writing & Presentation
- Project & Self Management

Scientific training
- Advanced Methods
- Critical Analysis of Literature
- Laboratory Skills

Career Development
- Application Training
- Funding Opportunities

Conferences
support for international scientific conferences & regional symposia

TACs
regularly **t**hesis **a**dvisory **c**ommittees to guarantee scientific excellence & success of each student

IMPRS-MCB at a glance

IMPRS-MCB
From Molecules to Biological Systems

IMPRS COORDINATOR

Dr. Kyle Austin

IMPRS Office
Lisa Breitner

More Information
www.imprs-mcb.mpg.de

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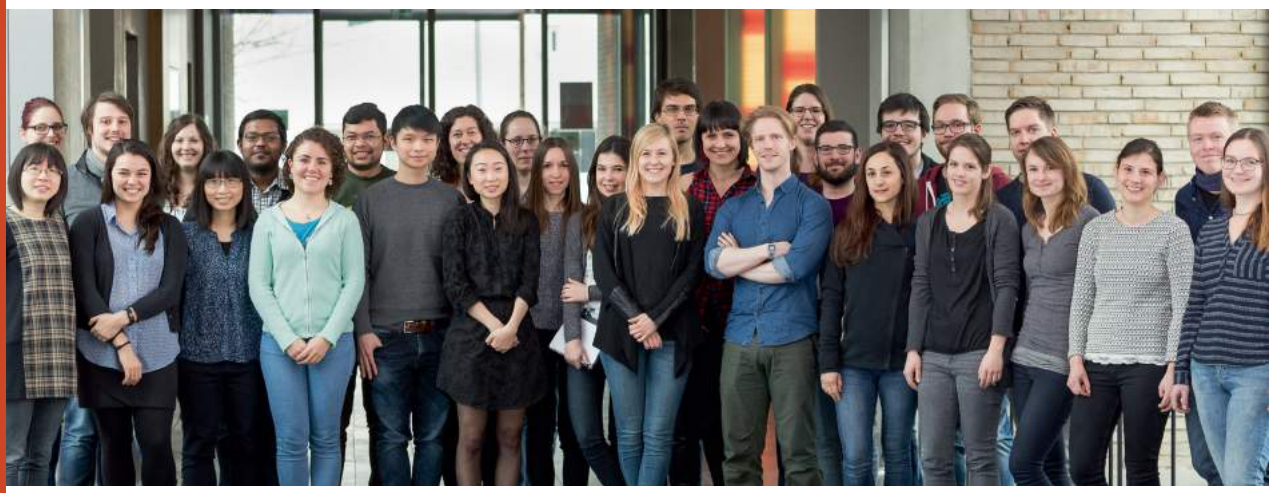


Mission of IMPRS-MCB

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 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 aspects 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.

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

Focus Immunobiology

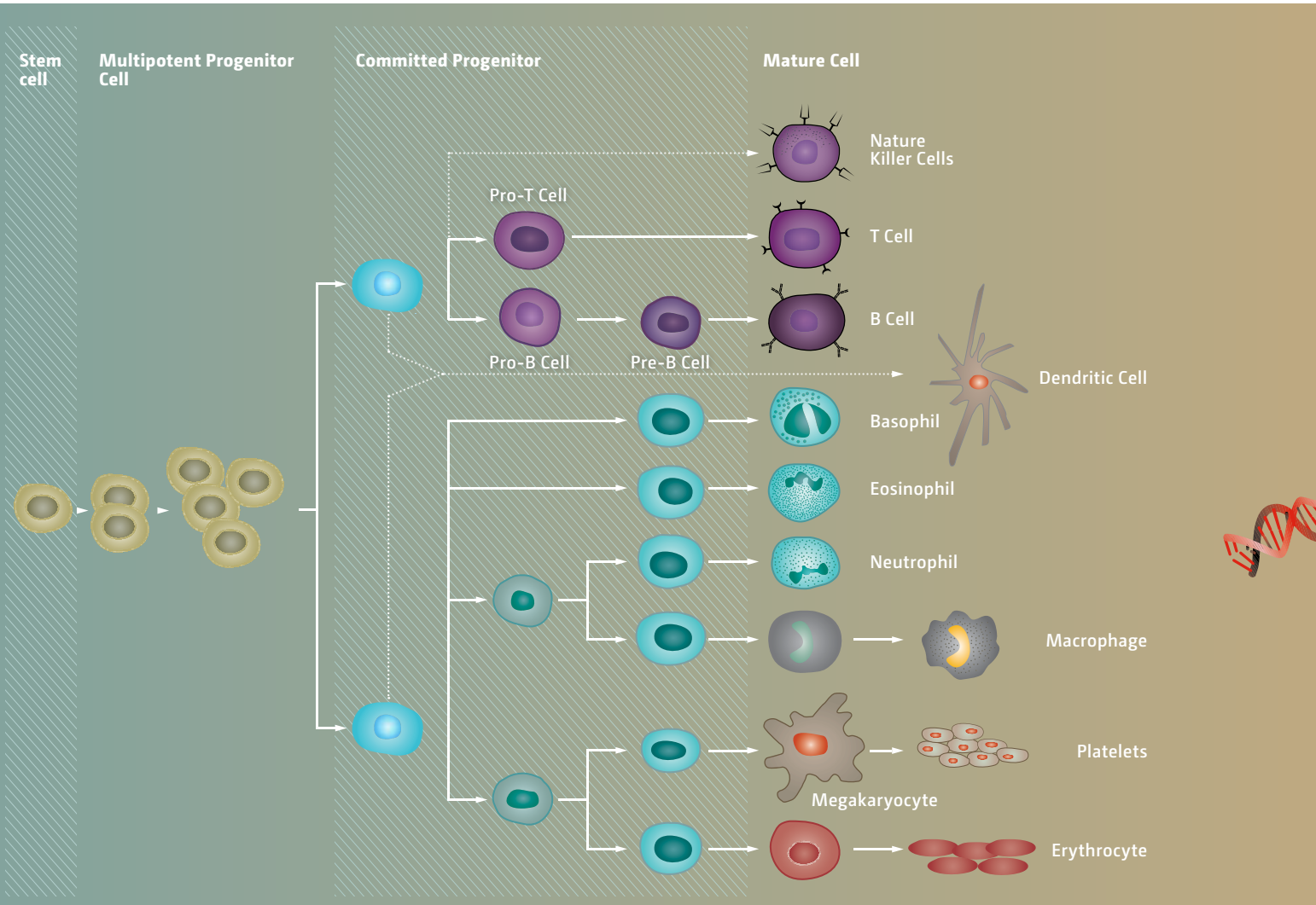
Immunobiology is concerned with the ways multicellular organisms defend themselves against the onslaught of pathogens. They have evolved a plethora of strategies to guard their bodily integrity, and to promote survival and reproduction.

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

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

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Focus Epigenetics

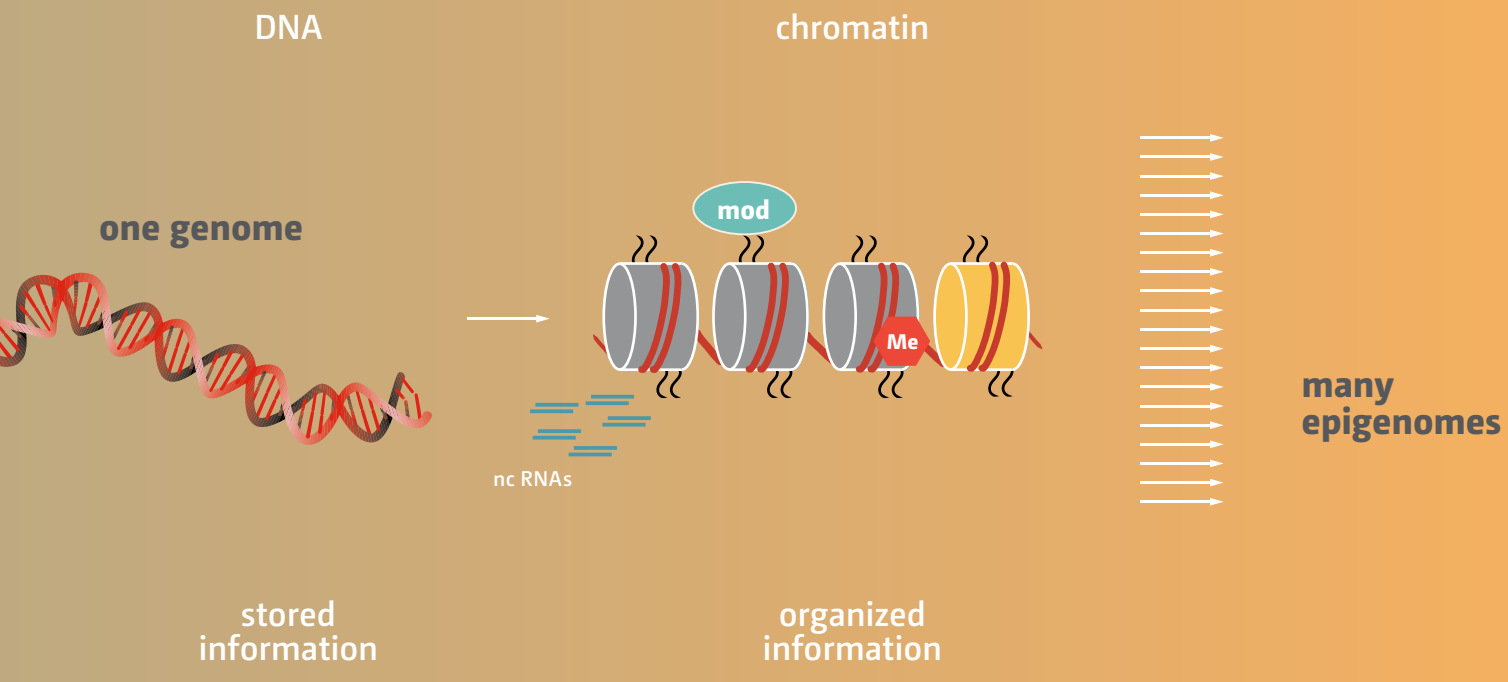
"Are we more than the sum of our genes and how can environmental cues alter gene expression?" While almost all cell types within a human body share an identical DNA sequence, its utilization will differ significantly according to the designated function of a cell. The DNA template within the cell nucleus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

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

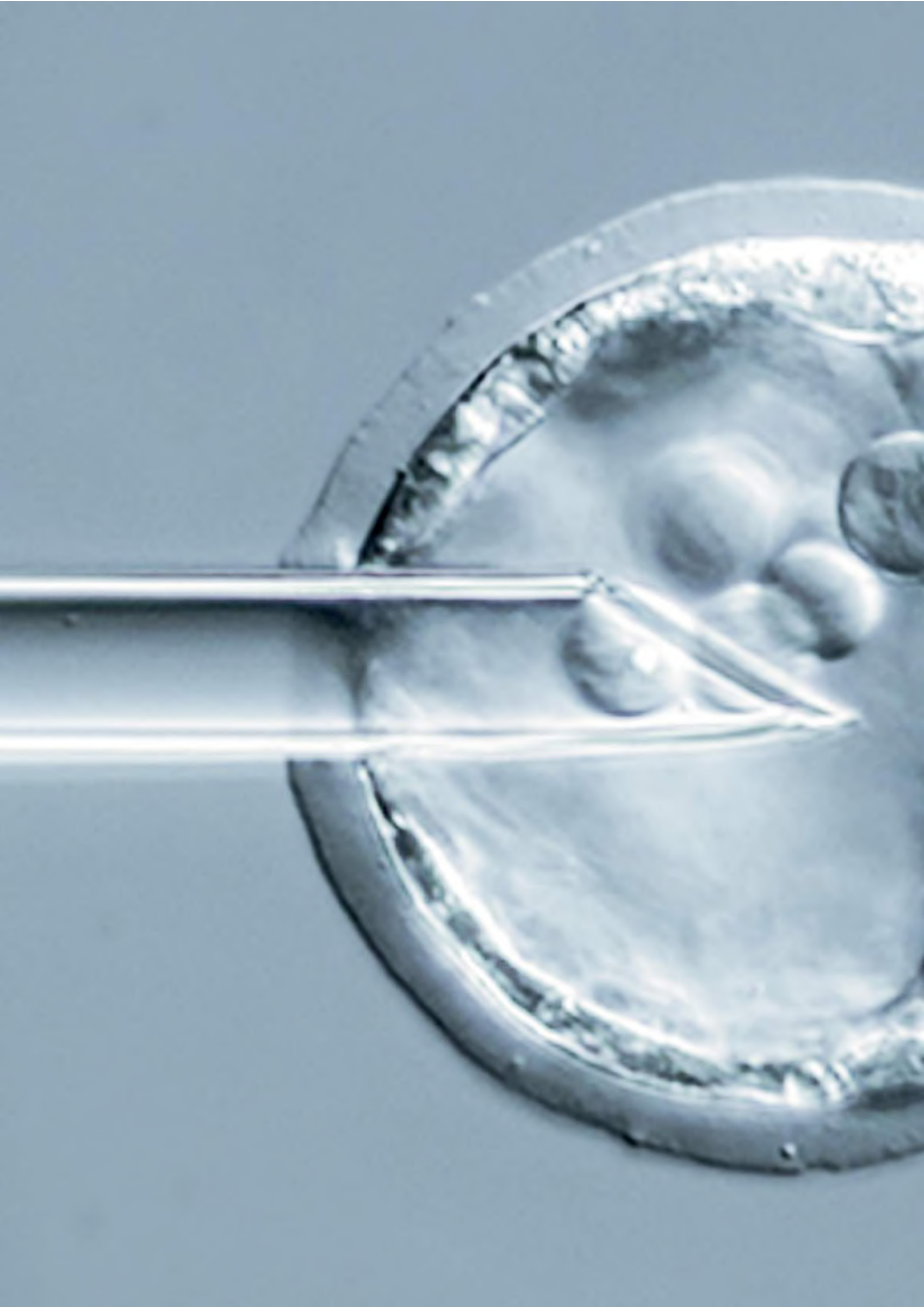
Although many diseases (e.g. cancer, neurodegenerative and metabolic disorders) are primarily caused by DNA mutations, epigenetic disregulation can significantly contribute to disease progression. Thus, epigenetic research promises far-reaching implications for new forms of therapy and diagnosis.

Epigenetic changes also allow responses to environmental influences such as nutrition, stress and hormones. Intriguingly, there is growing evidence that epigenetic alterations might even be inheritable over a few generations.

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



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



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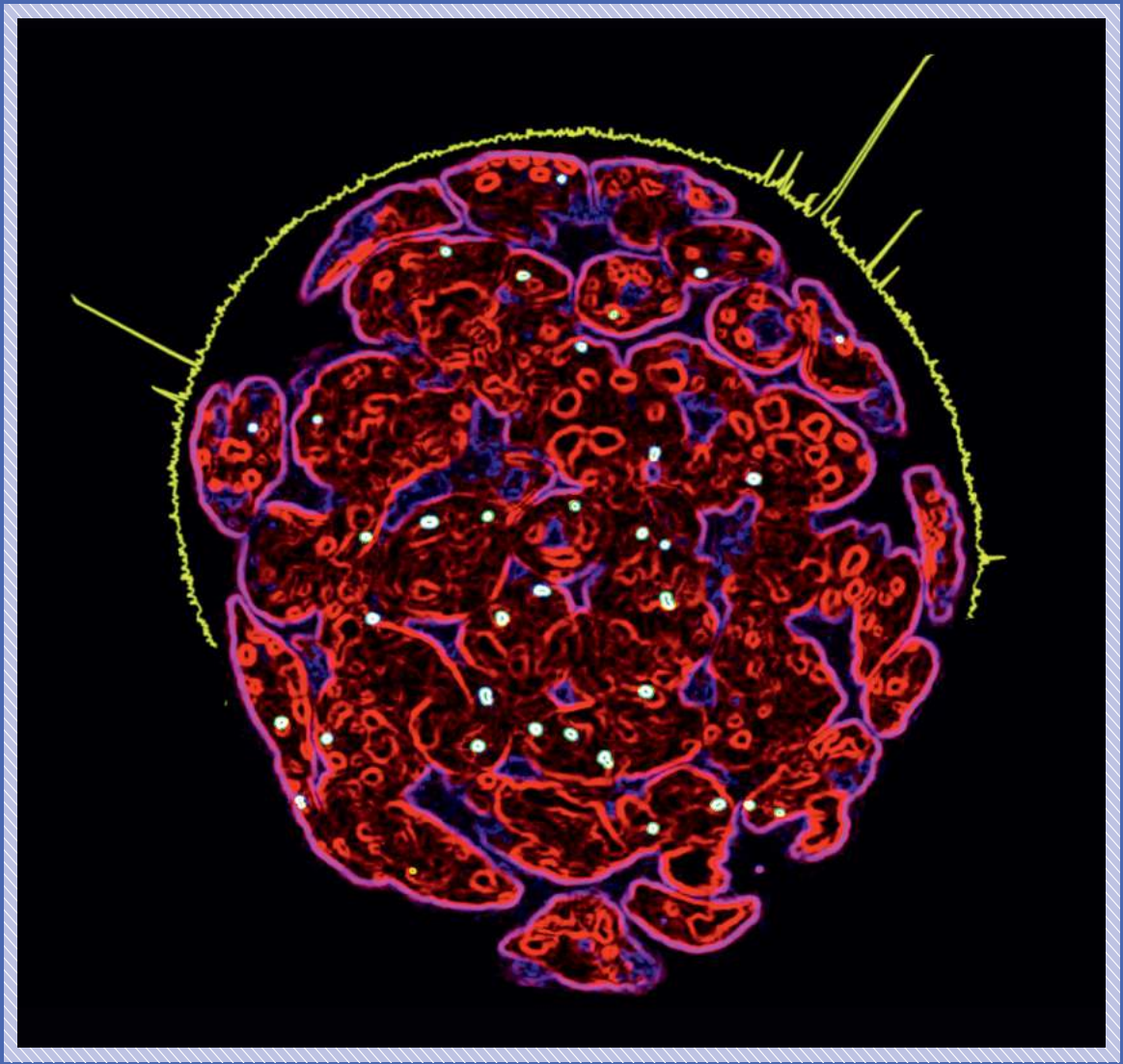
Research Activities



LAB ASIFA AKHTAR

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

02

Senior Group Asifa Akhtar

Chromatin Regulation

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GROUP LEADER ASIFA AKHTAR

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

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

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

2001-2009 Group Leader at EMBL, Heidelberg, Germany

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

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

2015-2017 Managing Director at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

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Basilicata MF, Bruel AL, Semplicio G, Valsecchi CIK, Aktaş T, Duffourd Y, Rumpf T, Morton J, Bache I, Szymanski WG, Gilissen C, Vanakker O, Õunap K, Mittler G, van der Burgt I, El Chehadeh-Djebbar S, Cho MT, Pfundt R, Tan TY, Kirchhoff M, Menten B, Vergult S, Lindstrom K, Reis A, Johnson DS, Fryer A, McKay V, DDD Study16, Fisher RB, Thauvin-Robinet C, Francis D, Roscioli T, Pajusalu S, Radtke K, Ganesh J, Brunner HG, Wilson M, Faivre L, Kalscheuer VM, Thevenon J and Akhtar A (2018). De novo variations of MSL3 cause a X-linked syndrome marked by impaired histone H4 lysine 16 acetylation. **Nature Genetics 50, 1442-1451**.

Valsecchi CIK, Basilicata MF, Semplicio G, Georgiev P, Gutierrez NM, Akhtar A (2018). Facultative dosage compensation of developmental genes on autosomes in Drosophila and mouse embryonic stem cells. **Nature Communications 9(1), 3626**.

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Senior Group Asifa Akhtar

EPIGENETIC MECHANISMS MEDIATED BY HISTONE ACETYLATION

Functional interplay between lncRNAs and epigenetic enzymes to understand transcriptional complexity

Figure 1 Genetic information is encoded in our chromosomes (shows 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.

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

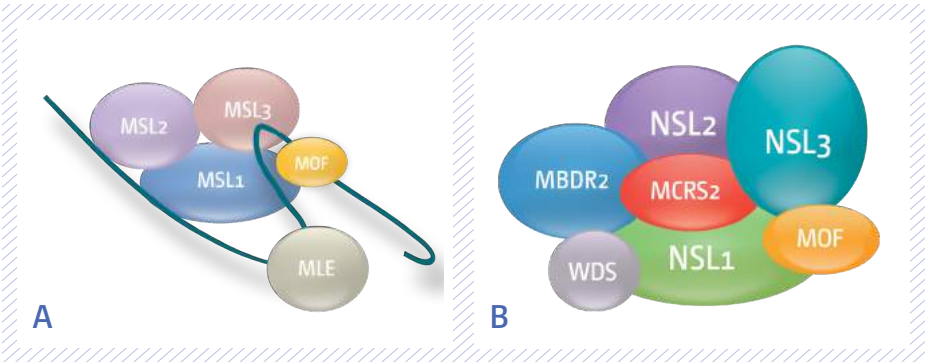


Figure 2 MOF histone acetyl transferase is part of two distinct multiprotein complexes

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 reterotransposable 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.

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, an antisense transcript controlling the expression of Xist long-non-coding RNA, the MSL complex members ensure that the X chromosome remains active in embryonic stem cells. In future, we plan to explore further the mechanism by which MSL proteins play a role during X chromosome inactivation in mammals. 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.

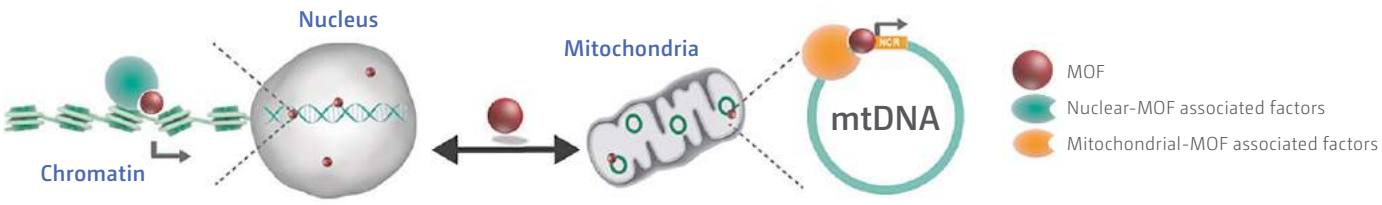
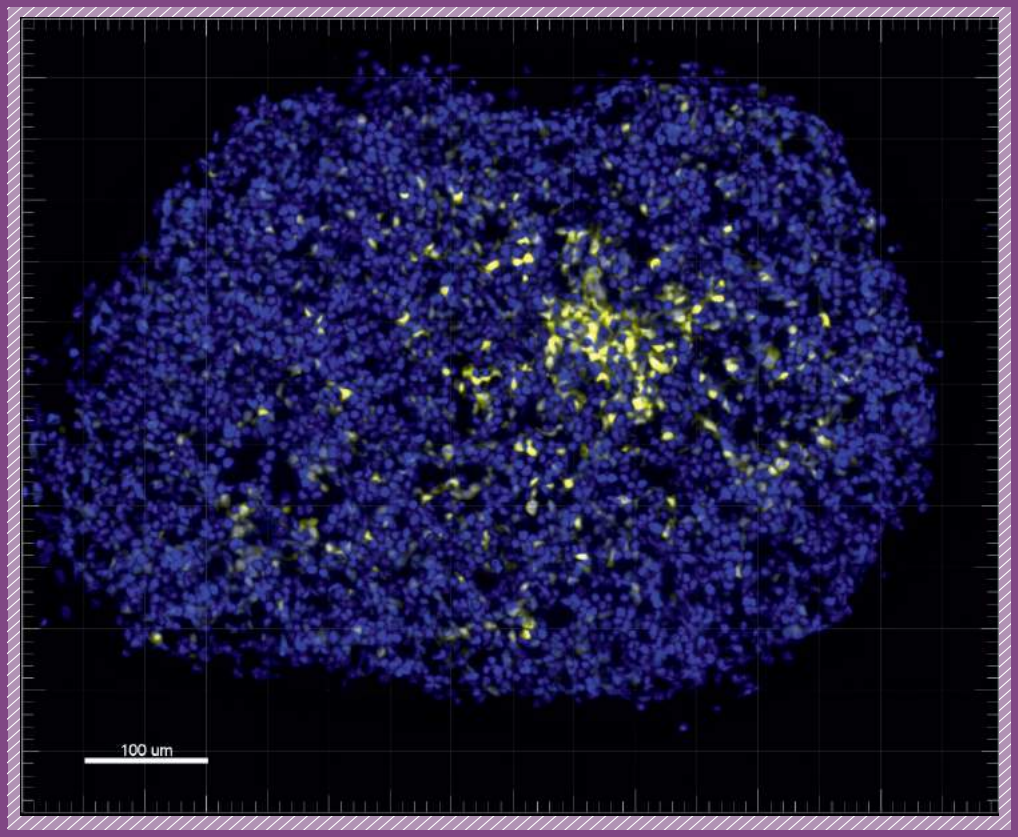


Figure 3 Schematic representation of the MOF's dual role in the regulation of nuclear and mitochondria, thus connecting epigenetics and metabolism.

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.



02

Senior Group Thomas Boehm

Design principles of adaptive immunity in vertebrates

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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

Iwanami N, Sikora K, Richter AS, Mönnich M, Guerri L, Soza-Ried C, Lawir D-F, Mateos F, Hess I, O´Meara OP, Schorpp M, Boehm T (2016). Forward genetic screens in zebrafish identify pre-mRNA processing pathways regulating early T cell development. **Cell Reports 17, 2259-2270.**

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Senior Group Thomas Boehm

THYMOPOIESIS: FROM EVOLUTIONARY ORIGINS TO FUTURE THERAPIES

Design Principles of adaptive immunity in vertebrates

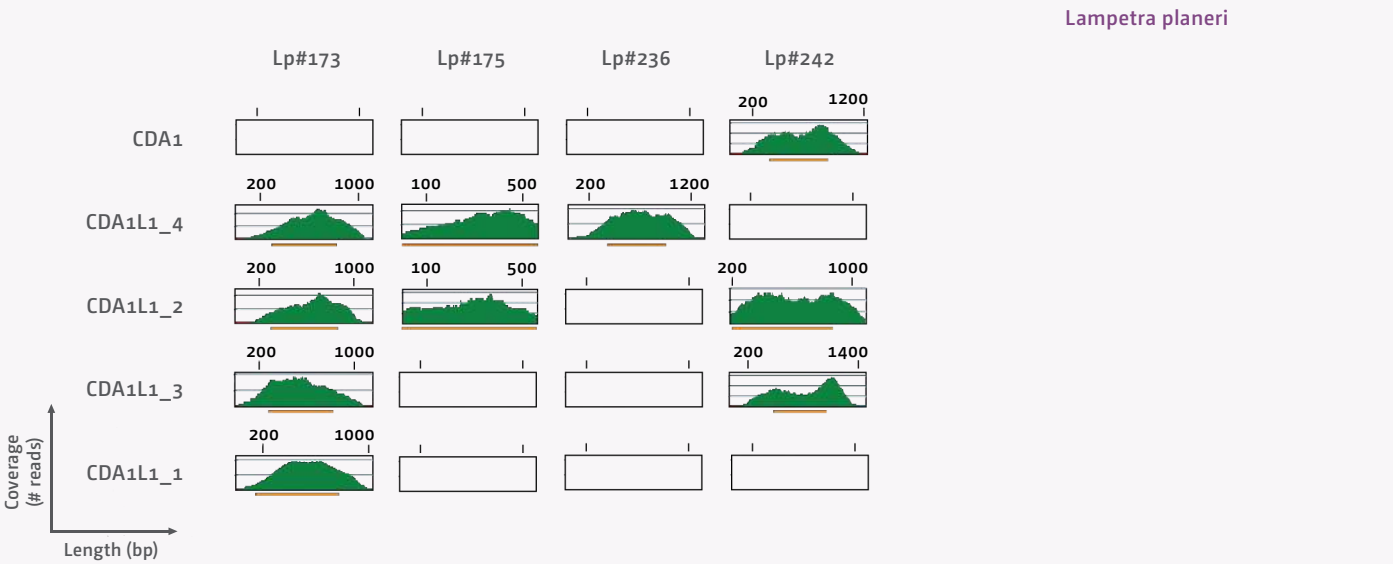


Figure 1 Identification and difference in copy number of *CDA1-like* genes in lamprey. Read coverage plots in whole genome sequences. Green color indicates a coverage by >5 reads; blue, 2-5 reads, and red, single read. Orange bars correspond to region of the contigs containing the open reading frames of the exons.

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

Thymus and T cell development in the mouse

The thymus is a primary lymphoid organ whose function is to provide mature and self-tolerant T lymphocytes that are required to fight infection and maintain tissue integrity. Thymopoiesis depends on the provision of a dedicated epithelial microenvironment that attracts, maintains and specifies T cell progenitors and supports their differentiation into mature, self-tolerant T cells. We are interested in the molecular basis of thymic epithelial development and the characterization of the epithelial progenitor cell, and are studying the molecular mechanisms of TEC specification, proliferation and differentiation. 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

02

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.

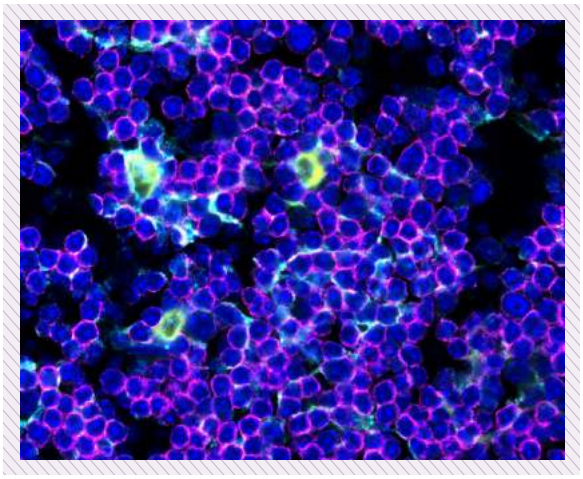


Figure 3 Histological section of a thymus of a 10-day old mouse transgenic for *Foxn1:mCherryNLS* (nuclei of thymic epithelia cells marked with yellow fluorescence) and *Foxn1:Turquoise*^{mem} (membranes of thymic epithelial cells marked with turquoise fluorescence) expression constructs, counterstained with an anti-CD4 antibody (red) and DAPI (blue). Note the intimate interaction of cortical thymocytes with the reticular network of thymic epithelial cells.

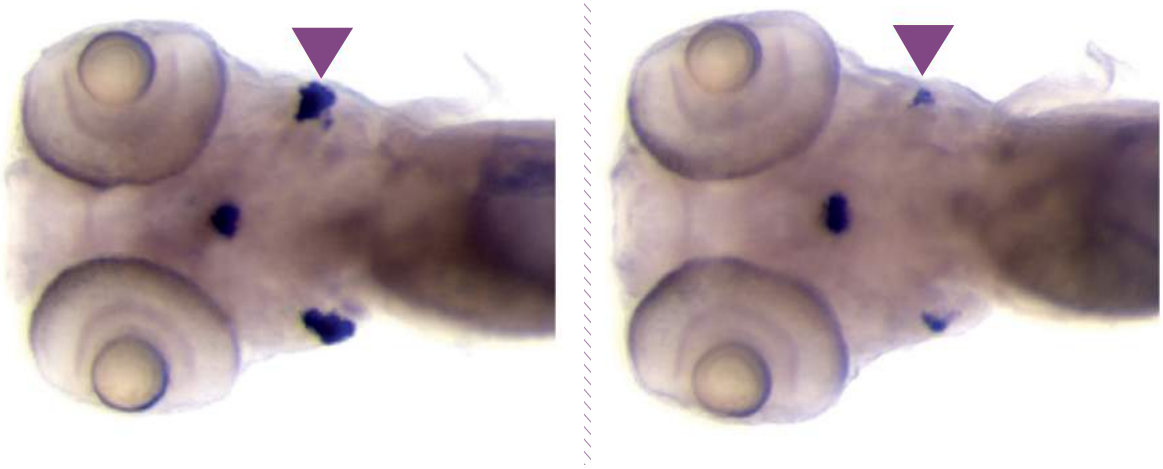
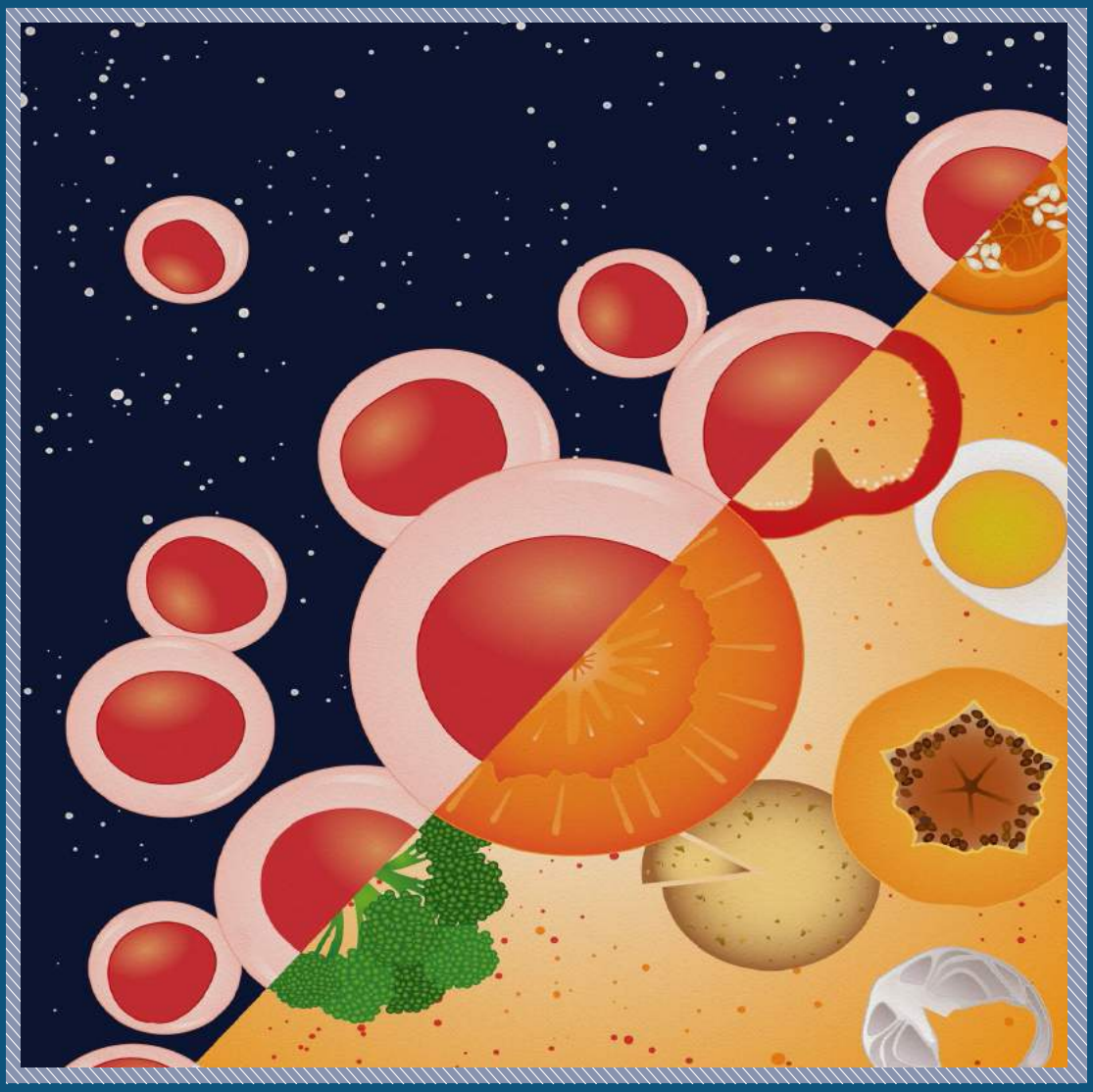


Figure 2 Thymocyte development in wild-type (left panel) and mutant (right panel) fish, visualized by RNA *in situ* hybridization. The thymus is marked with red arrowheads.

LAB NINA CABEZAS-WALLSCHEID

"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.



Cover design by Iris Joval.

02

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Hematopoietic Stem Cell

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SELECTED PUBLICATIONS

Schönberger K, Cabezas-Wallscheid N (2017). Vitamin C: C-ing a new way to fight leukemia. **Cell Stem Cell 21, 561-563**.

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Group Nina Cabezas-Wallscheid

REGULATION OF HEMATOPOIETIC STEM CELL QUIESCENCE

How distinct factors influence hematopoietic stem cell self-renewal

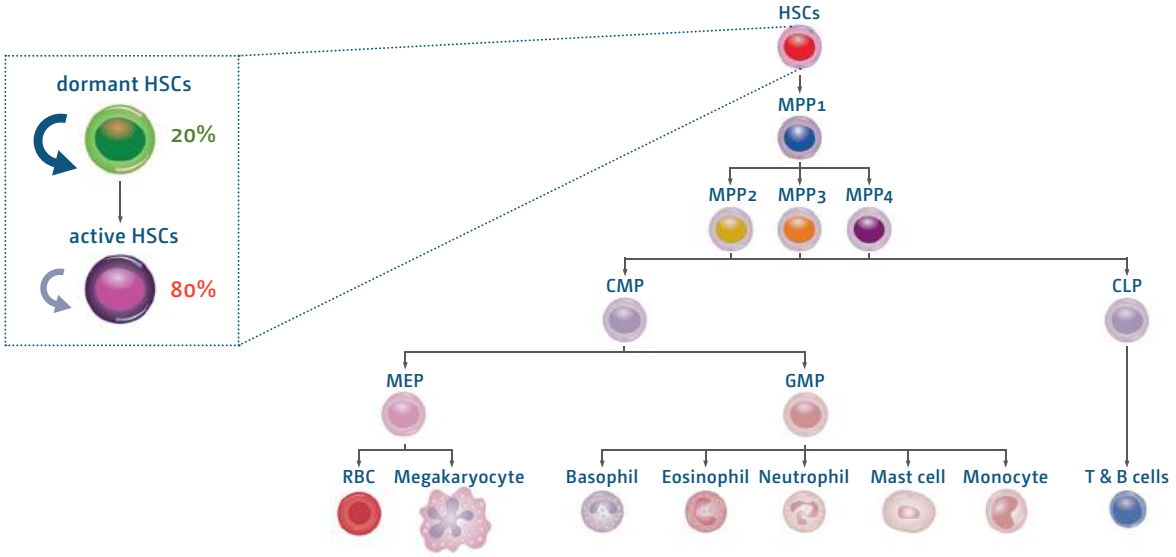


Figure 1 The murine hematopoietic system: **HSC** = Hematopoietic Stem Cell; **MPP** = Multipotent Progenitor; **CMP** = Common Myeloid Progenitor; **CLP** = Common Lymphoid Progenitor; **MEP** = Megakaryocyte Erythroid Progenitor; **GMP** = Granulocyte Macrophage Progenitor; **RBC** = Red Blood Cell.

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 and rapidly reacts to stress stimuli, for example 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

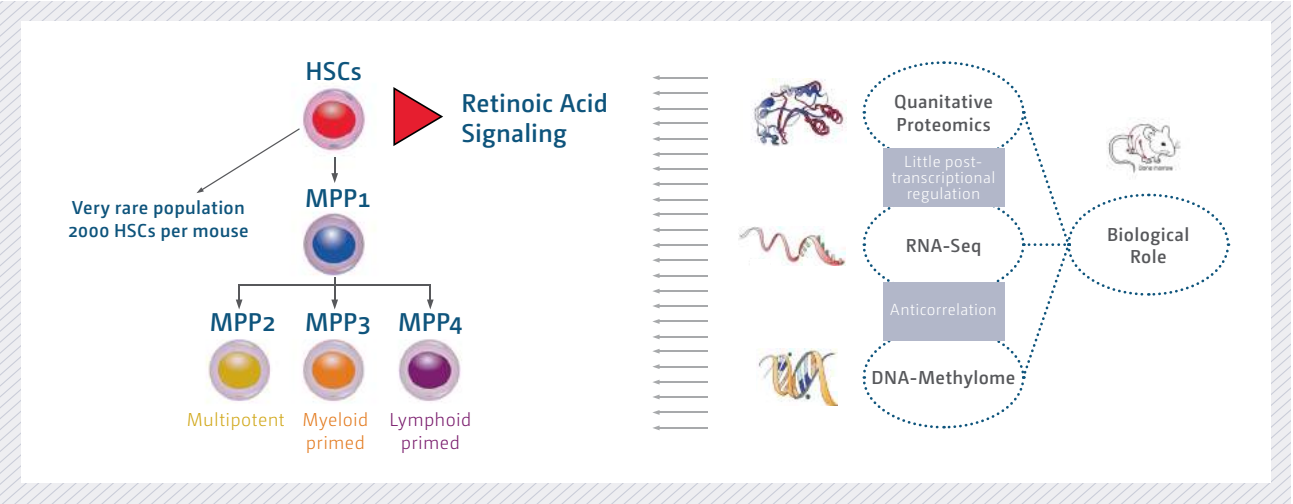


Figure 2 Identification of regulatory networks in HSCs and their immediate progeny via integrated proteome, transcriptome, and DNA methylome analysis. Main findings summarizing Cabezas-Wallscheid et al. in Cell Stem Cell 2014.

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

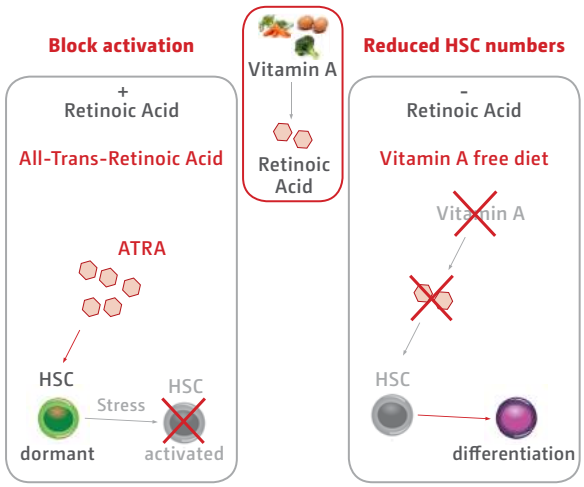


Figure 4 Vitamin A/retinoic acid signaling regulates HSC maintenance versus differentiation. Left panel shows high levels of vitamin A (All-trans-retinoic acid = agonist of vitamin A) retains HSC quiescent upon stress. Right panel shows vitamin A-free diet triggers HSCs to differentiate.

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.

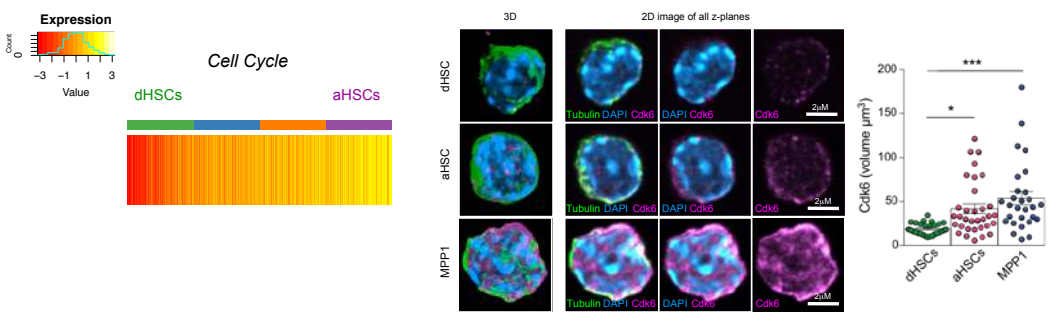
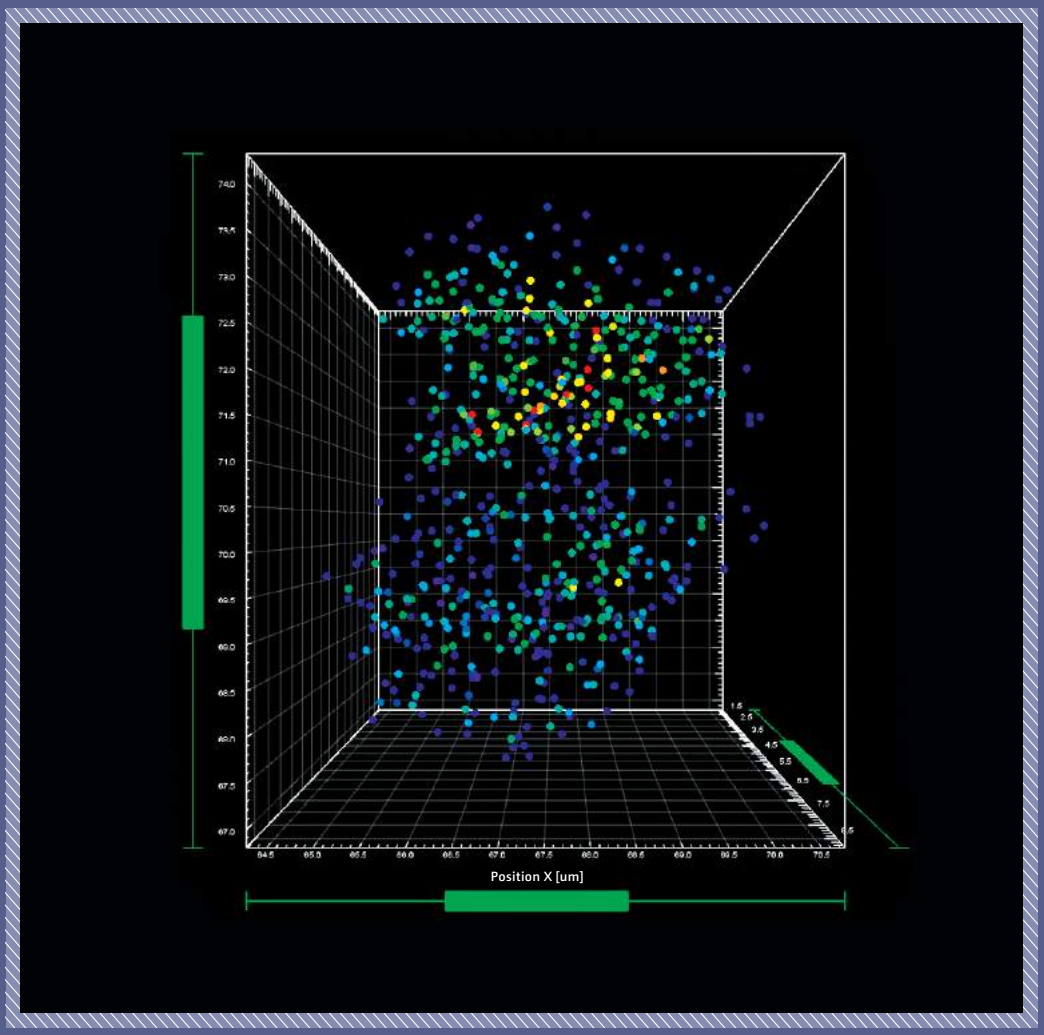


Figure 3 Active HSCs are cell cycle primed. Left panel: heatmap representing cell cycle genes based on single-cell RNA-seq data of dormant and active HSCs. Middle and right panel: Microscopy images and quantification of Cdk6 levels in dormant HSCs (dHSCs), active HSCs (aHSCs) and MPP1.



LAB RUDOLF GROSSCHEDL

mCherry-EBF1 localizes to distinct domains in the nucleus of B cells. Intensity analysis of mCherry-EBF1 domains inside B cell nuclei. Super-resolution images were processed via the combined Airyscan/Deconvolution module of Huygens. Domains of mCherry-EBF1 were subsequently identified using the IMARIS spot function, and domain intensities plotted using the IMARIS Vantage module.

02

Senior Group Rudolf Grosschedl

Regulatory Circuitries underlying B Lymphopoiesis

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Andreani V, Ramamoorthy S, Pandey A, Lupar, E, Nutt SL, Lämmermann T, Grosschedl R (2018). Cochaperone Mzb1 is a key effector of Blimp1 in plasma cell differentiation and β1-integrin function. **Proceedings of the National Academy of Sciences of the USA 115, E9630-E9639**.

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Senior Group Rudolf Grosschedl

REGULATORY CIRCUITRIES UNDERLYING B LYMPHOPOIESIS

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

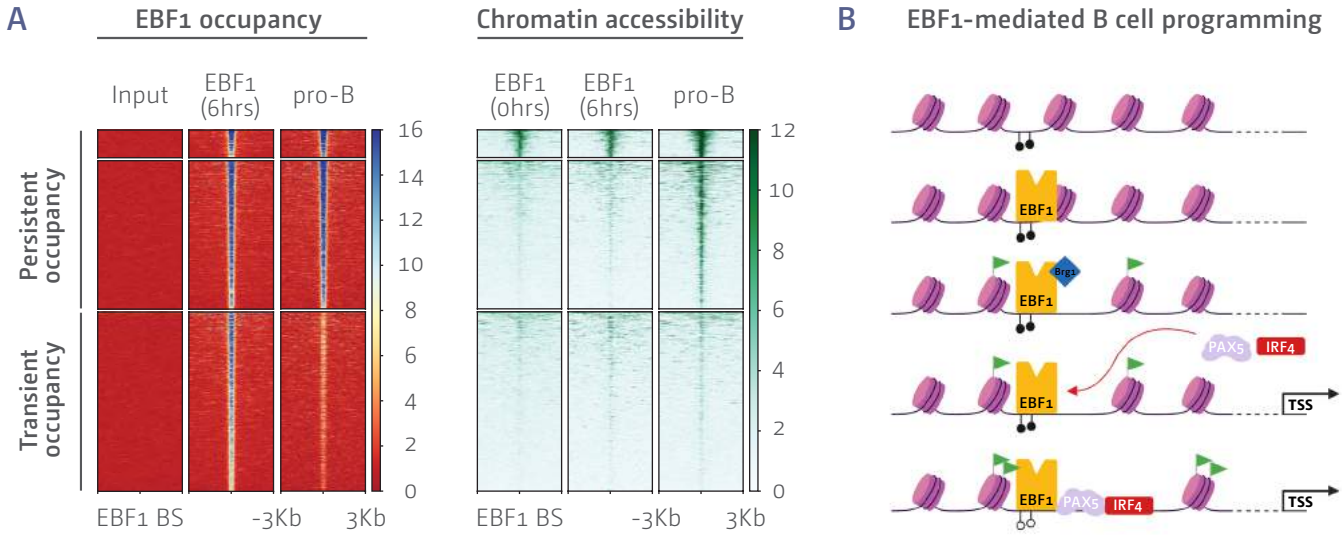

Figure 1 – A Heatmaps of EBF1 binding and formation of chromatin accessibility in Ebf1-deficient hematopoietic progenitor cells, as determined by ChIP-seq and ATAC-seq analyses, prior and 6 hours after doxycycline-induced expression of EBF1. **B** Scheme of the temporal order of EBF1-mediated programming. EBF1 can bind naïve progenitor chromatin prior to establishment of chromatin accessibility. Solid & open dots represent methylated and unmethylated CpGs, respectively.

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 stabilizes 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

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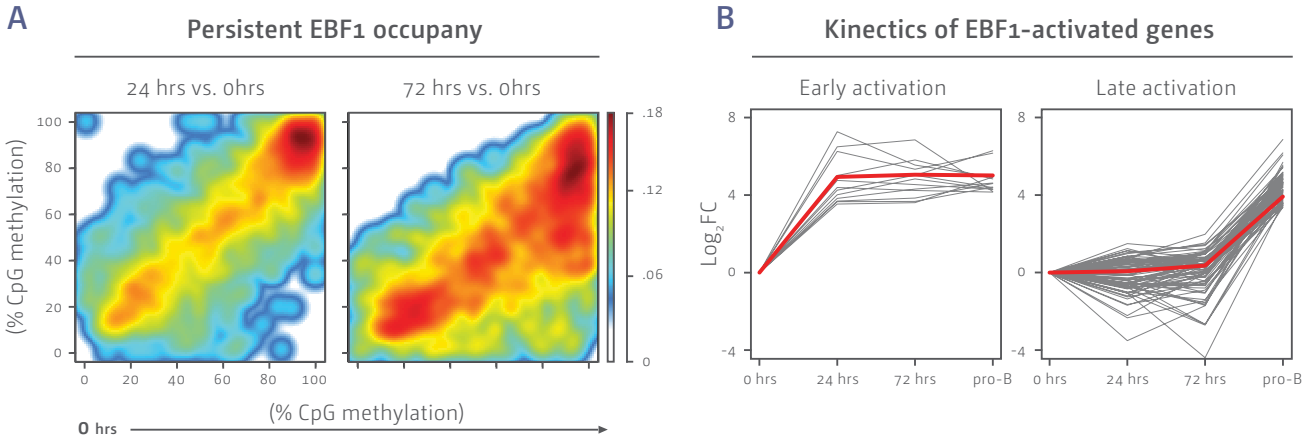

Figure 2 – A Dynamics of CpG DNA methylation around EBF1-occupied sites. Cloud maps presenting the levels of CpG methylation in ±100bp windows of persistent EBF1-occupied sites that are associated with low methylated regions. The levels at 24hrs and 72hrs after EBF1 induction and at the pro-B cell stage 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.

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 EBFI binds naïve 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.

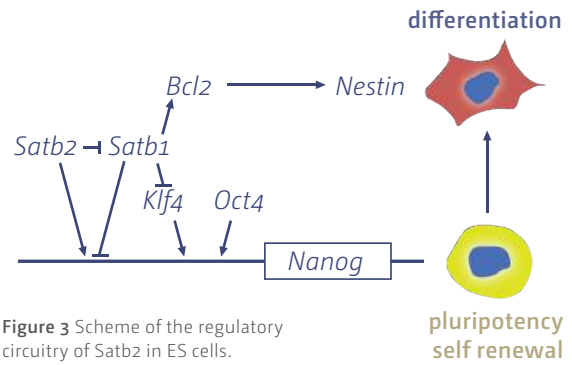

Figure 3 Scheme of the regulatory circuitry of Satb2 in ES cells.

LAB DOMINIC GRÜN

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

02

Group Dominic Grün

Quantitative single cell biology

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SELECTED PUBLICATIONS

Aizarani A, Saviano A, Sagar, Mailly L, Durand S, Herman JS, Pessaux P, Baumert TF, Grün D (2019). A Human Liver Cell Atlas: Revealing Cell Type Heterogeneity and Adult Liver Progenitors by Single-Cell RNA-sequencing. **Nature (in press)**.

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Group Dominic Grün

QUANTITATIVE SINGLE CELL BIOLOGY

Investigating cell fate decisions with high resolution

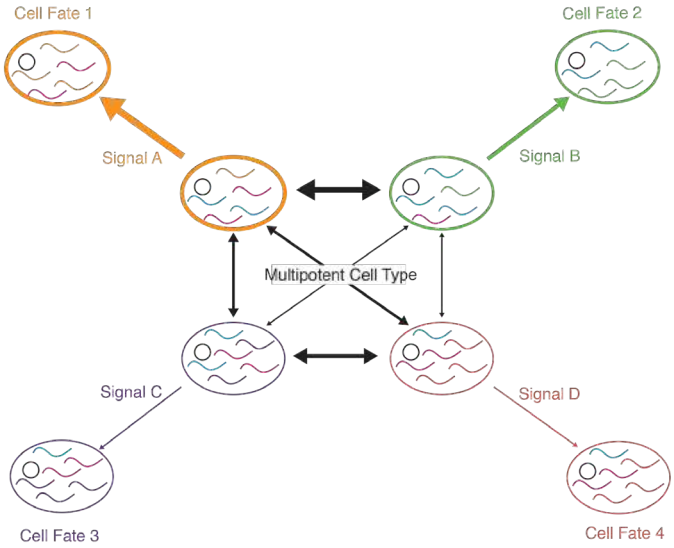


Figure 1 Model of stem cell differentiation. A multipotent cell is defined as an ensemble of metastable states. Transitions between these states are possible, until a cell commits to a terminal fate in response to a stochastic gene expression fluctuation or an external signal.

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,

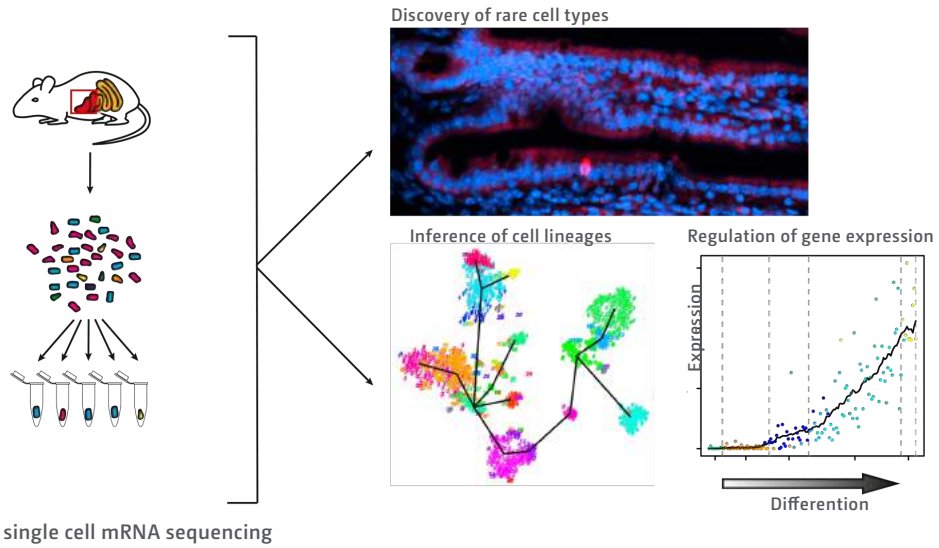


Figure 2 To understand organ differentiation cells are isolated from the tissue of interest, for example, the intestine, and profiled by single cell sequencing of all mRNAs expressed in a cell. The transcriptome of a cell can be interpreted as a fingerprint revealing its identity and computational methods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

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

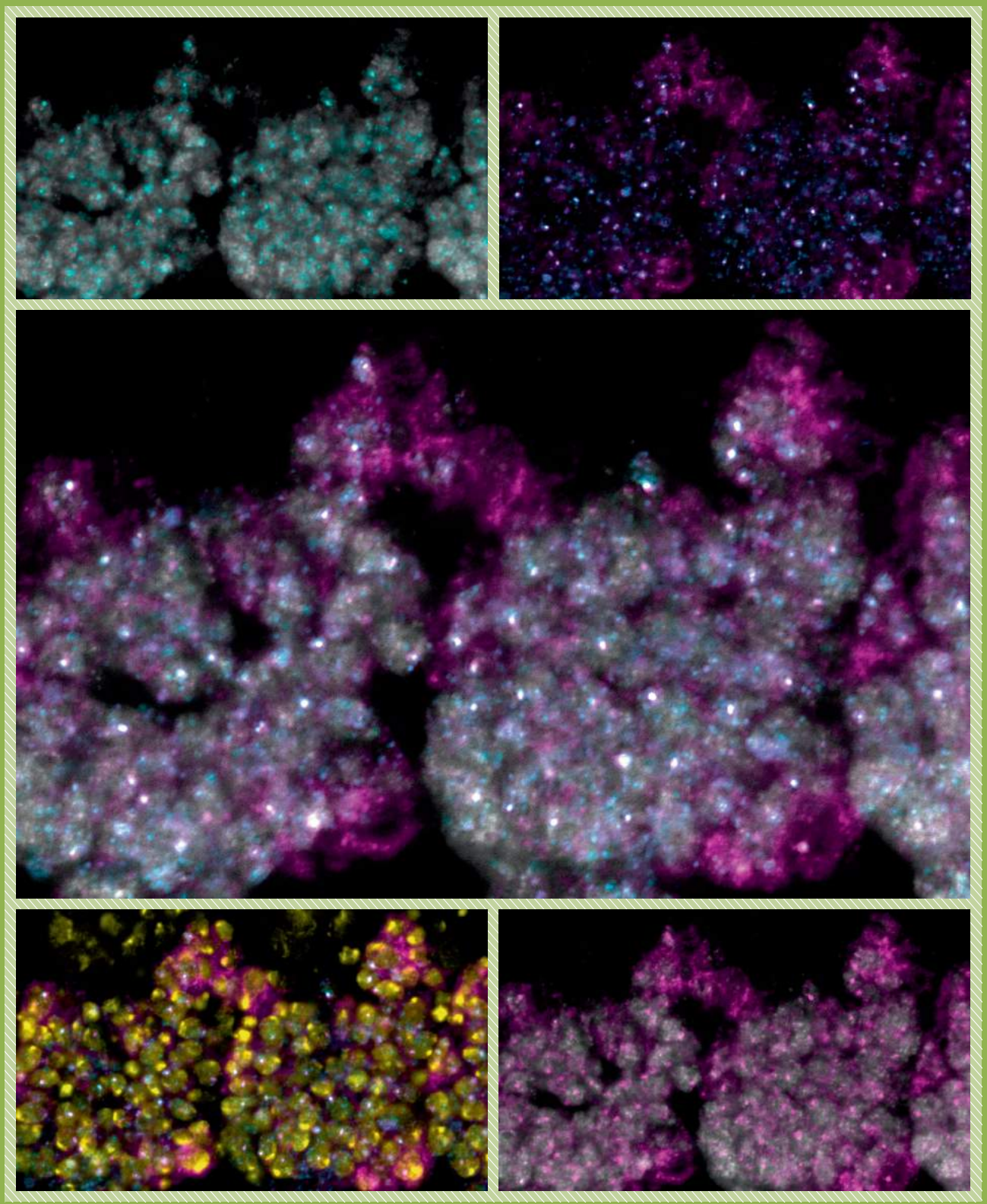
As a model of multi-lineage differentiation we are studying cells of the hematopoietic system with a focus on lymphocytes. Years of intense research have revealed the major hematopoietic cell types as well as multipotent progenitor populations with the help of flow cytometry on the basis of a relatively 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.

02

Group Valérie Hilgers

Alternative RNA processing in neurons

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SELECTED PUBLICATIONS

Wang Q, Taliaferro JM, Klibaite U, Hilgers V, Shaevitz JW, Rio DC (2016). The PSI-U1 snRNP interaction regulates male mating behavior in Drosophila. **Proceedings of the National Academy of Sciences 113, 5269-5274**.

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Hilgers V, Lemke SB, Levine M (2012): ELAV mediates 3' UTR extension in the Drosophila nervous system. **Genes and Development 26, 2259-2264**.

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Group Valérie Hilgers

ALTERNATIVE RNA PROCESSING IN NEURONS

Lessons to learn from ultra-long non-coding RNA sequences

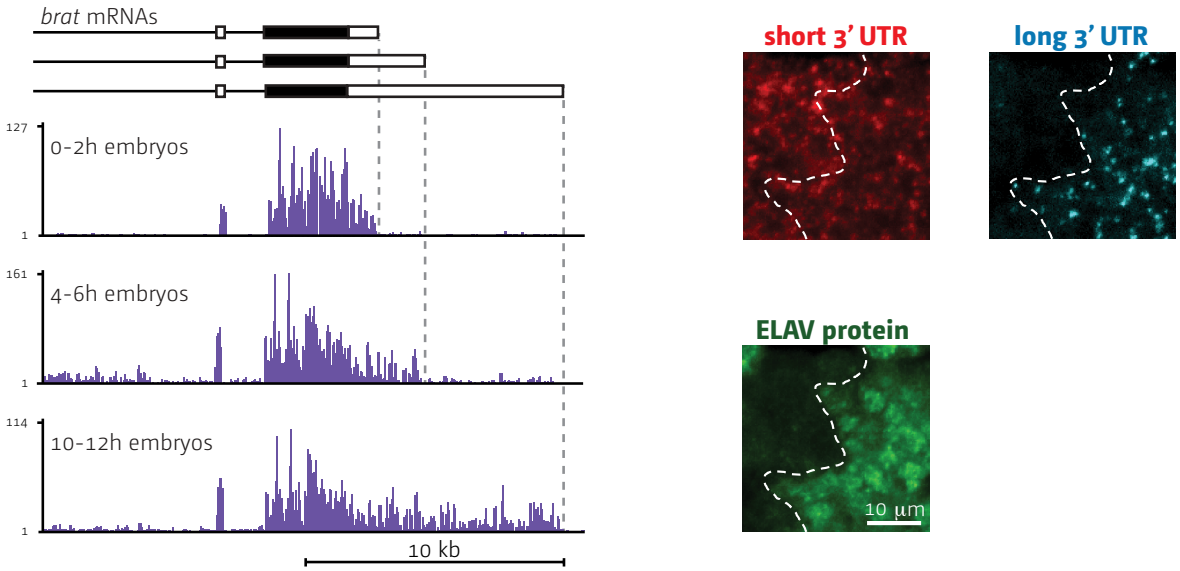


Figure 1 Alternative 3' processing specifically in the nervous system. Left: RNA-Seq data illustrate the progressive 3' UTR extension that occurs during embryonic development. Right: Confocal imaging of mRNAs carrying short and long 3' UTRs shows neuron-specific expression of the extended 3' UTR. Neurons are marked with the protein ELAV. Hundreds of genes are subjected to 3' UTR extension. In this figure, brain tumor (brat) is shown as an example.

Neurons are uniquely complex cells whose function heavily relies on gene regulatory mechanisms such as alternative splicing, alternative polyadenylation, and post-transcriptional processes. Although relatively little is known about how these mechanisms control neuronal development and function, the importance of RNA-directed regulation in the brain is exemplified by its implication in neurological diseases. Our long-term goal is to gain a better understanding of the regulatory mechanisms that drive neural development and disease.

We focus on a particularly distinctive, recently discovered RNA processing mechanism: the lengthening of the 3' UTRs in hundreds of mRNAs during embryonic development, which occurs exclusively in neurons: '3' UTR extension'. This process causes specific mRNAs to carry alternative, extraordinarily long 3' UTRs.

Neuronal 3' UTR extension also occurs in humans; its function is unknown. This novel topic is of particular interest since neural RNA regulation has emerged as a crucial contributing factor in neurodegeneration pathologies. Mechanistic and functional insight is lacking, especially from animal models.

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.

02

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.

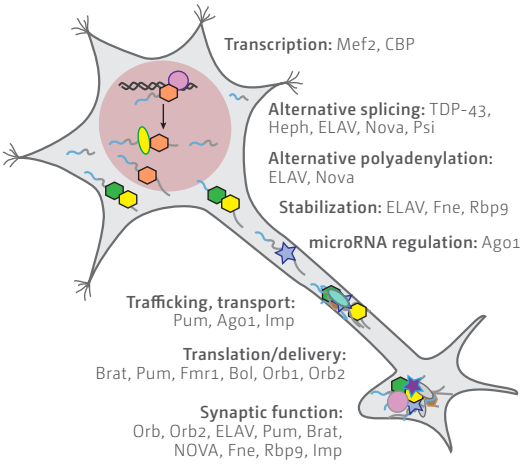
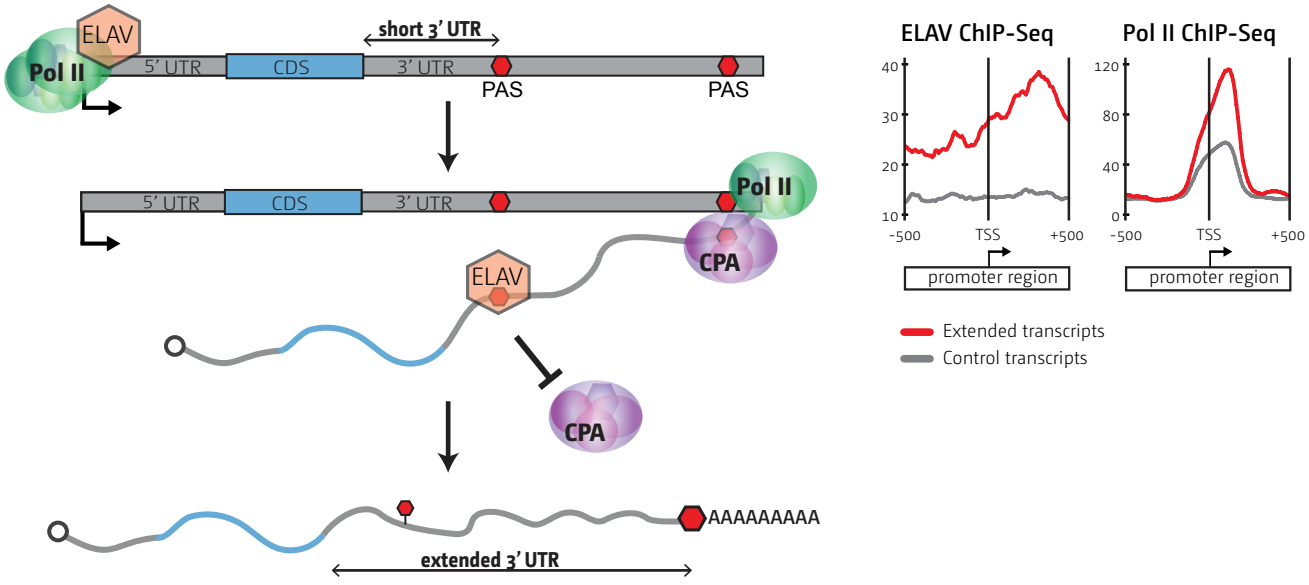
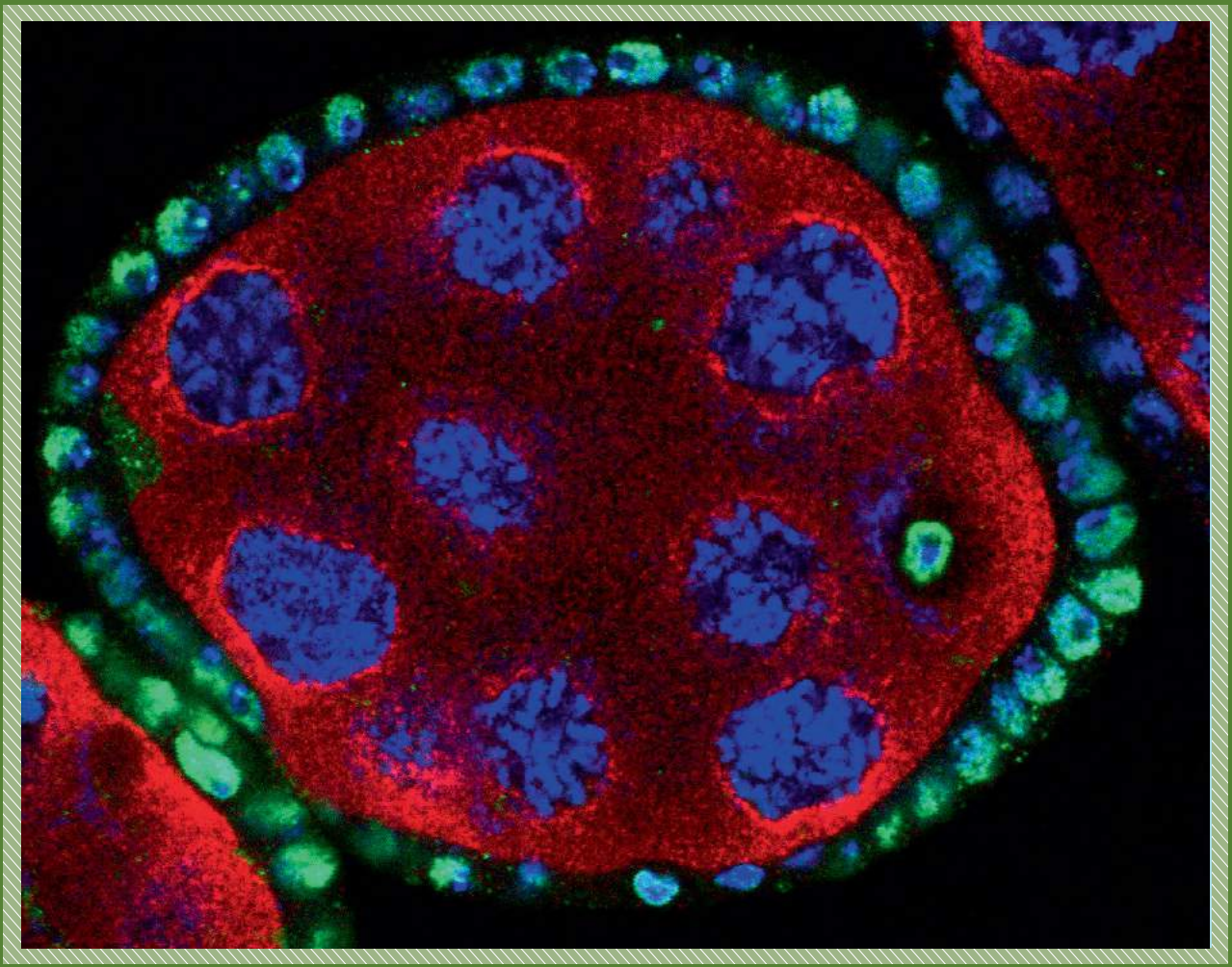


Figure 3 Alternative mRNA processing linked to neuronal function. Transcripts with alternative, extended 3′ UTRs typically encode important effector proteins of various steps of mRNA metabolism. The represented example proteins play crucial roles in neuron development, morphogenesis and survival as well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

Figure 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





LAB NICOLA IOVINO

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

02

Group Nicola Iovino

Epigenetic reprogramming and infertility

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SELECTED PUBLICATIONS

Zenk F, Loeser E, Schiavo R, Kilpert F, Bogdanović O, Iovino N (2017). Germ line–inherited H3K27me3 restricts enhancer function during maternal-to-zygotic transition. **Science 357 (6347), 212-216.**

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Group Nicola Iovino

EPIGENETIC REPROGRAMMING AND INFERTILITY

Epigenetic control of early embryogenesis

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

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

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

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

Maternal gametes and epigenetic inheritance

Ova are generated by oogenesis in the female germline, the ovary. During the differentiation process, their chromatin undergoes profound condensation, transcriptional shutdown and reductive division to haploidy. Defects in any of these processes can lead to full sterility with complete lack of functional gametes. Chromatin condensation and transcriptional shut down in the ovum are mainly achieved trough 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

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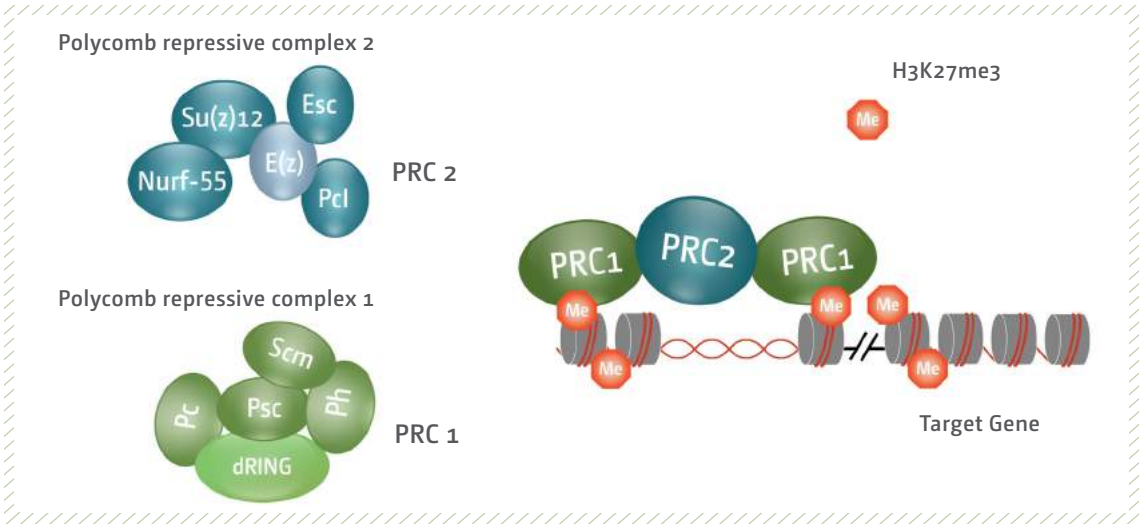


Figure 2 Schematic representation of the Polycomb repressive complex 1 and 2 (PRC1/2) with the respective subunits.

Repressive Complex 2 (PRC2) as a critical chromatin determinant of Drosophila oocyte cell fate (Figure 1). PRC2 is the major complex that catalyzes trimethylation of histone 3 at lysine 27 (H3K27me3), a mark usually associated with facultative heterochromatin and transcriptional repression (Figure 2). In the absence of PRC2 components, the oocyte undergoes a trans-determination process and becomes a polyploid cell. The resulting lack of a functional haploid gamete causes full sterility. 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).

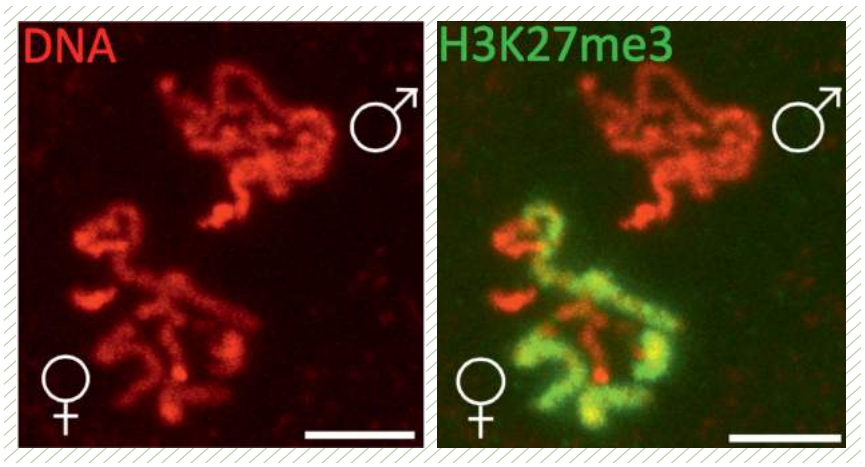
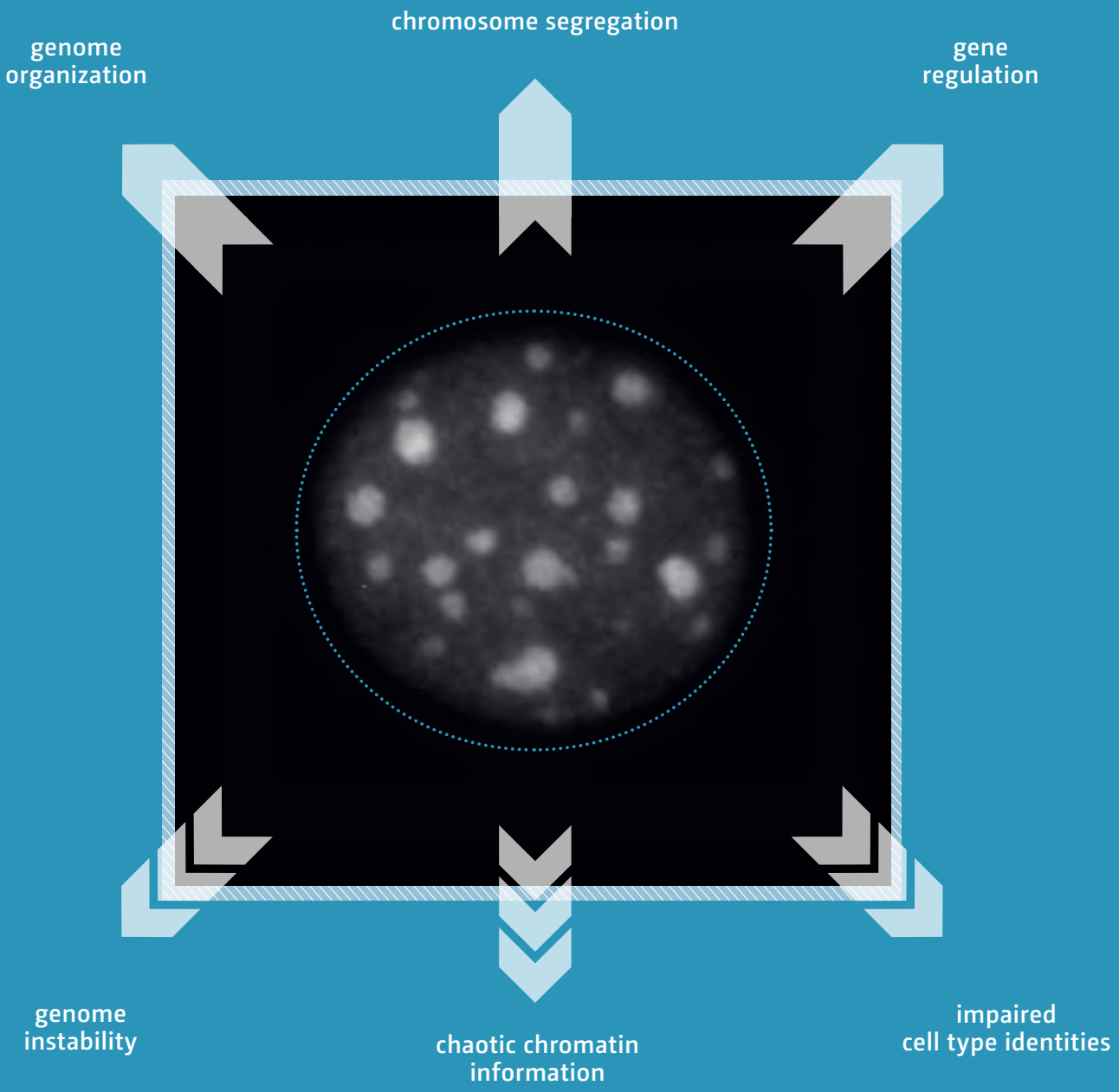


Figure 3 Drosophila early embryo nucleus showing asymmetric distribution for H3K27me3 between paternal (upper right) and maternal (lower left) chromosomes.



LAB THOMAS JENUWEIN

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

02

Senior Group Thomas Jenuwein

Epigenetic Control by Histone Lysine Methylation

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SELECTED PUBLICATIONS

Nicetto D, Donahue G, Jain T, Peng T, Sidoli S, Sheng L, Montavon T, Becker JS, Grindheim JM, Blahnik K, Garcia BA, Tan K, Bonasio R, Jenuwein T, Zaret KS (2019). H3K9me3-heterochromatin loss at protein-coding genes enables developmental lineage specification. **Science 363, 294-297**.

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Bulut-Karslioglu A, Perrera V, Scaranaro M, De La Rosa-Velazquez IA, Van De Nobelen S, Shukeir N, Popow J, Gerle B, Opravil S, Pagani M, Meidhof S, Brabletz T, Manke T, Lachner M and Jenuwein T (2012). A transcription factor-based mechanism for mouse heterochromatin formation. **Nature Structural Molecular Biolology 19, 1023-1030**.

Senior Group Thomas Jenuwein

EPIGENETIC CONTROL BY HISTONE LYSINE METHYLATION

Establishment and maintenance of mammalian heterochromatin

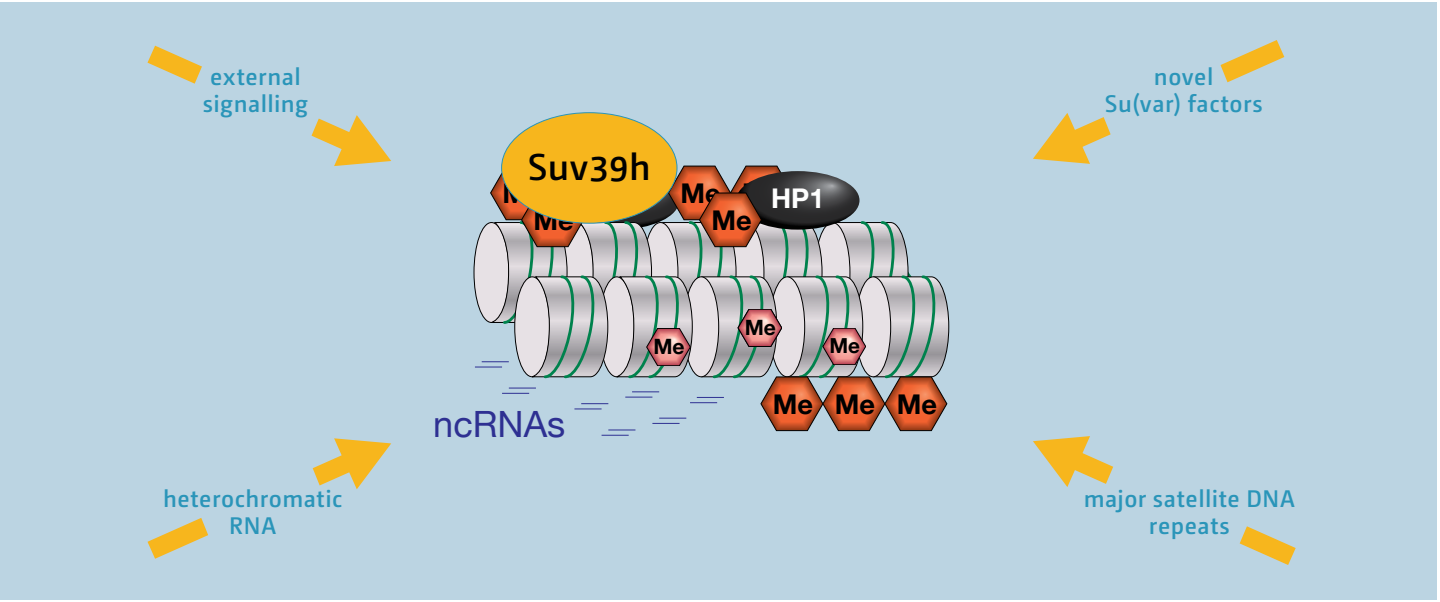


Figure 1 Molecular definition of mouse heterochromatin. The Figure highlights the basic mechanisms that build mammalian heterochromatin and shows a compacted array of nucleosomes that are enriched for DNA methylation (small Me hexagon), H3K9me3 histone methylation (big Me hexagon), binding of the Suv39h KMT and of heterochromatin protein 1 (HP1), and chromatin association of non-coding repeat RNA. Ongoing research projects are indicated by the yellow arrows.

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 LINE elements are the primary chromatin regions to be silenced by the Suv39h enzymes in mouse ES cells and permutated or truncated MSR or LINE 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)

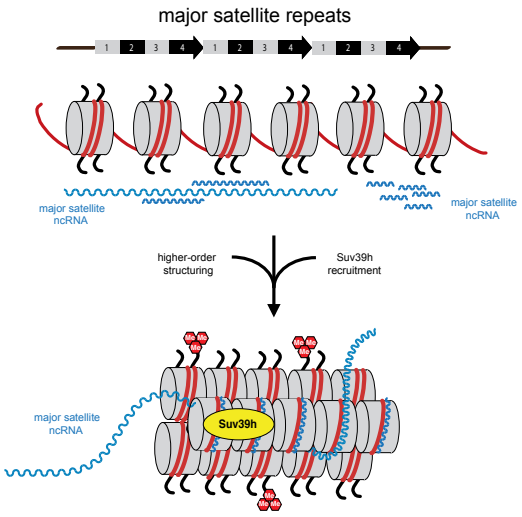
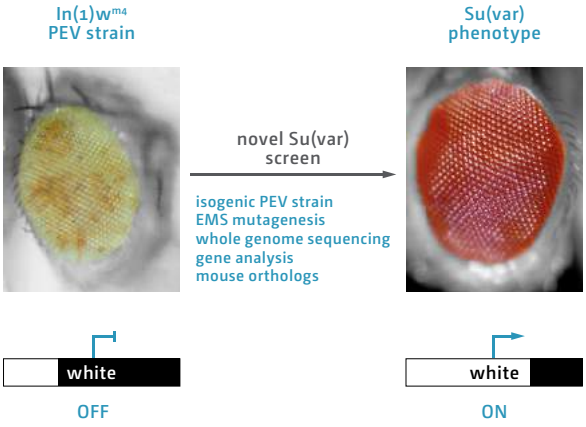


Figure 2 Major satellite repeat transcripts organize an RNA-nucleosome scaffold at mouse heterochromatin. Model depicting a higher-order RNA nucleosome scaffold that is formed by chromatin association of major satellite repeat (MSR) transcripts. In addition, MSR transcripts also facilitate recruitment and secure retention of the Suv39h enzymes to heterochromatin.

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 chromatin (Velazquez Camacho et al. 2017) (Figure 2). We are now extending these studies to examine secondary structures of MSR transcripts that allow the formation of RNA:DNA hybrids or fold into distinct stem loops for the possible RNA interaction of chromatin factors. We will also investigate 5meC and me6A RNA methylation of repeat-derived transcripts and modulate the expression of endogenous MSR repeats by targeting of TALEN and/or dCas9 directed transcriptional activators and transcriptional repressors.



around 60 novel Su(var) genes predicted
25 Su(var) genes identified

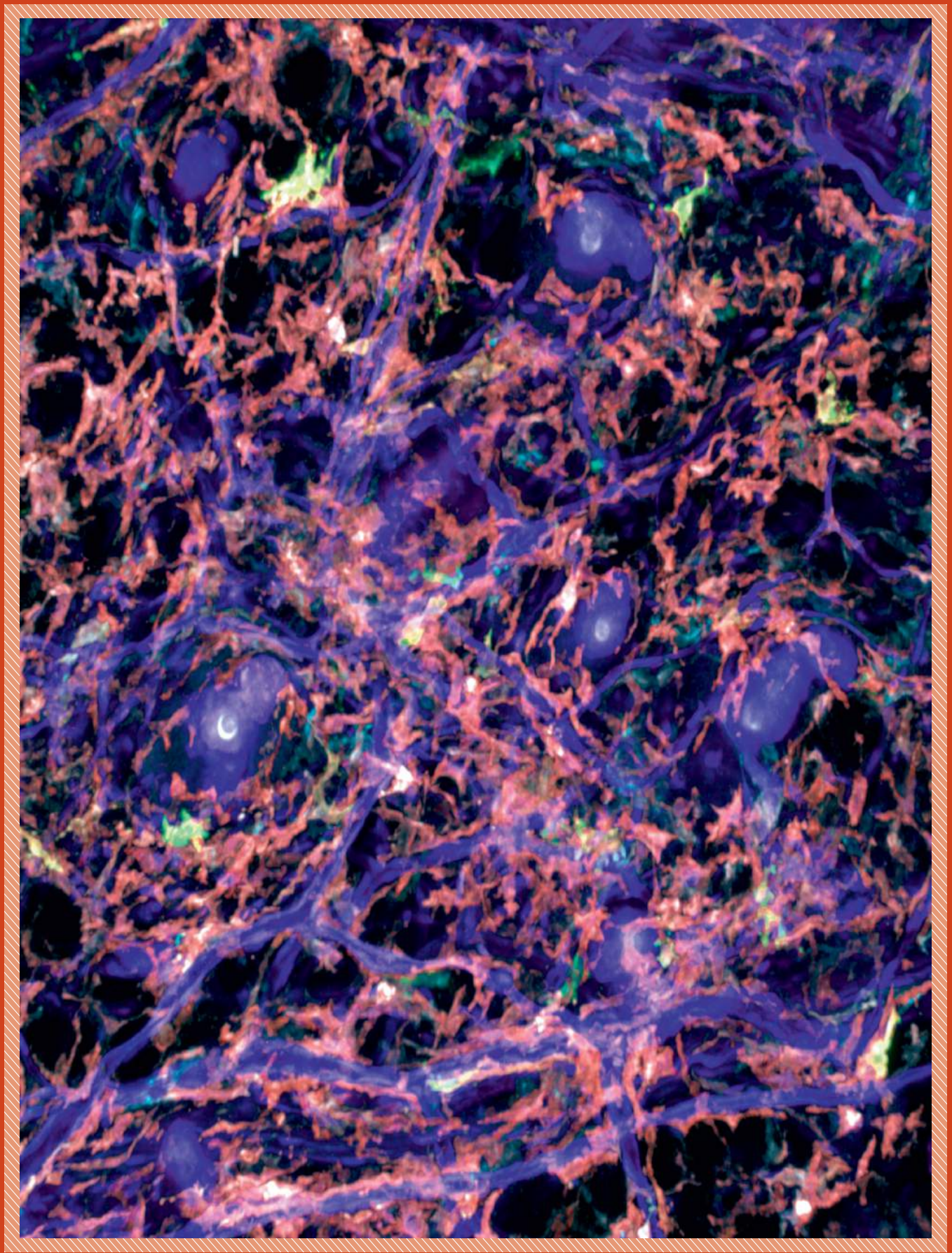
Figure 3 A new *Su(var)* screen in *Drosophila* identifies novel Su(var) factors. The Figure depicts the classic system of position effect variegation (PEV) in *Drosophila*, in which the *white* gene (which codes for a red eye pigment) is translocated into a block of heterochromatin and stochastically silenced, resulting in variegated eye pigmentation. Suppressors of variegation, so-called *Su(var)* genes, encode chromatin factors and enzymes that stabilize a heterochromatic state. *Su(var)* gene mutants therefore weaken heterochromatin and lead to transcriptional activation of the translocated *In(1)wm4^h white* gene that is detected by a full red eye colouring.

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.

LAB TIM LÄMMERMANN

Innate immune cells as sentinels of our body periphery



02

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Cellular dynamics of innate immune responses

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SELECTED PUBLICATIONS

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Group Tim Lämmermann

CELLULAR DYNAMICS OF INNATE IMMUNE RESPONSES

Signals guiding immune cell movement and positioning

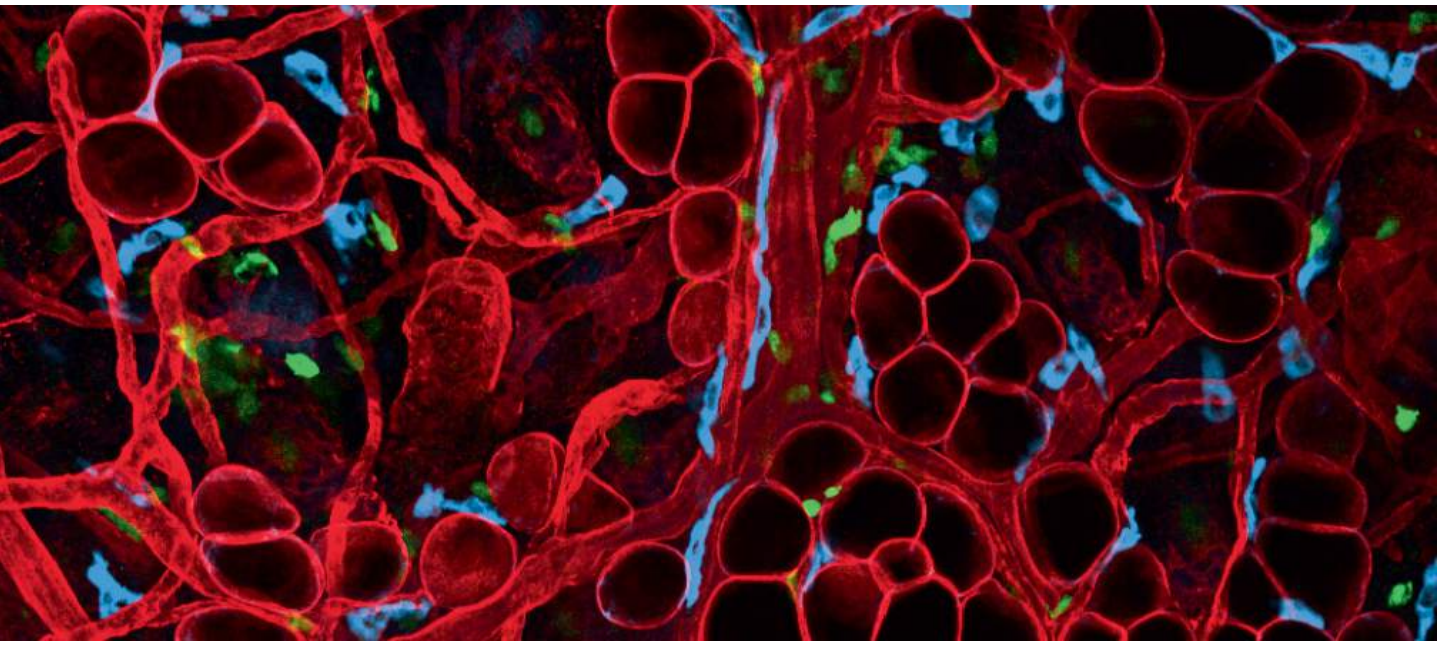


Figure 1 Strategically positioned immune cells in the heterogenous and complex tissue environment of the skin dermis, immunofluorescence staining of skin whole mount tissue.

Immune responses are highly dynamic and require coordinated migration, tissue positioning and interactions of immune cells possessing diverse effector functions. Most cells of the immune system stand in contrast to other body cell types owing to their extremely fast movement and capacity to invade and migrate within diverse organs. In particular, within minutes of tissue damage or incipient infection, cells of our innate (non-specific) immune system undertake a coordinated, multi-cellular and multi-layered response to isolate sites of tissue damage and microbial invasion from healthy tissue. Phagocytes (neutrophils, macrophages, monocytes) are the major cell types involved in this front line of immune defense. 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-

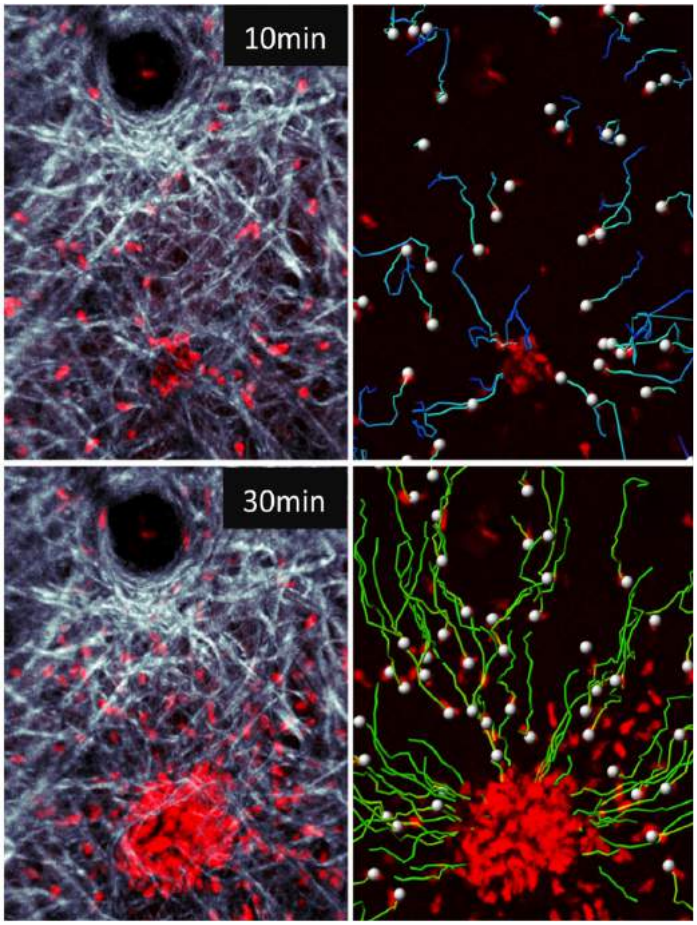


Figure 2 Neutrophil swarming upon local sterile injury. Upon local tissue injury, neutrophils (red) close to the damage site sense short-range attractant factors. Cell death occurring at the injury site initiates amplified neutrophil recruitment from distant tissue regions. Hereby, single neutrophils 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.

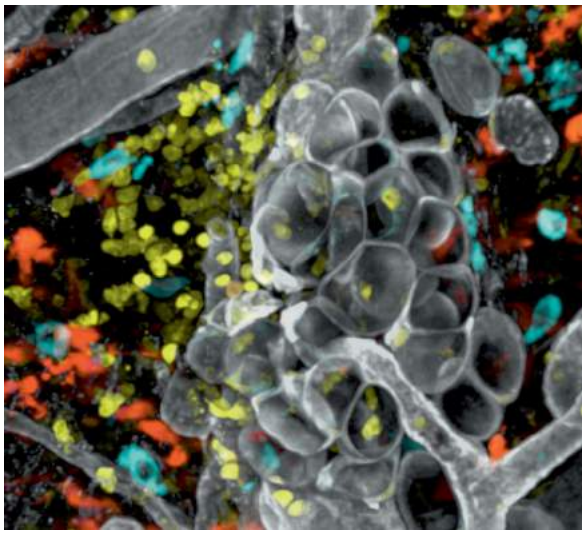


Figure 3 Coordination of immune cell functions between several immune cell types (differentially colored) of the innate immune response, immunofluorescence staining of skin whole mount tissue.

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.

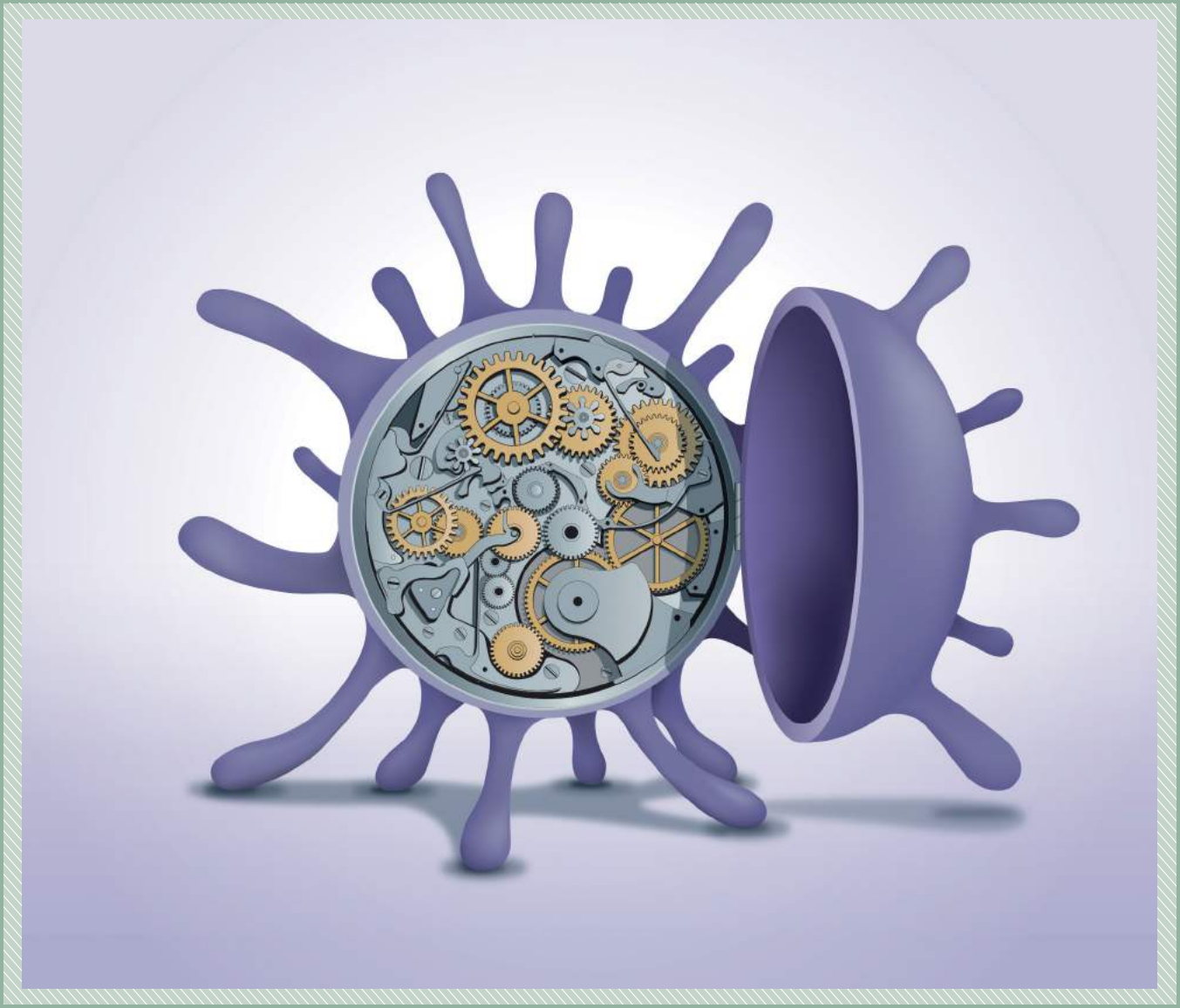
Positioning & migration patterns of other innate immune cells

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 chemoattractants 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.

LAB EDWARD PEARCE

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



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

02

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University/MPI-IE Senior Group Edward Pearce

REPROGRAMMING METABOLISM FOR IMMUNITY

Understanding the links between the immune system and metabolism

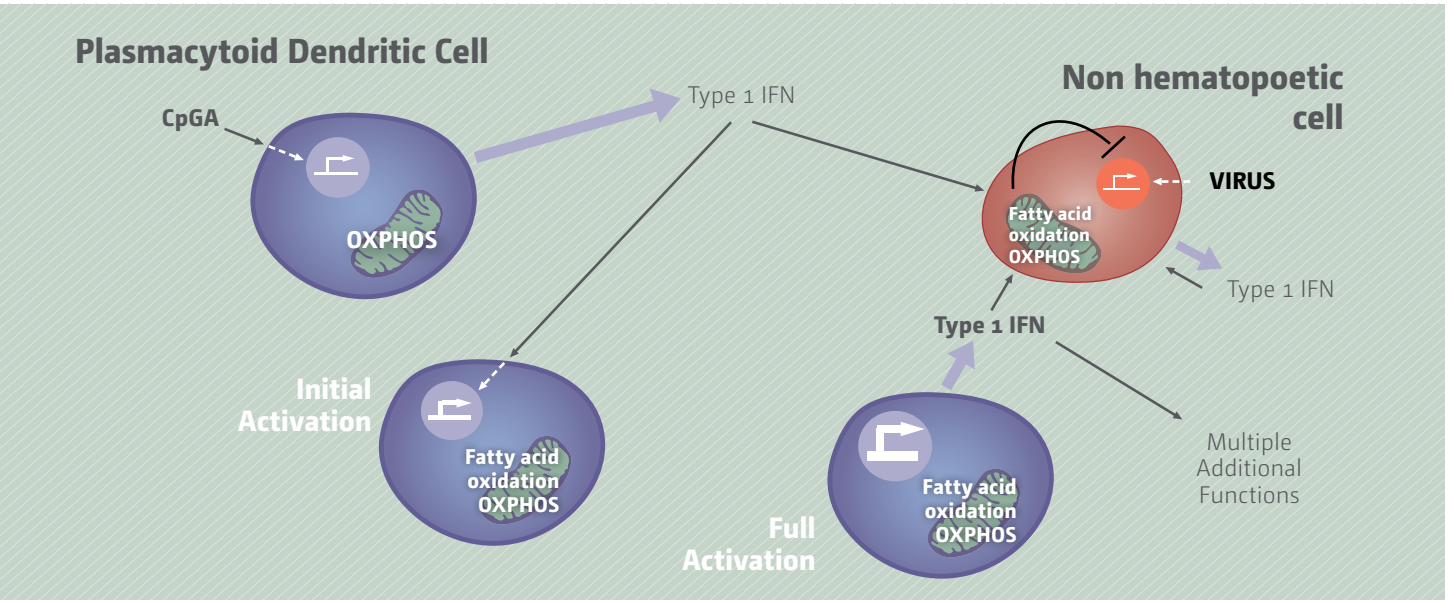


Figure 1 In pDCs stimulated via TLR9, fatty acid oxidation is enhanced due to autocrine type 1 IFN signaling. Type 1 IFNs promote fatty acid oxidation and oxidative phosphorylation and Increased fatty acid oxidation induced in pDCs by TLR9 ligation is essential for cellular activation. Type 1 IFN-induced fatty acid oxidation in pDCs is partly PPARα-dependent and has anti-viral properties.

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.

De Immune 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- , macrophages become pro-inflammatory and assume microbicidal properties. In contrast, the cytokines IL-4 and IL-13 promote alternative macrophgage 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-

ternatively activated macrophages a significant change in expression of a set of genes that includes Retnla, which encodes RELM . 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.

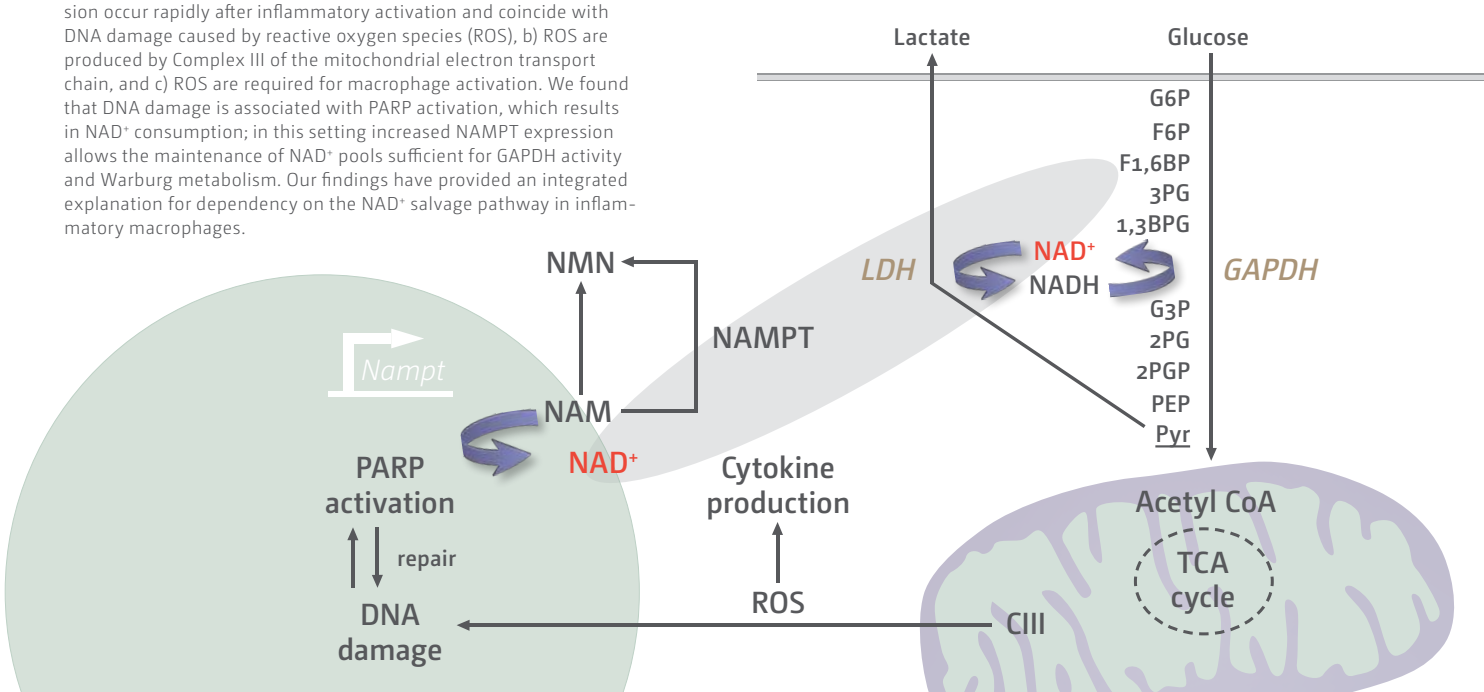
Warburg metabolism, a process by which ATP is produced from glucose independently of mitochondrial oxidative phosphorylation, is dependent on NAD^+, which is a required cofactor for GAPDH, a central enzyme in glycolysis. NAD^+ is produced by the oxidation of NADH, and the interconversion of NADH to NADv 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.

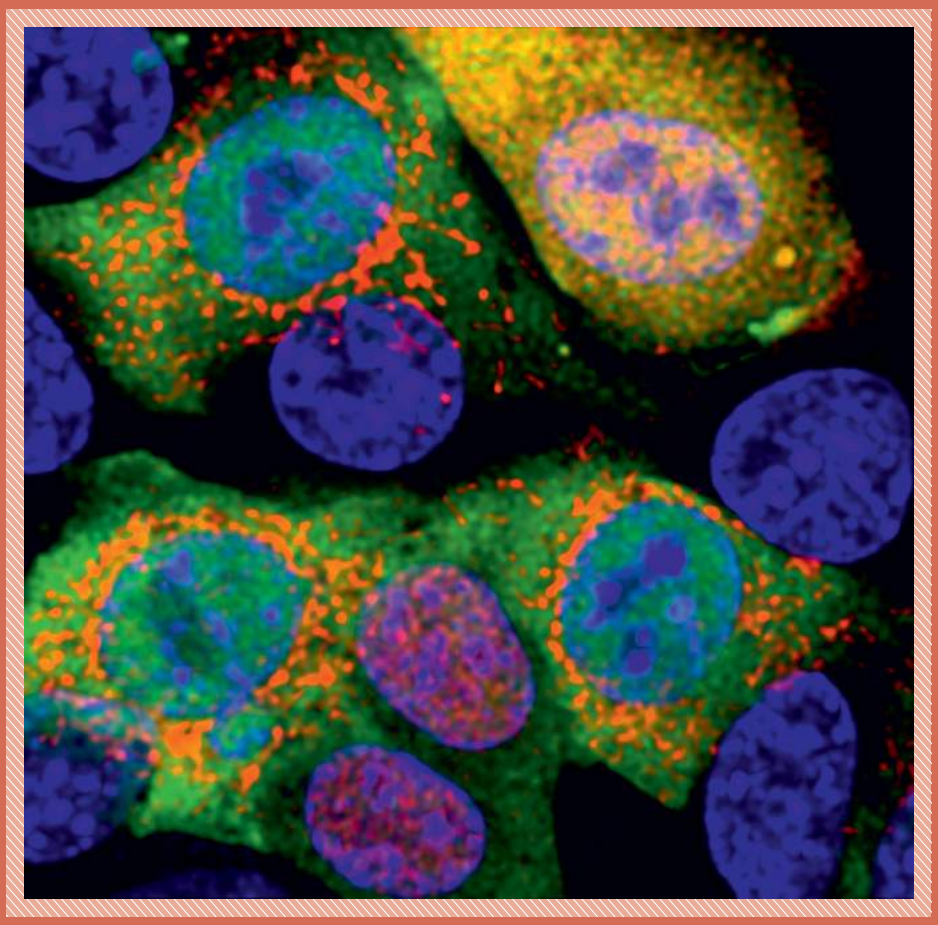
Figure 2 Adoption of Warburg metabolism is critical for macrophage activation in response to lipopolysaccharide (LPS). Macrophages stimulated with LPS increase expression of nicotinamide phosphoribosyltransferase (NAMPT), a key enzyme in NAD^+ salvage, and loss of NAMPT activity alters their inflammatory 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 interations

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 hemostasis 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 parastic 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.





LAB ERIKA PEARCE

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.

02

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Immunometabolism

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Senior Group Erika Pearce

METABOLIC REGULATION OF T CELL FUNCTION AND FATE

Understanding metabolic reprogramming in T cells during infection and cancer

T cell response to acute infection

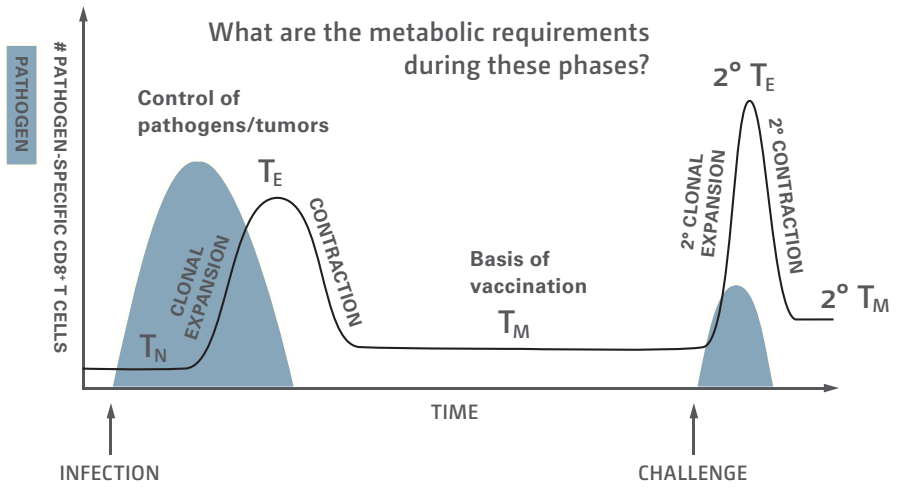

Figure 1 In response to antigen and costimulation, T cells become activated, proliferate, and gain effector functions important for clearing pathogens or tumors. Long-lived memory T cells are also generated during an immune response and are important for protecting the host against future re-infection. T cells must adapt to a wide array of environmental stressors as part of their normal development and undergo dramatic metabolic remodeling in the process.

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 enforces either the effector or memory fate of a T cell.

Immune cell metabolism in the tumor microenvironment

During a productive immune response to cancer, naive tumor antigen-specific T cells become activated and produce a variety of effector molecules that mediate tumor clearance. However, T cells often experience a progres-

02

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

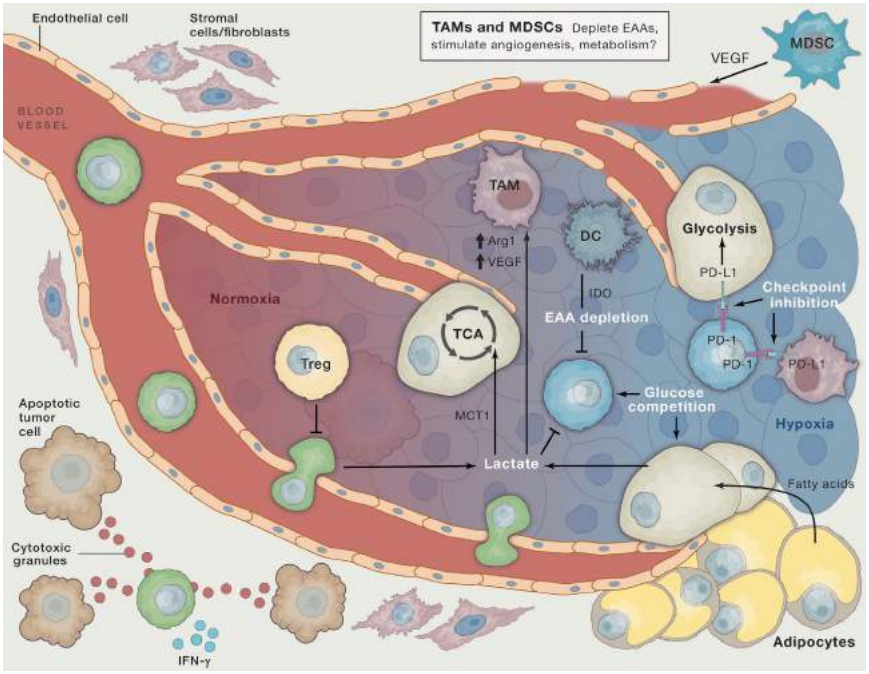

Figure 2 Metabolic tug-of-war within the tumor microenvironment. The balance of nutrients and oxygen within the tumor microenvironment controls immune cell function. Glucose and amino acid consumption by tumor cells can outpace that of infiltrating immune cells, specifically depriving them of nutrients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

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 highly 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^{H} in overcoming ribosome stalling. We found that eIF5A^{H} is required for the differential activation of bone marrow-derived macrophages (BMMϕ), 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.

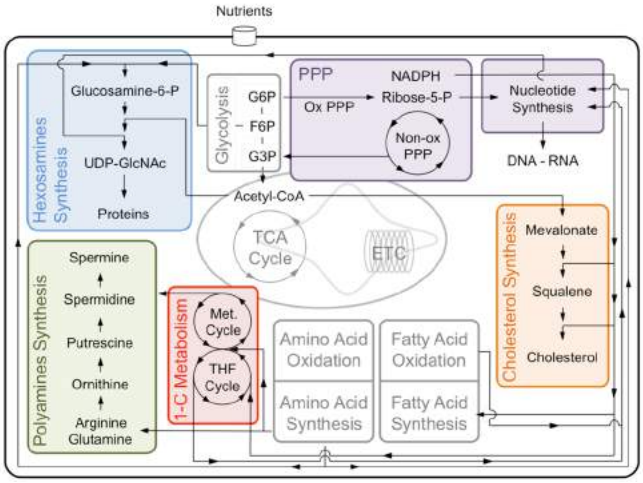

Figure 3 Ancillary Metabolic Pathways Are Intimately Intertwined with Core Metabolism Core metabolic pathways (gray shaded) use most of the carbon equivalents derived from nutrients for the production of energy, to control redox balance, and to generate biomass. "Peripheral" pathways are also intertwined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

LAB ANDREA PICHLER

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

02

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SUMO & Ubiquitin control

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Group Andrea Pichler

REGULATION OF SUMO CONJUGATION

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

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

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

SUMO is a small protein that regulates protein functions like stability, activity, intracellular localization etc. The covalent attachment of SUMO to its substrate, called sumoylation, is essential for viability in most organisms. Sumoylation is executed by the hierarchical action of E1, E2 and E3 enzymes that results in mono- or multi-sumoylation of a target protein or attachment of a SUMO chain (Figure 1 and Figure 2, upper panel). Deregulation of this system is implicated in various diseases ranging from diverse types of cancer to several neuropathological diseases. We aim to understand the molecular mechanisms of how conjugation of SUMO is regulated. In our studies, we put strong emphasis on biochemical approaches in combination with general cell biology to gain novel mechanistic insights in the powerful complexity of such regulatory SUMO enzymes.

Regulation of sumoylation by E2 enzyme (Ubc9) sumoylation

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

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

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

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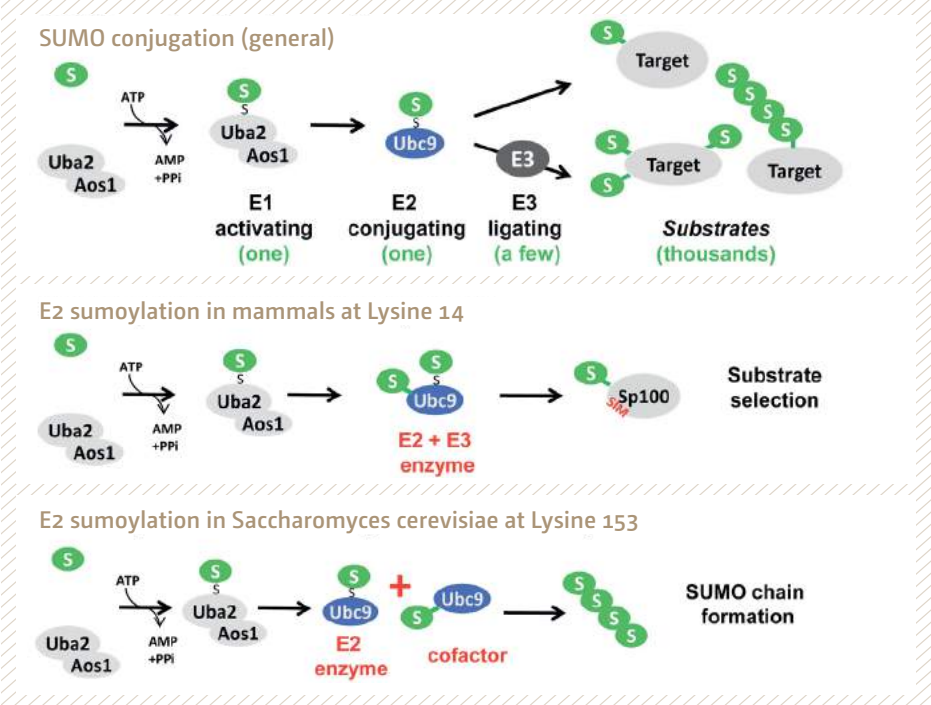


Figure 2 SUMO conjugation is regulated via E1, E2 and E3 enzymes (upper panel). E2 (Ubc9) sumoylation contributes to regulation of SUMO conjugation: in mammals Ubc9 is modified at Lysine 14 and this contributes to substrate selection (middle panel), in yeast Ubc9 is modified at Lysine 153, which inactivates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

The ZNF451 family, a novel class of SUMO enzymes

Very recently, we discovered a novel family of SUMO conjugating enzymes with E3 ligase and E4 elongase (specialized E3s for SUMO chain elongation) functions (Figure 3, upper panel). We show that ZNF451, a mainly uncharacterized zinc finger protein, has SUMO E3 ligase activity and efficiently assembles SUMO2/3 chains. Detailed biochemical analysis demonstrates that ZNF451 functions distinct to all known E3 ligases described for SUMO and ubiquitin conjugation: ZNF451 executes catalysis via a tandem-SIM and its interSIM region. One SIM orients the donor-SUMO, while a second SIM binds SUMO on the backside of the E2 enzyme. Intriguingly, this short tandem-SIM region is sufficient to extend a backside-anchored SUMO chain (E4 elongase activity) in contrast to chain initiation, which in addition requires a zinc finger region to recruit the initial acceptor SUMO (E3 ligase activity, Figure 3, lower left panel). Four human proteins share this E4-elongase activity (Figure 3, lower right panel) and are involved in stress-induced global sumo(2,3)ylation after DNA double strand break induction or proteasome inhibition in vivo (Eisenhardt, Chaugule et al 2015 and Cappadocia et al 2015).

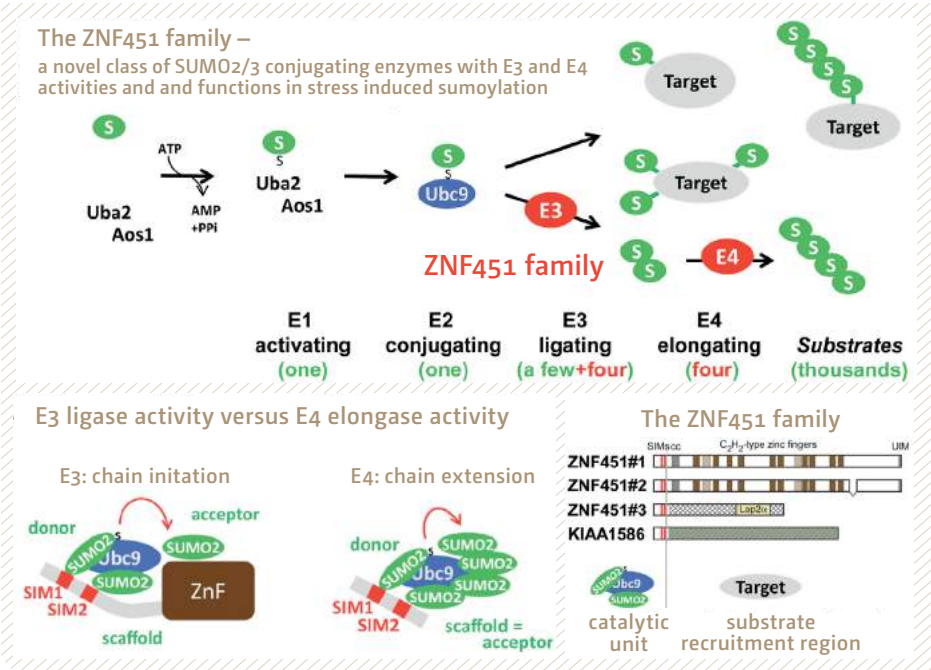
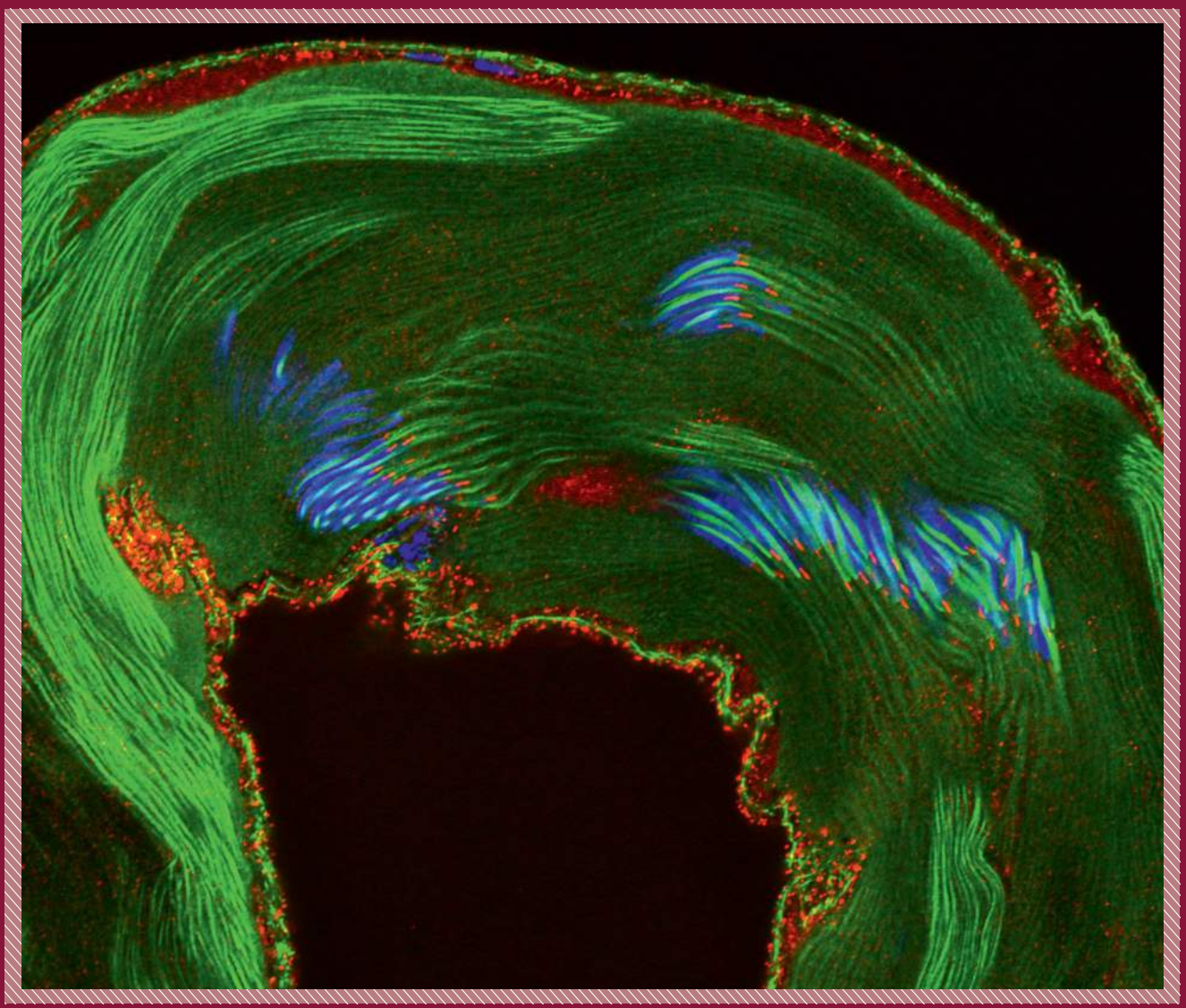


Figure 3 The ZNF451 family presents a novel class of SUMO conjugating enzymes with E3 and E4 activities (upper panel). A tandem SIM and the interSIM region display the catalytic unit of the enzymes. E3 and E4 activities involve distinct substrate (acceptor) binding interfaces (lower, left panel). The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



LAB J. ANDREW POSPISILIK

Immunoflurescence 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 ‚epi'genetic information capable of stably influencing disease phenotypes lifelong in the offspring.

02

Group J. Andrew Pospisilik

Epigenetic Control of Complex Disease

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Group J. Andrew Pospisilik

EPIGENETIC CONTROL OF COMPLEX DISEASE

Chromatin plasticity in metabolic disease

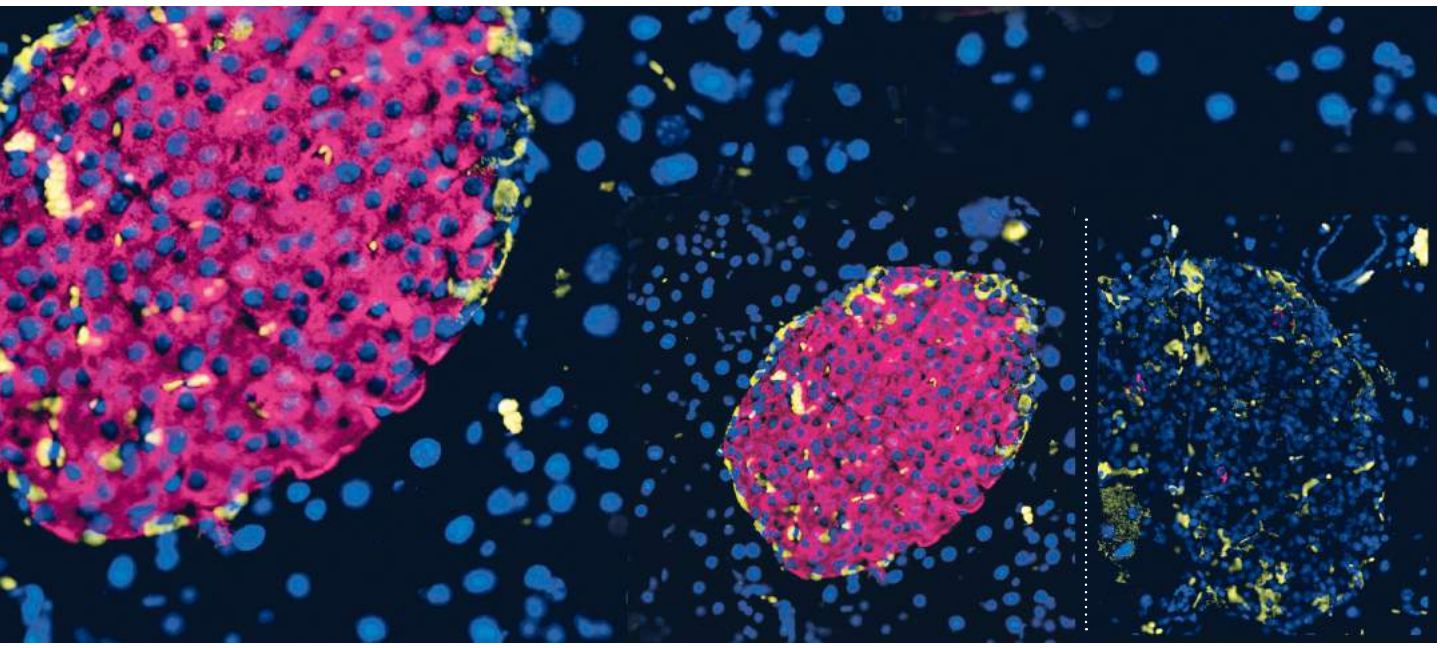

Figure 1 Recent studies have shown beta-cell de-differentiation to be a hallmark of diabetes progression. We find that PcG disruption can induce normal islets (left) to de-differentiate (right), without altering key cellular processes such as proliferation or apoptosis.

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:

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.

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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.

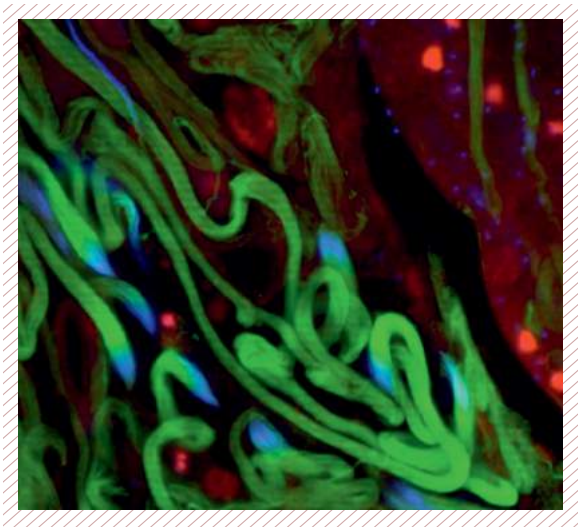


Figure 2 The fruit fly, Drosophila melanogaster, has some of the longest sperm cells of any species. Here one can see bundles of blue headed, green tailed sperm during late sperm development. These single cells are able to carry epigenetic memories of parental experience or stress and re-shape complex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

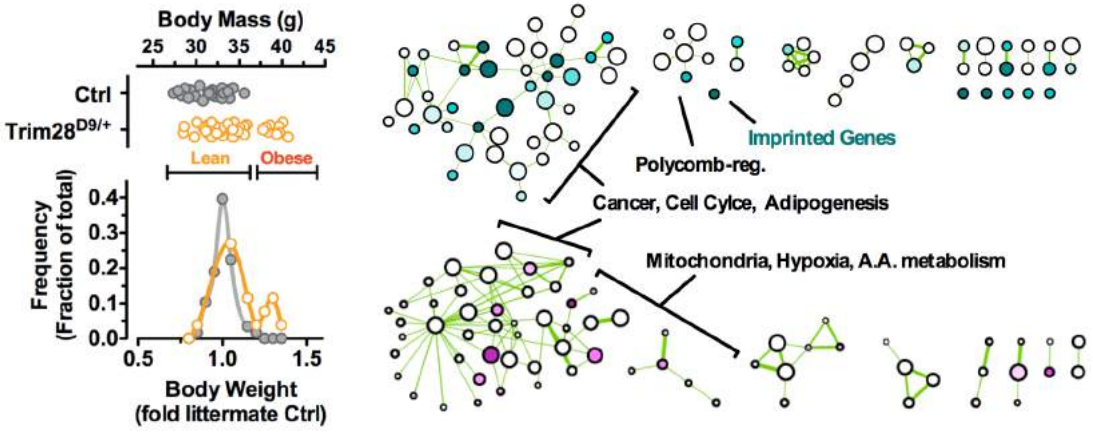
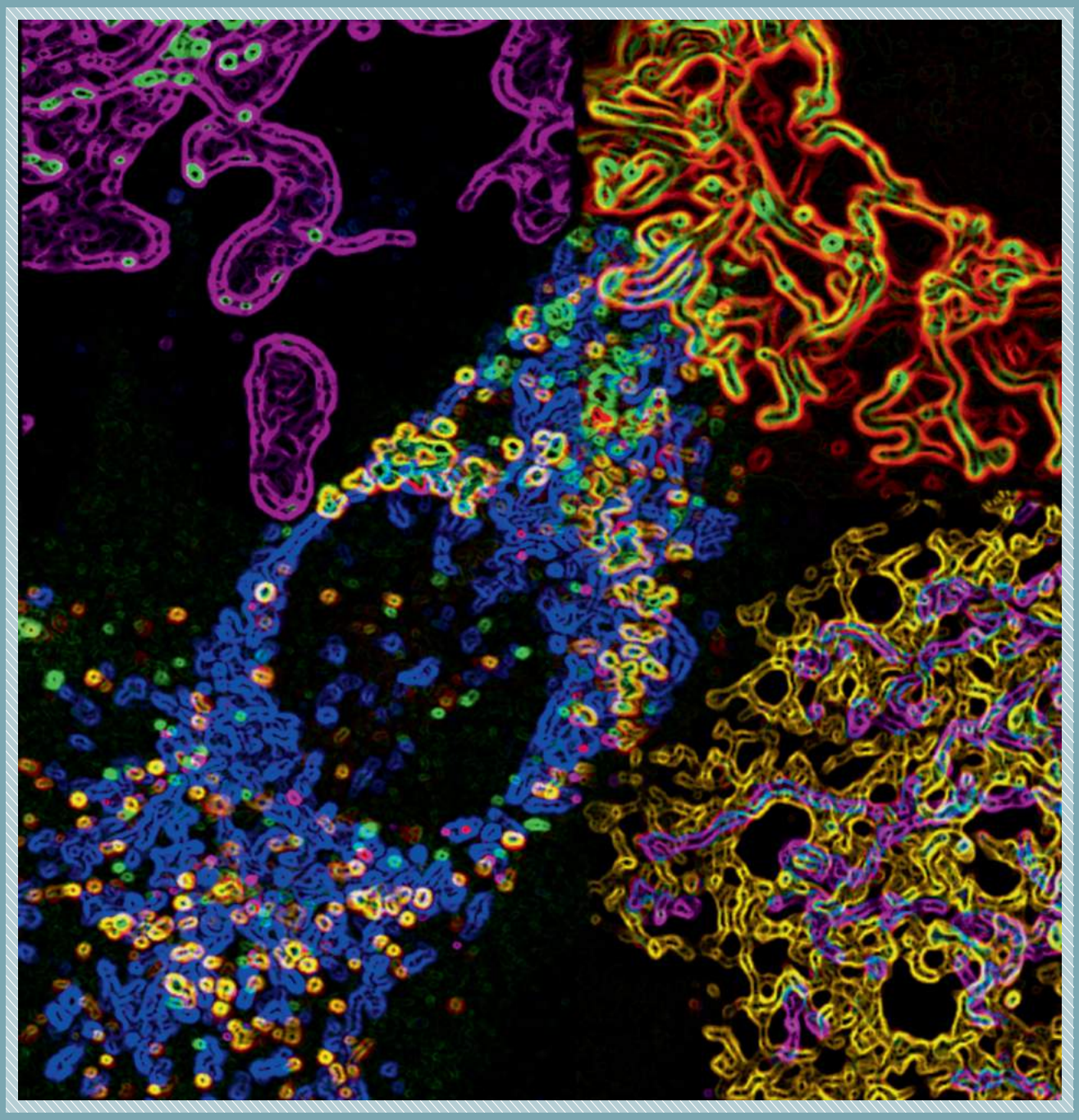


Figure 3 By examining epigenetically sensitized mouse strains, we have found evidence of polyphenism in mammals. Polyphenism is a phenomenon where genetically identical individuals can emerge from development in one of two or more forms. In the case shown, mice emerge into either Lean or Obese states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



LAB ANGELIKA RAMBOLD

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

02

Group Angelika Rambold

Organelle networks in cellular immunity

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

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Group Angelika Rambold

ORGANELLE NETWORKS IN CELLULAR IMMUNITY

Communication between cell organelles shape the function of immune cells

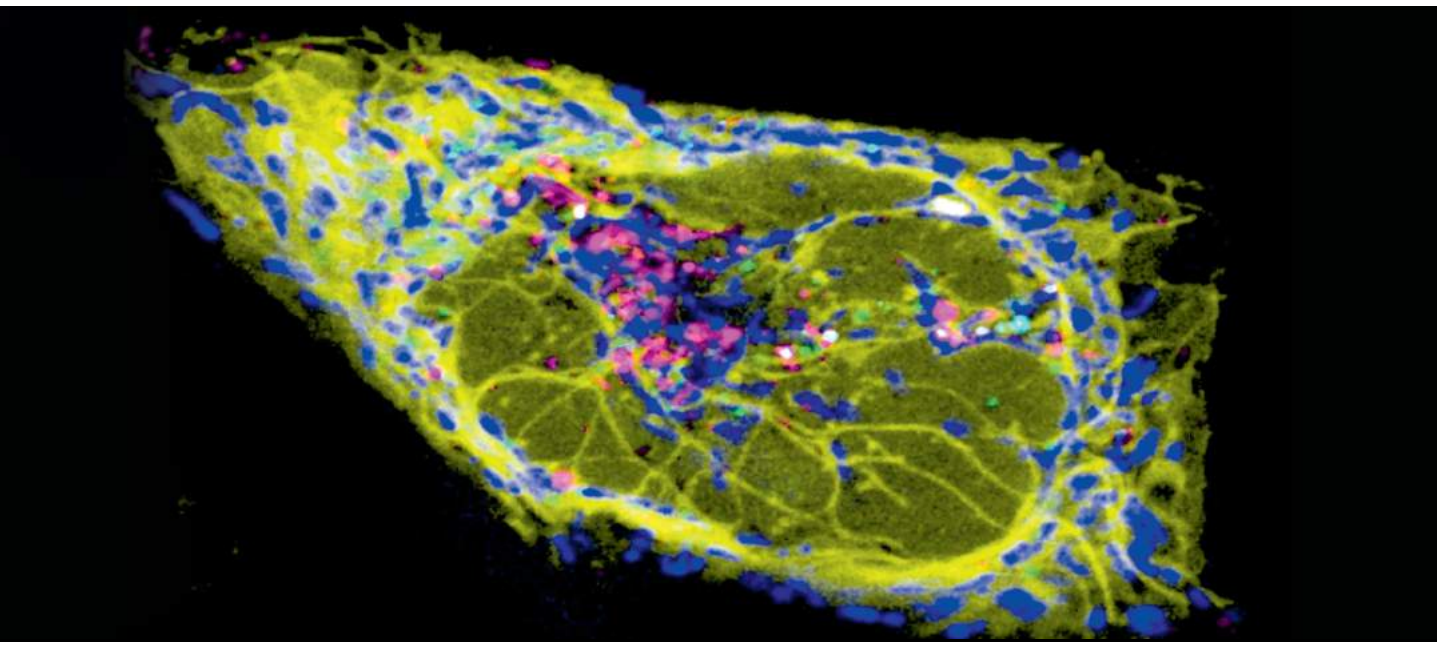

Figure 1 Organelles (differentially colored) selectively arranging their position and functional interaction in the dense interior of the cell.

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

Organelle networks as drivers of metabolic cell activation

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

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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 organellar networks and, through them, control the metabolism and effector functions of different immune cells (T cells, macrophages). Integrated into the Center for Chronic Immunodeficiency (CCI) of the University of Freiburg, we investigate if metabolic alterations contribute to the onset and progression of lysosome-related immune disorders, such as CHS.

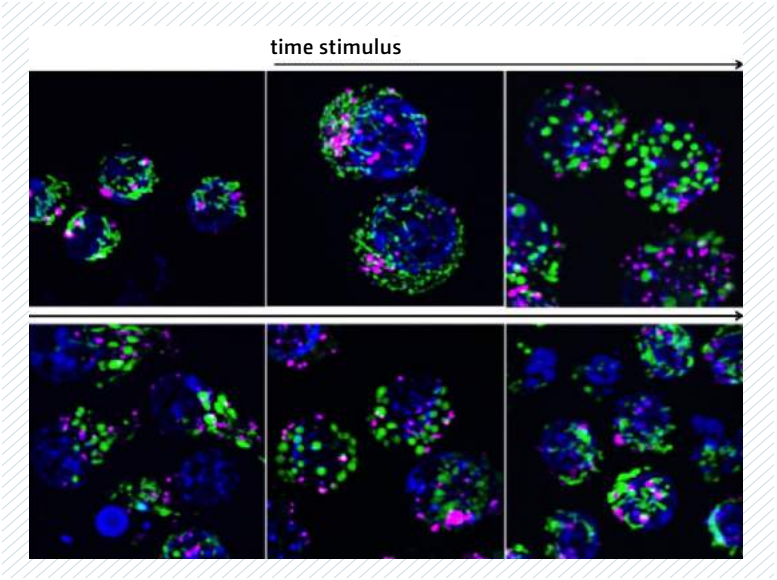


Figure 2 During an immune response different organelles (differentially colored) adapt their morphology and inter-organelle interaction position and functional interaction.

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.

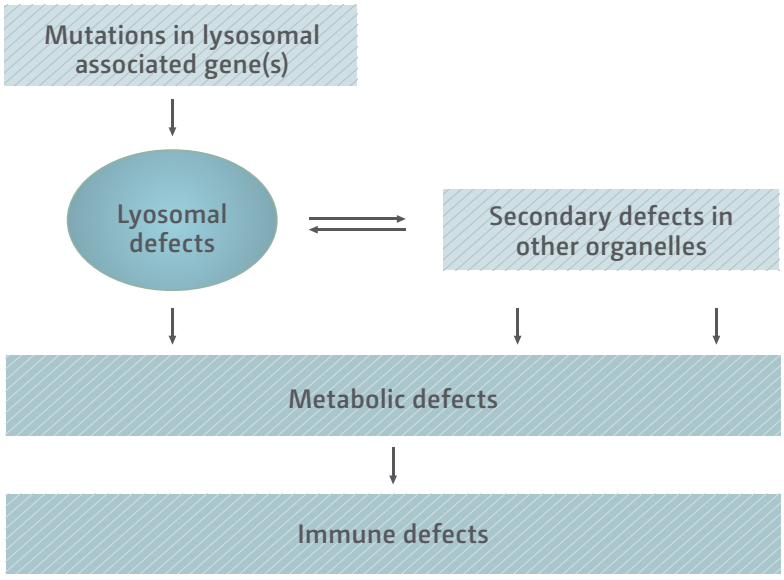


Figure 4 Model of organelle networks contributing to the onset and/or progression of organelle-related immunodeficiencies. Defects in metabolically active organelles, such as lysosomes, can affect the cell's metablic flexibility, a process essential for the proper function of most immune cells. Organelle-driven 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.

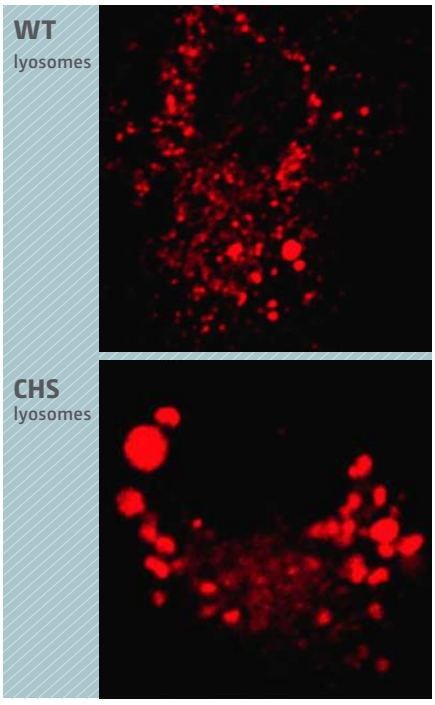
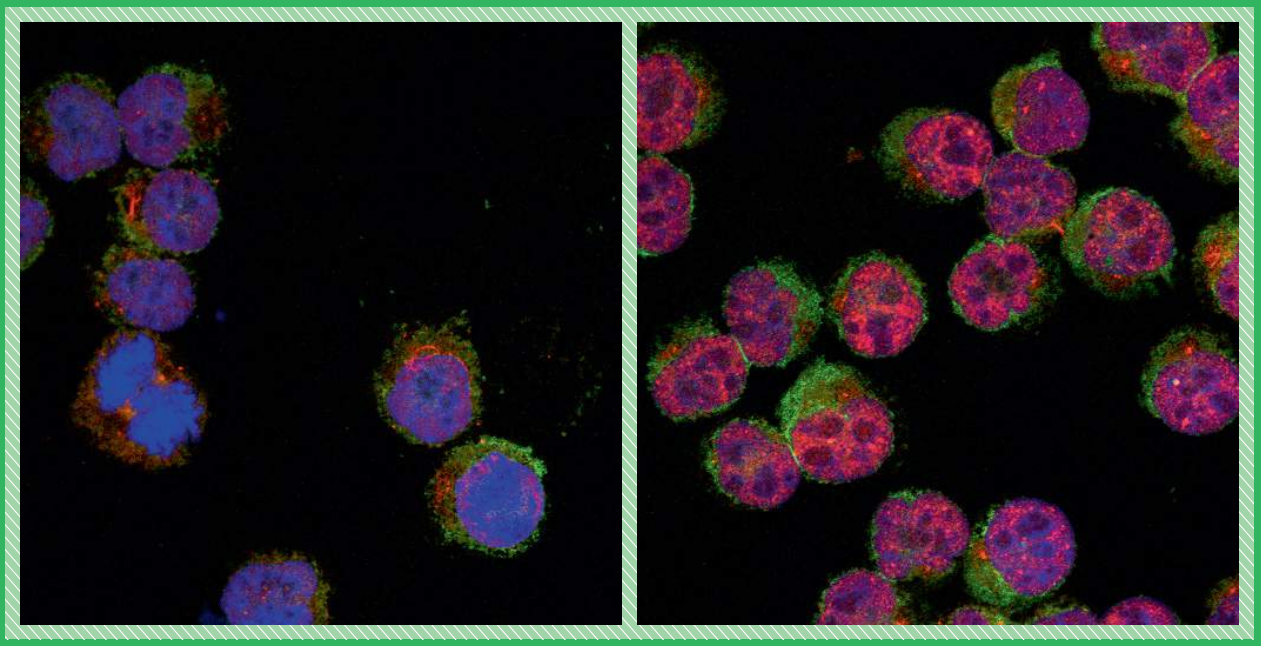


Figure 5 Aberrant lysosomal size and positioning in macrophages generated from Wildtype (top) or a murine Chediak Higashi Syndrome model (bottom).



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LAB RITWICK SAWARKAR

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

02

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Regulation of transcription by protein homeostasis

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Group Ritwick Sawarkar

REGULATION OF TRANSCRIPTION BY PROTEIN HOMEOSTASIS

The role of molecular chaperones and proteasomes in gene expression

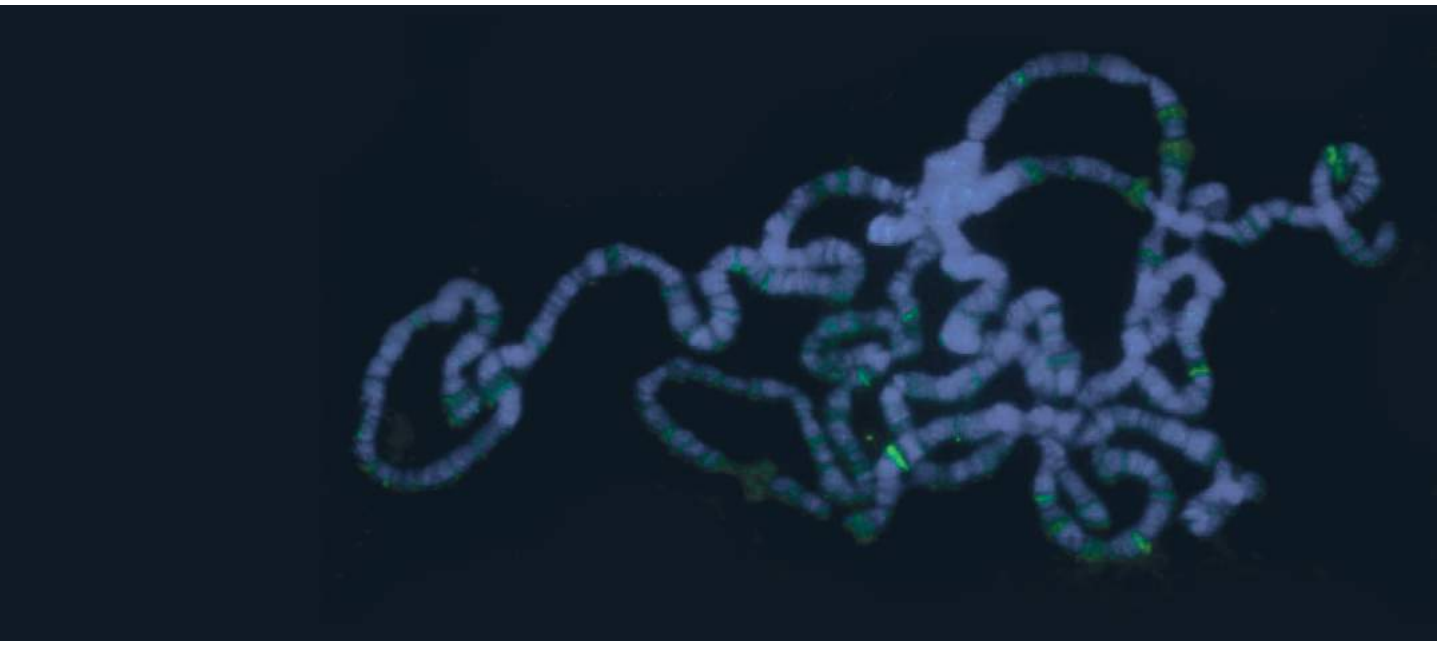


Figure 1 Activity of individual genes can be visualized by staining the giant chromosomes of salivary glands of Drosophila. In this picture, Cdc37 co-chaperone of heat-shock protein 90 (Hsp90) is shown to bind to specific loci on polytene chromosomes. This co-chaperone helps Hsp90 in stabilizing protein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

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

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

Chromatin-based proteostatic network

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

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

framework to understand how cytosolic proteins reach specific chromatin locations.

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

Response of chromatin to environmental stress

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

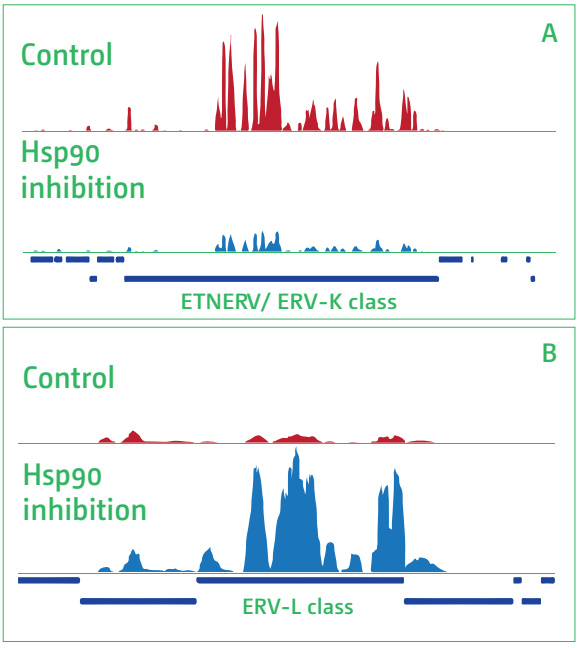


Figure 2 Genome browser snapshots of RNA sequencing data from mouse cells treated with or without a highly specific inhibitor of the chaperone heat-shock protein 90 (Hsp90). (**A**) Changes in RNA-seq reads at one of the loci representing mouse endogenous retrovirus type K (ERV-K); (**B**) Changes in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

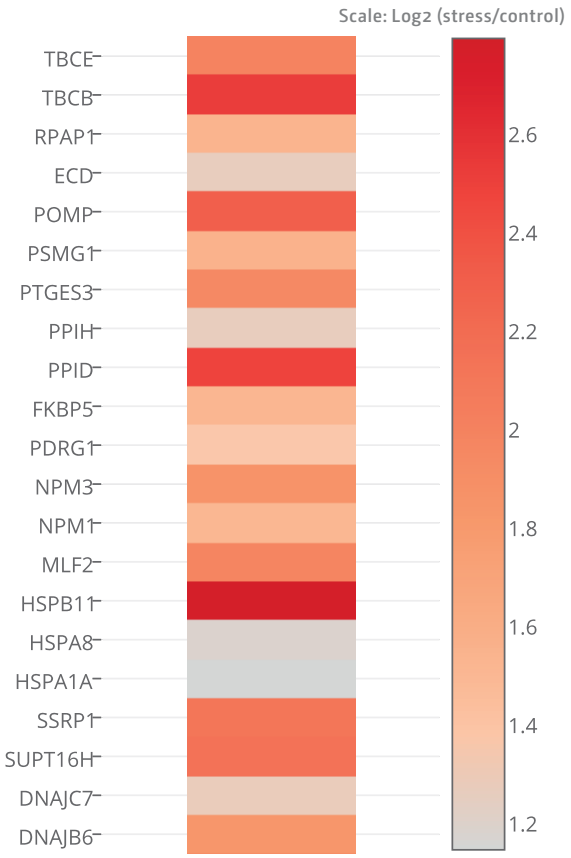
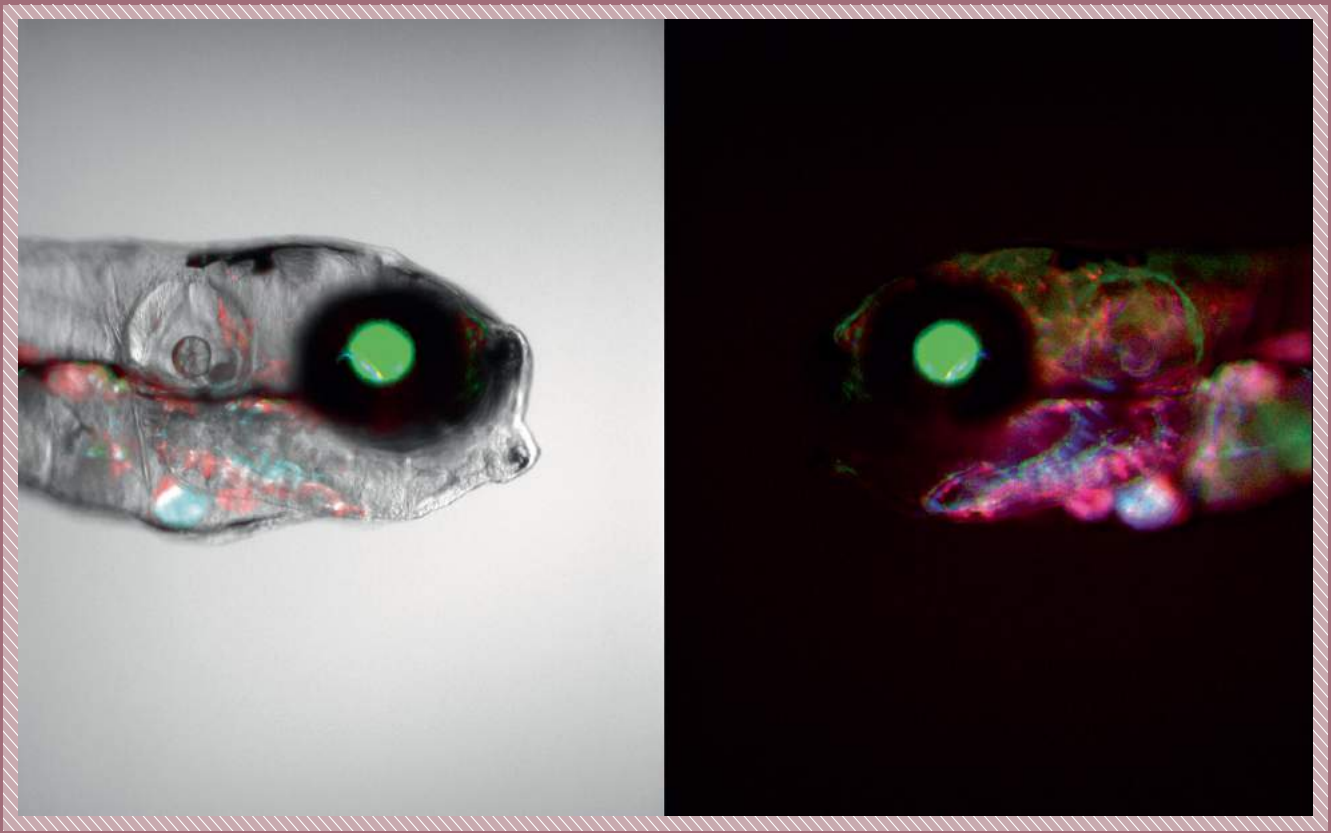


Figure 3 A heat map depicting quantitative changes in abundance of chromatin-associated proteins upon stress in human cells. SILAC-mass spectrometry done on chromatin of human cells subjected to thermal stress revealed an increase in the amount of several ubiquitin ligases, suggesting an increase in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

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

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



LAB EIRINI TROMPOUKI

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

02

Group Eirini Trompouki

Bloody Signals

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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

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Group Eirini Trompouki

BLOODY SIGNALS

Regulation of embryonic and adult hematopoiesis

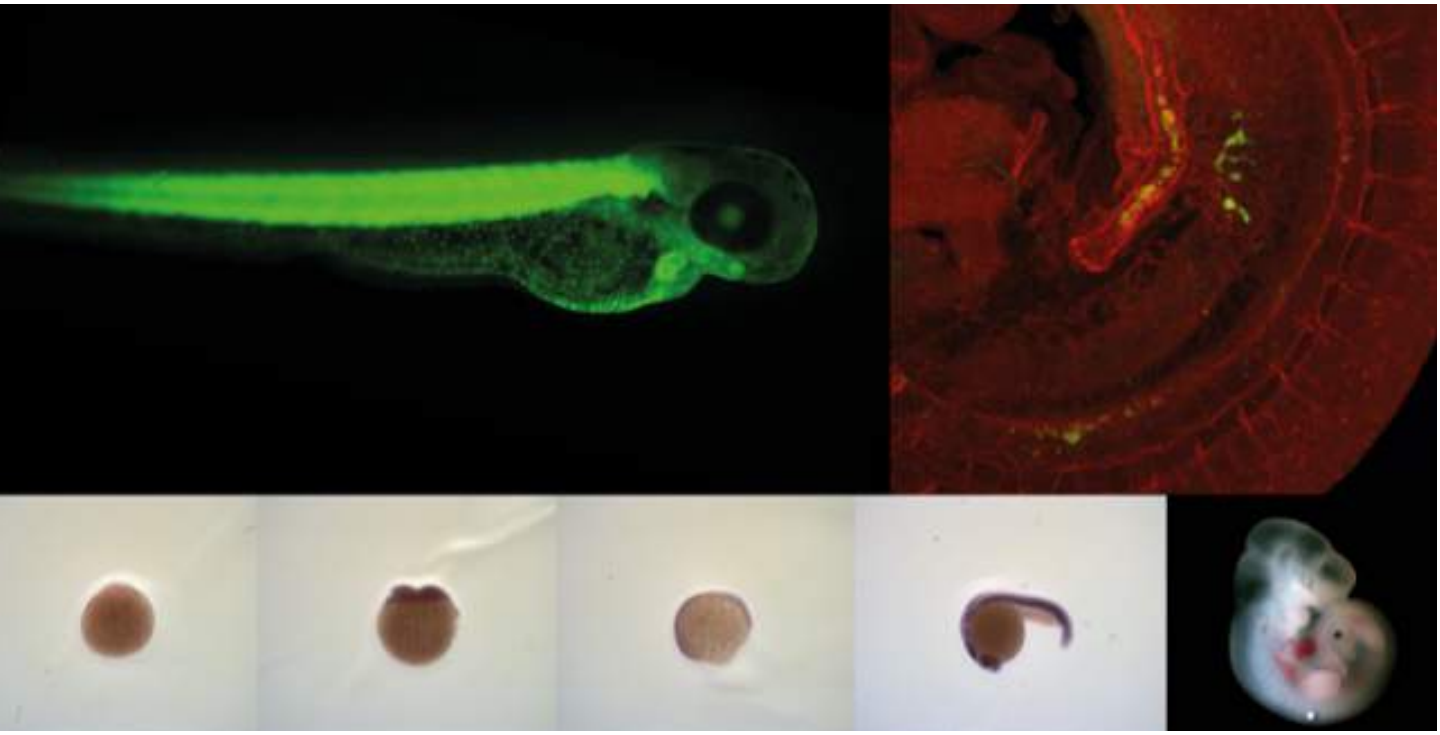

Figure 1 Different model organisms help to answer complex questions. In this figure zebrafish and mouse embryos are used to study hematopoiesis.

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

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

02

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).

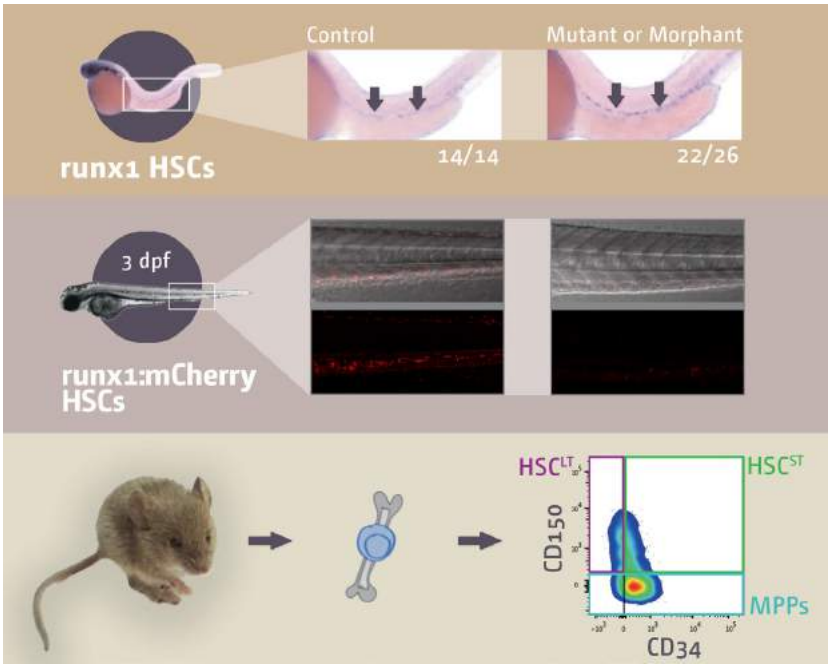

Figure 2 Identification of novel regulators of HSC emergence and maintenance in zebrafish and mice. HSCs are detected by whole mount in situ hybridization, live imaging or FACs sorting.

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.

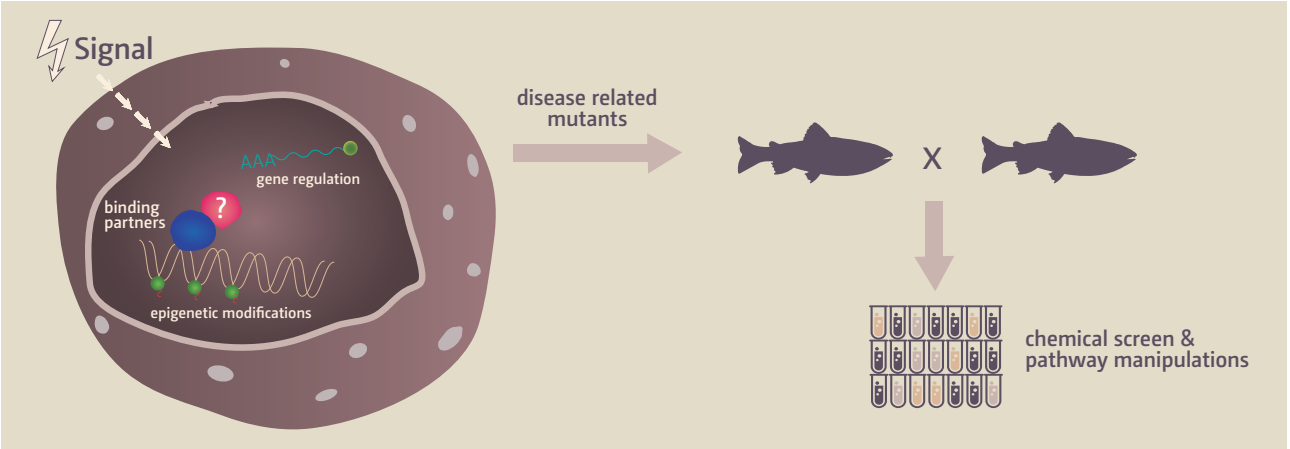

Figure 3 Transcription factor networks and disease modeling in zebrafish

03

Research Facilities

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.

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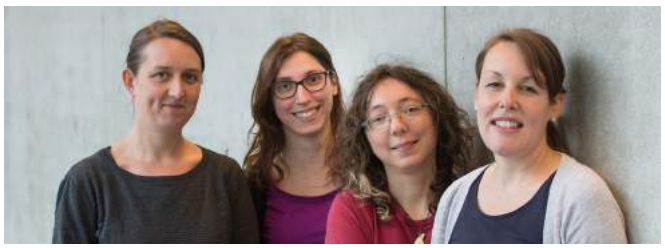
03

MEMBERS



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Postdoctoral Fellow
Laura Arrigoni

ULRIKE BÖNISCH

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2007–2012 Ph.D. Student, Department of Microsystems Engineering, University of Freiburg, Freiburg, Germany

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

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).

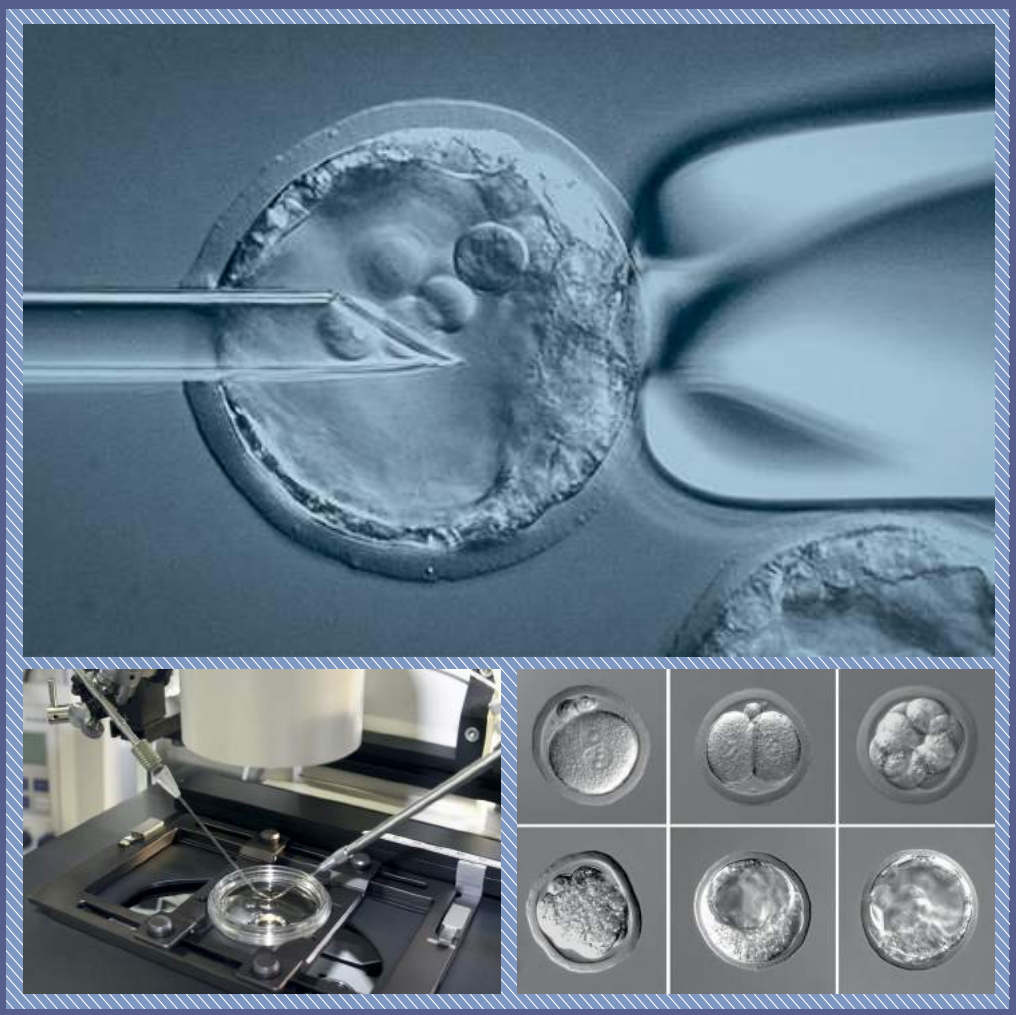
Technology

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.

Methological 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



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

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03

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Elsa Lopez

BENOÎT KANZLER

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1996-1999 Postdoctoral Fellow, Max Planck Institute, Freiburg, Germany, Laboratory of Dr. Moisés Mallo (Department of Developmental Biology)

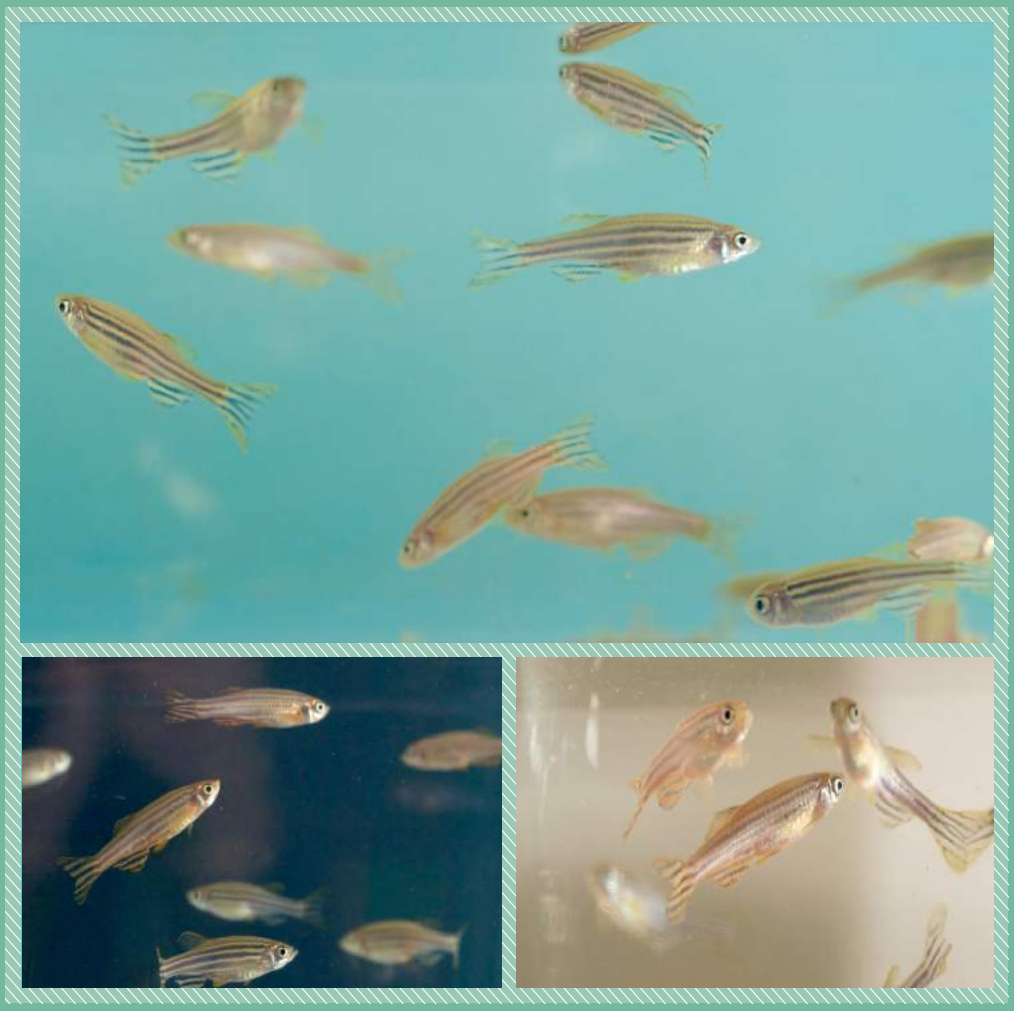
Since 1999 Head of the Transgenic Mouse Facility at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

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.

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Hess I, Boehm T (2016): Stable multilineage xenogeneic of definitive hematopoiesis in adult zebrafish. **Science Reports 6, 19634**.

03

MEMBERS

Technicians
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MICHAEL SCHORPP

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1996-2000 Postdoctoral research fellow at the German Cancer Research Center, Heidelberg and at the Max Planck Institute of Immunobiology, Freiburg, Germany

Since 2000 Project Leader at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

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

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

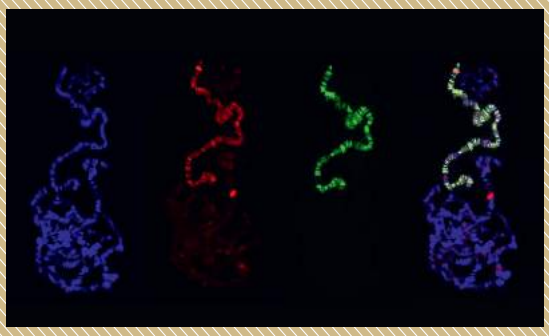
Service

The fish facility at the Max Planck Institute of Immunobiology and Epigenetics was opened in 2007. It houses zebrafish (Danio rerio) and Medaka (Oryzias latipes) fish. Each room is equipped with an independent water treatment unit. Access to the facility is restricted and automatically recorded; technical parameters of the entire facility are remotely controlled. A separate quarantine room provides opportunity for short-term experiments without compromising the high hygienic standards of the main facility. Currently, about 50 different lines carrying 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

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

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Ilik IA, Maticzka D, Georgiev P, Gutierrez NM, Backofen R, Akhtar A (2017). A mutually exclusive stem-loop arrangement in roX2 RNA is essential for X-chromosome regulation in Drosophila. **Genes & Development 31, 1973-1987.**

03

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Elena Wiesler

PLAMEN GEORGIEV

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

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

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

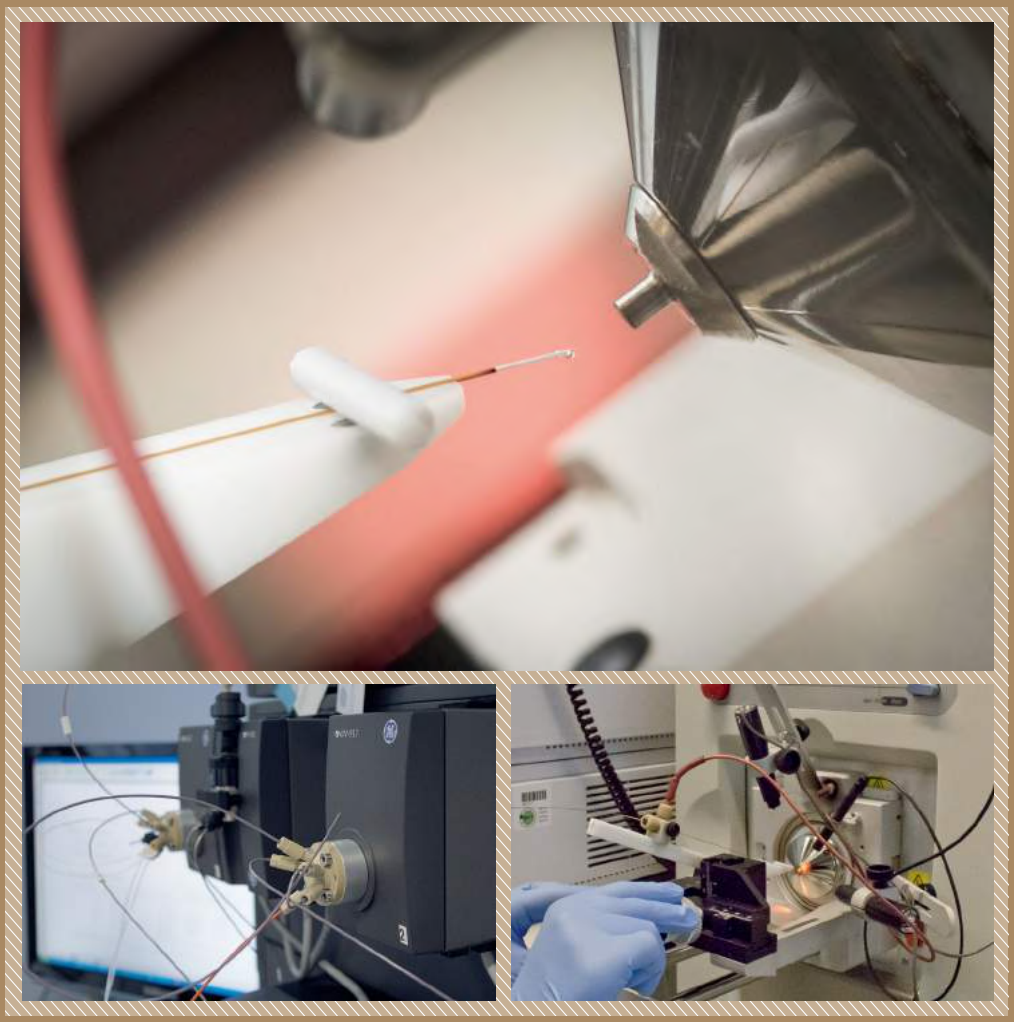
The fruit fly Drosophila is one of the most extensively characterized metazoan organisms. Over more than 100 years since it was introduced as an experimental animal model for biological research, it has allowed for key biological concepts and phenomena to be elucidated, leading to some of biology's fundamental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

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

The Fly facility was set up in 2010 upon recruitment of Dr Asifa Akhtar. The facility contains a fly room with 7 dissection microscopes and CO_2 supply for day-to-day fly pushing and a stereo microscope for fluorescent imaging. Attached to the fly room are 25°C and 18°C light-, temperature- and humidity-controlled rooms for fly stocks. In addition, there are separate rooms allocated for large population cages allowing embryo collections for chromatin isolation and an 18°C room for a collection of mutant and transgenic lines maintained to facilitate rapid amplification upon request. In addition, a fly food preparation kitchen with a large production capacity has been established for the maintenance of these flies as well as for the daily needs of the fly groups. A state-of-the-art microinjection unit equipped with an inverted microscope, Eppendorf Femtojet, micromanipulator and embryo aligning stage, allows Drosophila embryo transformation and generation of transgenic animals using transposase-mediated random insertion as well 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



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

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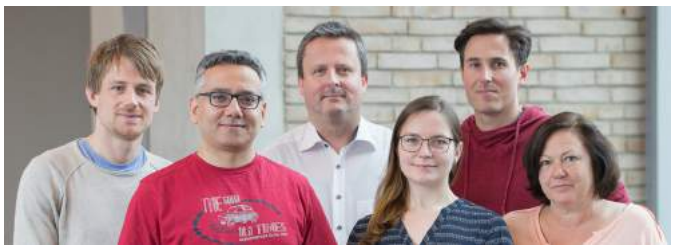
03

MEMBERS



Technician
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Postdoctoral Fellows
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Witold Szymanski

Ph.D. Student
Monika Puchalska

Data Engineer
Daniel Eilertz
(joint appointment with Metabolomics)

GERHARD MITTLER

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

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

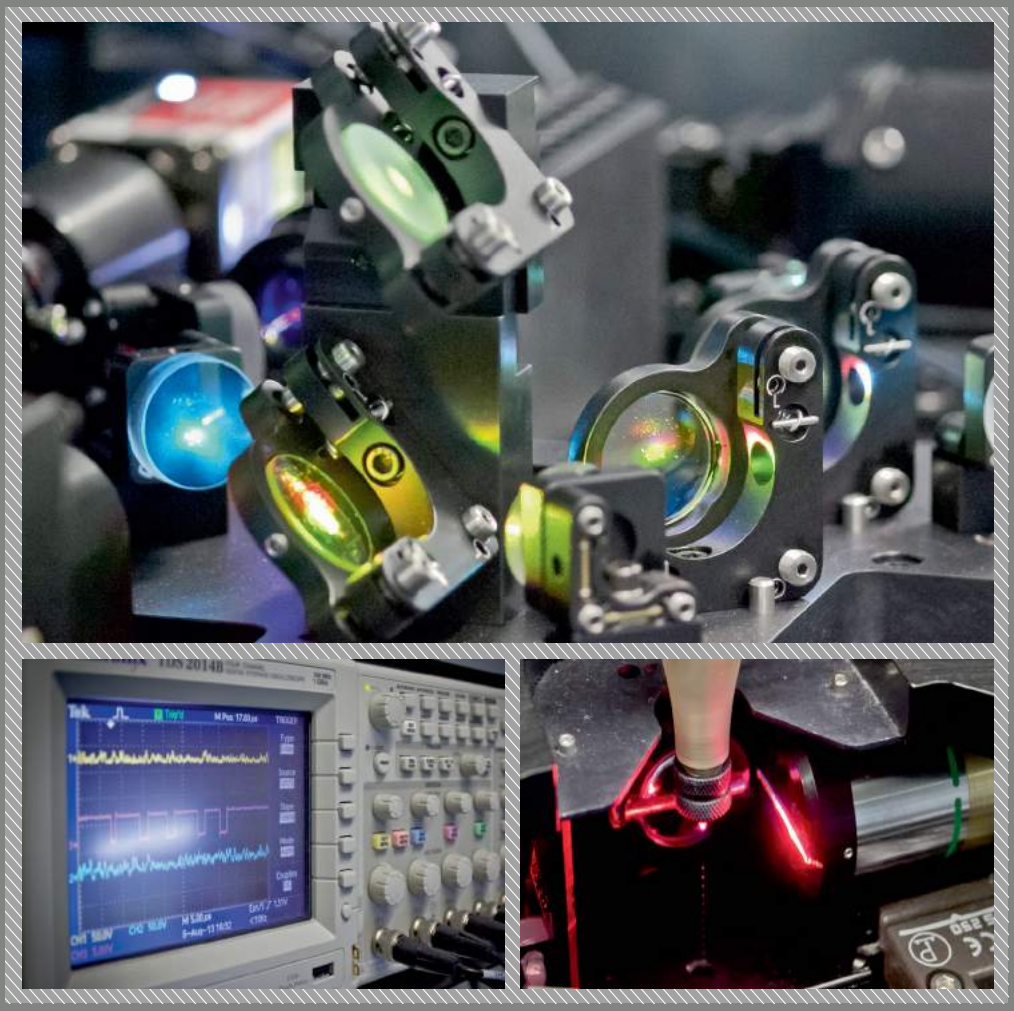
2002–2005 Postdoctoral fellow, Center of Experimental Bioinformatics, University of Southern Denmark, Odense, Denmark

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

The unit 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

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03

MEMBERS

Technicians
Sebastian Hobitz
Maike Knoblauch
Konrad Schuldes

ANDREAS WÜRCH

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

1993-1999 Research Assistant at the Department of Cellular and Molecular Immunology, Prof. Dr. Klaus Eichmann, Max Planck Institute of Immunobiology, Freiburg, Germany

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

The Flow Cytometry Unit, utilizing high quality flow cytometry instrumentation, provides comprehensive and technically sophisticated cell analysis and sorting services as well as flow cytometry training and education for new users. Flow cytometry is a method that allows measurements of various characteristics of individual cells by using fluorescent probes. Our laboratory has the capability to run many advanced flow cytometry applications.
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 tweenty 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.

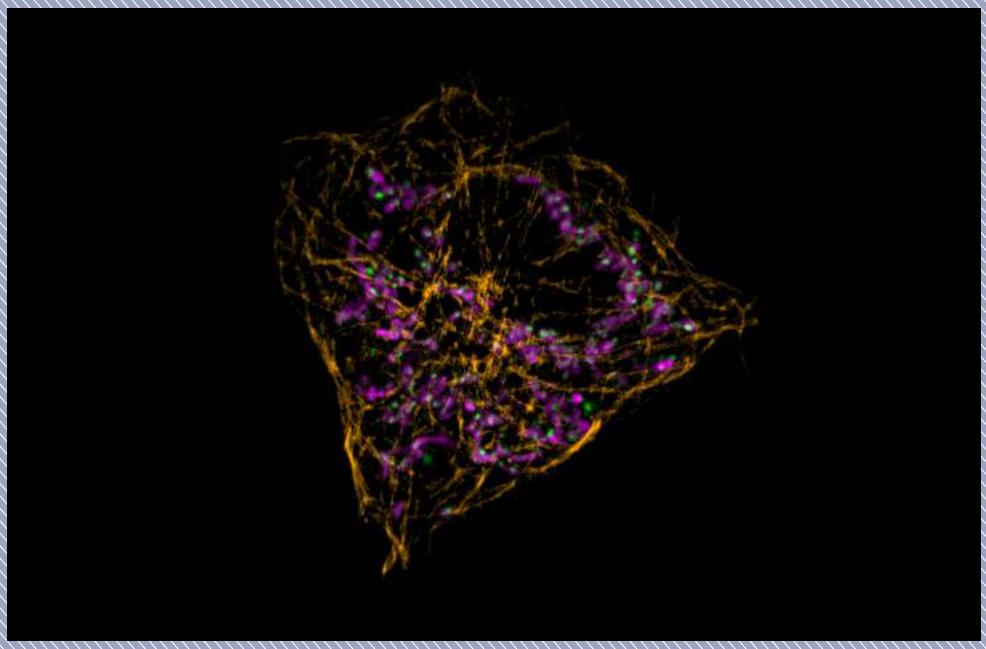
Currently available applications include:

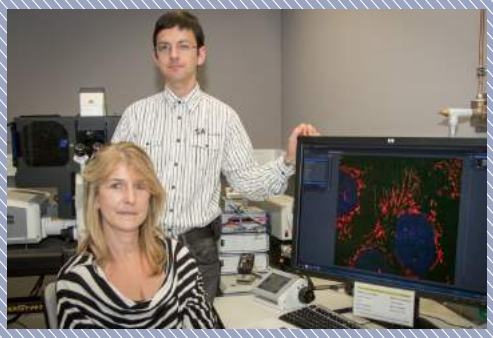
- cloning and cell sorting
- multi-color immunofluorescence
- DNA cell cycle analysis
- measurements of apoptosis
- calcium flux
- fluorescence resonance energy transfer (FRET)

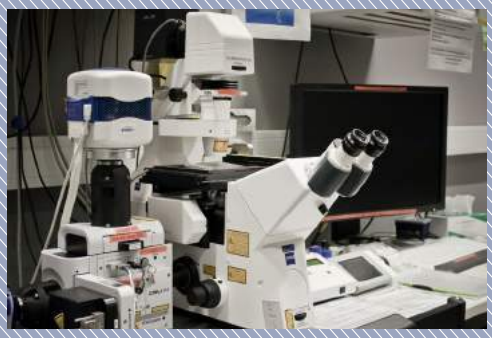
Sequencing Facility

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

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Avilov SV and Aleksandrova N (2018) Fluorescence protein complementation in microscopy: applications beyond detecting bi-molecular interactions. **Methods and Applications in Fluorescence 7, 012001**.

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03

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

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 hi-end confocal microscope with super-resolution capacity, has been 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.

LABORATORY ANIMAL FACILITY

Top: Mouse (Mus musculus) of our facility with polycarbonat enrichment showing typical explorative behaviour. Bottom: IVC-Housing of our mice.

03

PETER KLOTZ

2008-2014 Studies in veterinary medicine at the University of Giessen, Germany; License to practice veterinary medicine

2014-2018 Ph.D. studies at the Institute of Hygiene and Infectious Diseases of animals, University of Giessen, Germany

2018-2019 Designated Veterinarian at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

since April 2019 Head of the Laboratory Animal Facility at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

The Animal Facility at the Max Planck Institute of Immunobiology and Epigenetics has supported research for more than 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.

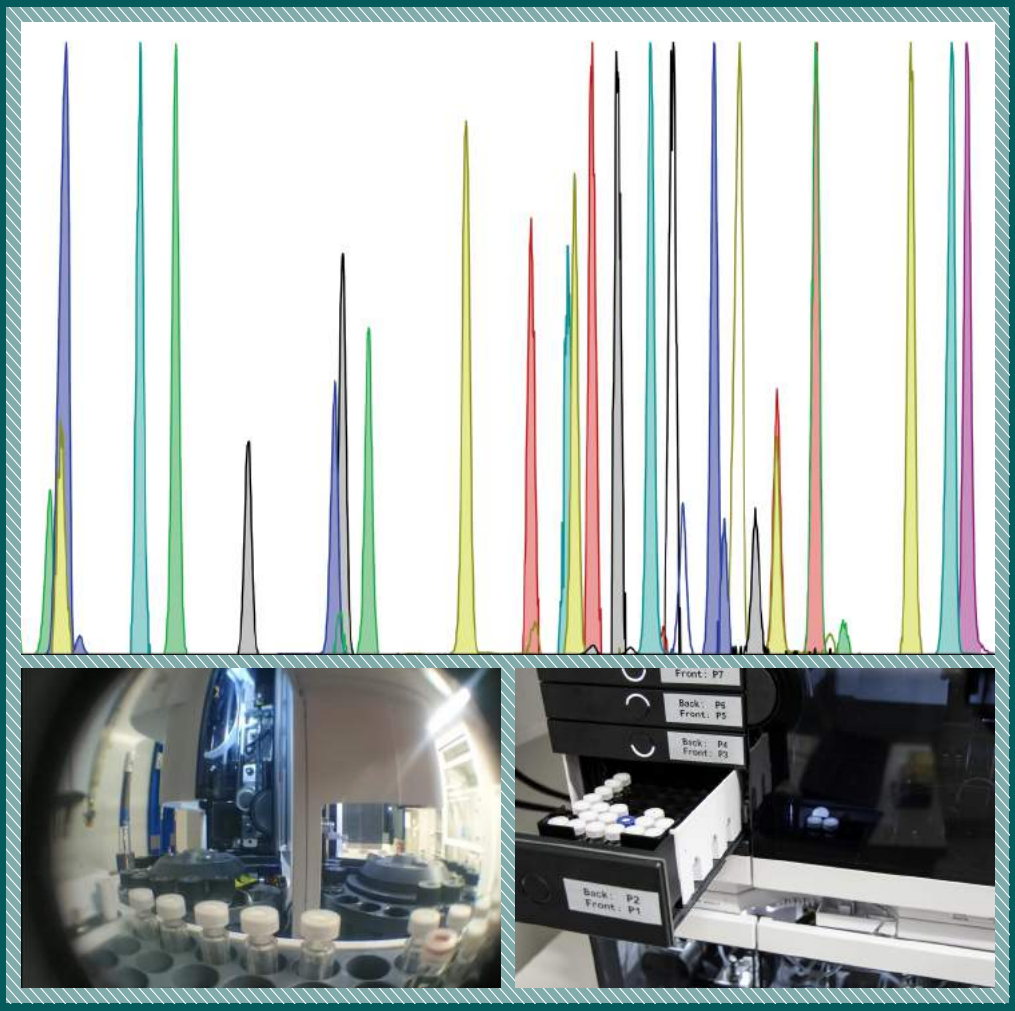
During September 2017, the construction of the new building for animal holding and breeding of mice exceeding the new European animal welfare regulations and standards has started.

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.

METABOLOMICS FACILITY



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.

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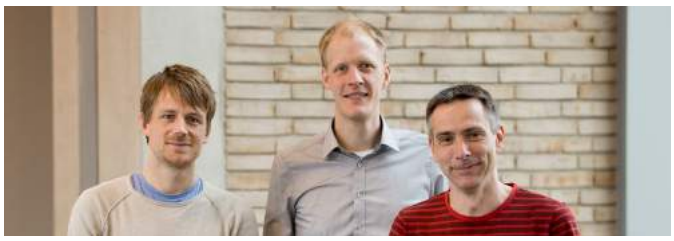
Klein Geltink RI, O'Sullivan D, Corrado M, Bremser A, Buck MD, Buescher JM, Firat E, Zhu X, Niedermann G, Caputa G, Kelly B, Warthorst U, Rensing-Ehl A, Kyle RL, Vandersarren L, Curtis JD, Patterson AE, Lawless S, Grzes K, Qiu J, Sanin DE, Kretz O, Huber TB, Janssens S, Lambrecht BN, Rambold AS, Pearce EJ, Pearce EL (2017). Mitochondrial Priming by CD28. **Cell 171(2), 385-397.**

03

MEMBERS



Technicians
Michael Mitterer

Data Engineer
Daniel Eilertz
(joint appointment with Proteomics)

JÖRG BÜSCHER

2000–2006 studies in Biotechnology at the Technical University Braunschweig, Germany

2006–2010 PhD studies at the Institute of Molecular Systems Biology, ETH Zurich, Switzerland

2011–2016 Scientist and project manager at Brain AG, Zwingenberg, Germany

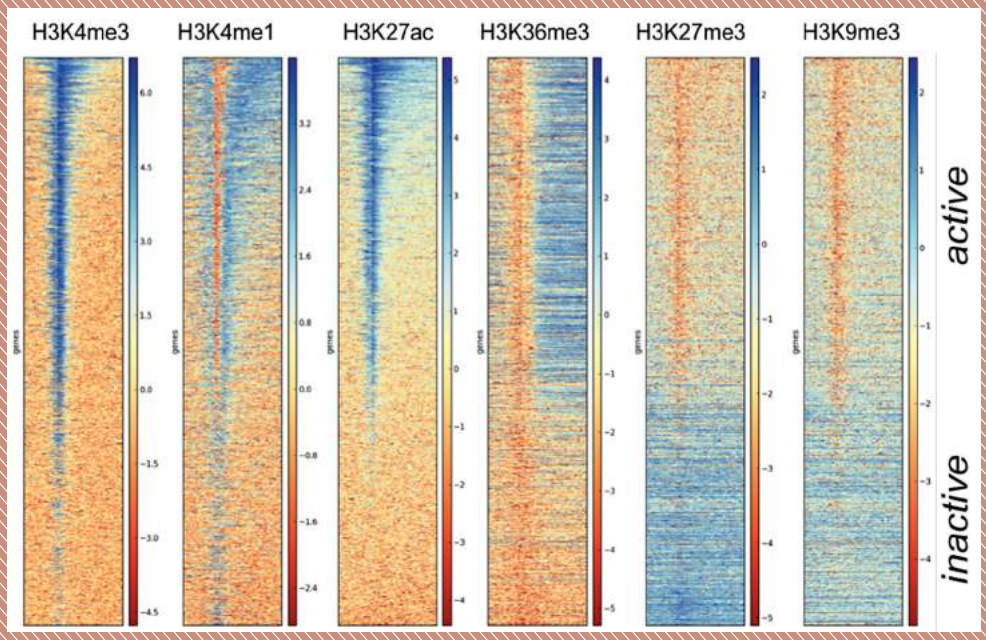
Since October 2016 Head of the Metabolomics Facility at the Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

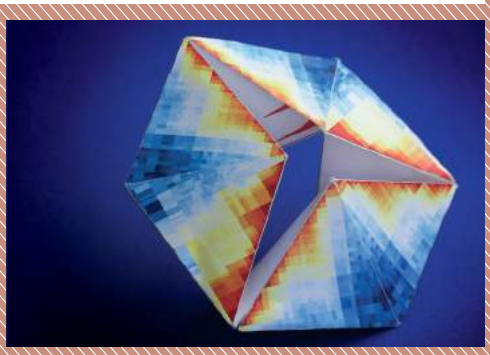
All living cells are metabolically active, i.e. they take up substrates and use them to fuel a network of biochemical reactions. The intermediates of this network are called metabolites. Their entirety is the metabolome. Metabolomics is the study of these chemical fingerprints. Like the genetic code, 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.

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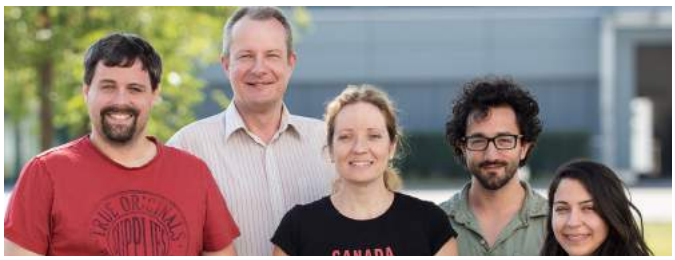
03

MEMBERS



Bioinformaticians
Leily Rabbani
Devon Ryan
Katarzyna Sikora

Ph.D. student
Francesco Ferrari

THOMAS MANKE

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

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

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

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

Data Center

We operate a state-of-the-art data center with a dedicated cooling system, more than 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.

Entrance and lobby of the MPI-IE

04

Around the Institute

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.

04

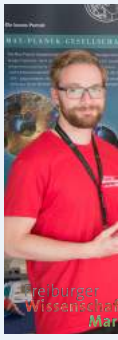
maxday mpi-ie

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, facilites 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 multifacted 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 sucessfully 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 releveance of immunobiology and epigenetics to the public.



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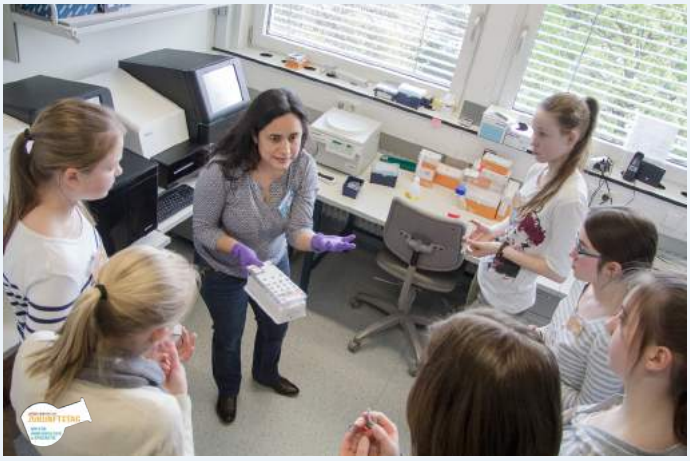
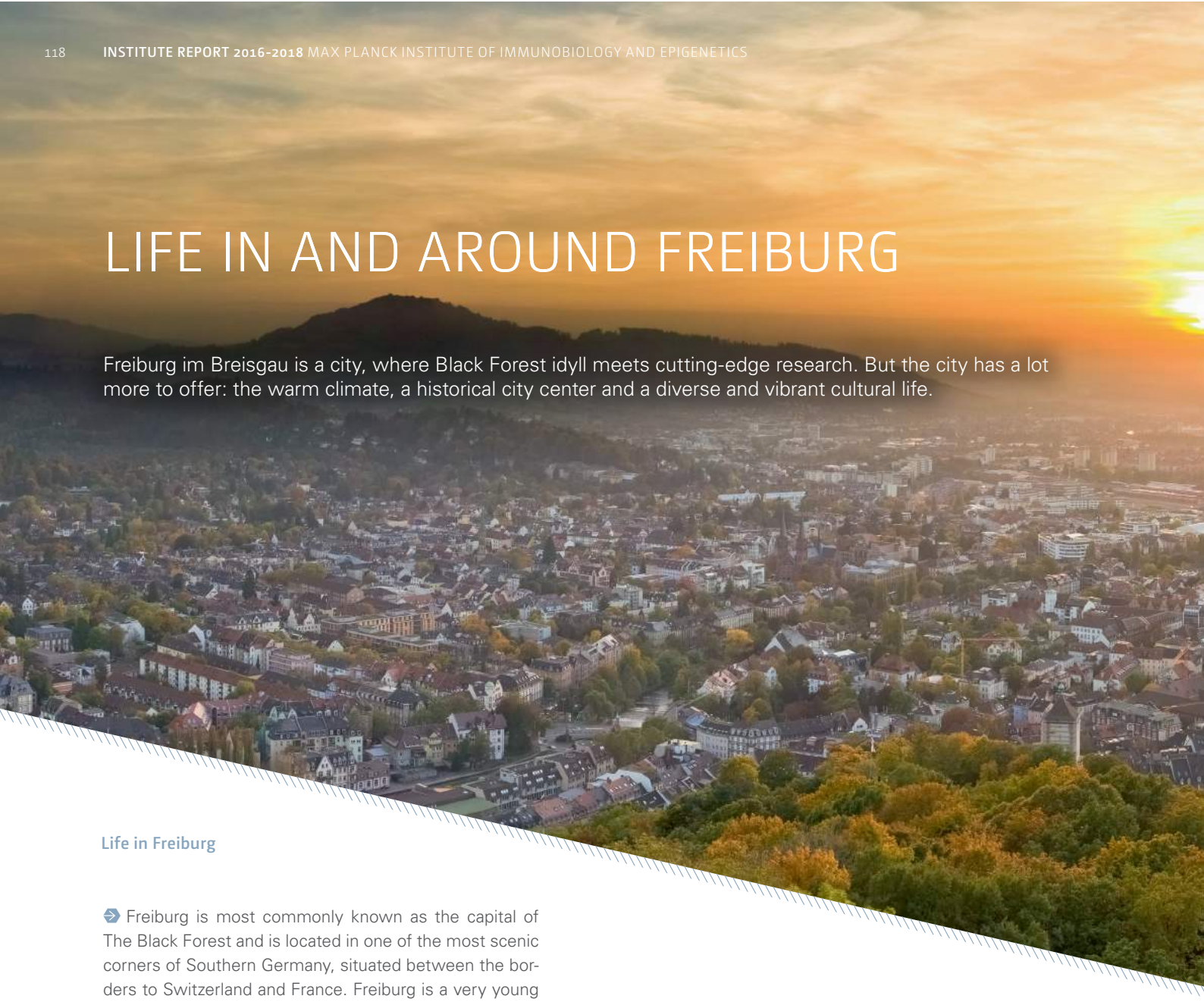


OUTREACH EVENTS

Making science compelling and accessible to the public is done by a range of formats for different audiences. Regulary 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 releveance of immunobiology and epigenetics. Occupational orientation is in focus at the MPI-IE "Zukunftstag" 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.



Life in Freiburg

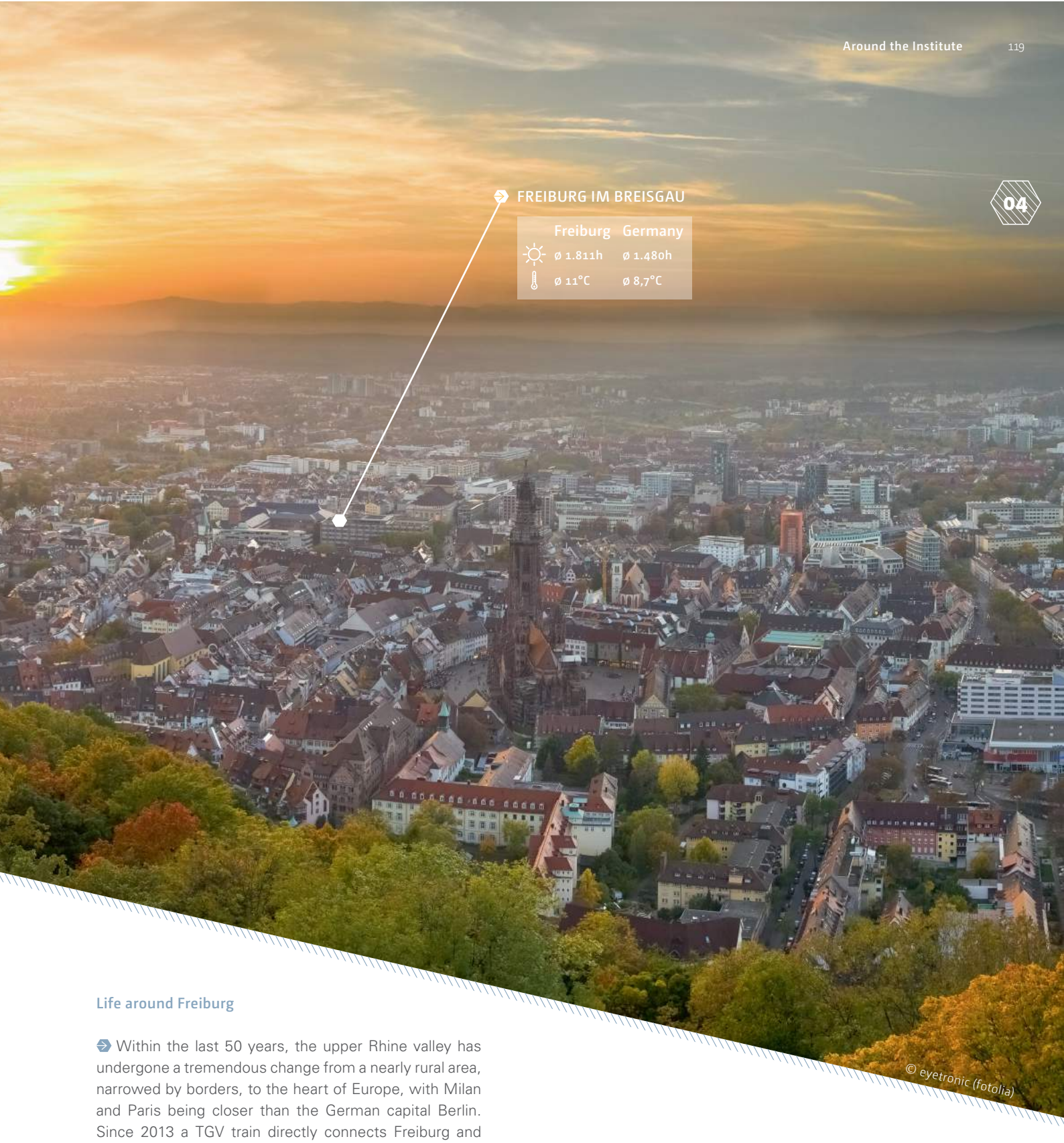
Freiburg is most commonly known as the capital of The Black Forest and is located in one of the most scenic corners of Southern Germany, situated between the borders to Switzerland and France. Freiburg is a very young and lively city due to a high percentage of students. The town with its more than 220.000 inhabitants is characterized by 25.000 students at the University of Freiburg. 16 percent among them are from abroad, half European and non-European, which adds to the international flair of the town.

The 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

31. March 2016

Reina Mebius
VU University Medical Cente, Amsterdam, The Netherlands

Lymph node stromal cells in control of immune cells

07. April 2016

Sebastian Amigorena
Immunity and Cancer Dept. Curie Institute, Paris, France

Antigen cross presentation and T cell activation by dendritic cells

14. April 2016

Kees Murre
University of California, San Diego, USA

The contraction of space and time in gene regulation

14. July 2016

Marc Schmidt-Supprian
Ludwig Maximilians University, Munich, Germany

Investigating lymphomagenesis, NF-kB activation and beyond

13. October 2016

Meinrad Busslinger
Research Institute of Molecular Pathology, Vienna, Austria

Control of B cell immunity and leukemia by the transcription factor Pax5

17. October 2016

Mina Bissell
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

2017

09. January 2017

Ben Lehner
Centre for Genomic Regulation, Barcelona, Spain

Trans-generational epigenetic inheritance of environmental information in C. elegans

28. February 2017

Douglas Green
St. Jude Children's Research Hospital, Memphis, USA

To the edge of necroptosis, and back

24. March 2017

Harinder Singh
Cincinnati Children's Hospital Medical Center, Cincinnati, 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

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 Lambrech
VIB UGent Center for Inflammation Research, Gent, Belgium

Dendritic cell – Epithelial cell interactions at the heart of the allergy epidemic

Harinder Singh, Special Guest in March 2017

04

2018

06. July 2017

Danny Reinberg
Howard Hughes Medical Institute, New York, USA

Epigenetics: One genome, multiple phenotypes

12. September 2018

Julius Brennecke
Institute of Molecular Biotechnology, Vienna, Austria

Ubiquitin sets the timer: Impacts on stress response and aging

12. September 2018

Jens Brüning
MPI for Metabolism Research, Cologne, Germany

Inflammatory signaling in obesity and metabolic disease

12. October 2017

Emmanouil Dermitzakis
University of Geneva, Switzerland

Contribution of non-coding DNA to complex disease and cancer

23. November 2017

Paul Martin
University of Bristol, UK

Studying inflammation in wound healing and cancer

05. December 2017

John O'Shea
NIH, Bethesda, USA

Genomic views of lymphocyte differentiation and activation

15. February 2018

Ivan Đikic
Goethe University, Frankfurt am Main, Germany

Ubiquitin and autophagy networks in health and disease

02. March 2018

Alexander Stark
Research Institute of Molecular Pathology, Vienna, Austria

Decoding transcriptional regulation

15. March 2018

Luca Scorrano
Venetian Institute of Molecular Medicine, Padova, Italy

Keeping mitochondria in shape: a matter of life and death

22. March 2018

Richard Morimoto
Northwestern University, Illinois, USA

The biology of proteostasis in aging and neurodegenerative disease

05. April 2018

Nikolaus Rajewsky
Max-Delbrück-Center for Molecular Medicine Berlin-Buch, Berlin, Germany

Single cell sequencing in space and time

26. April 2018

Ralf Sommer
MPI for Developmental Biology, Tübingen, Germany

Nature vs. Nurture: Genetics and epigenetics of phenotypic plasticity and the first self-recognition system in nematodes

17. May 2018

José Enríquez
National Center for Cardiovascular Research, Madrid, Spain

Genomic views of lymphocyte differentiation and activation

06. June 2018

Andrea Musacchio
MPI of Molecular Physiology, Dortmund, Germany

Feedback control of mitosis: down to mechanisms

26. June 2018

Doreen Cantrell
Dundee University, UK

Different environmental sensing pathways and responses to mTORC1 in naïve versus effector T cells

12. July 2018

Bertie Gottgens
University of Cambridge, UK

Regulatory Networks and Cellular States of Normal and Malignant Blood Development

23. July 2018

Andreas Trumpp
DKFZ, Heidelberg, Germany

Stem Cells during Normal Physiology and Cancer

13. September 2018

Brigitta Stockinger
Crick Institute, London, UK

Environmental influences via diet on intestinal homeostasis

25. September 2018

Alex Schier
University of Basel, Switerland

Single-cell reconstruction of vertebrate development

27. September 2018

Anne DeJean
Institut Pasteur, Paris, France

Chromatin roles for SUMO in innate immunity and cell identity

27. September 2018

Harmit Malik
Fred Hutchinson Cancer Research Center, Seattle, USA

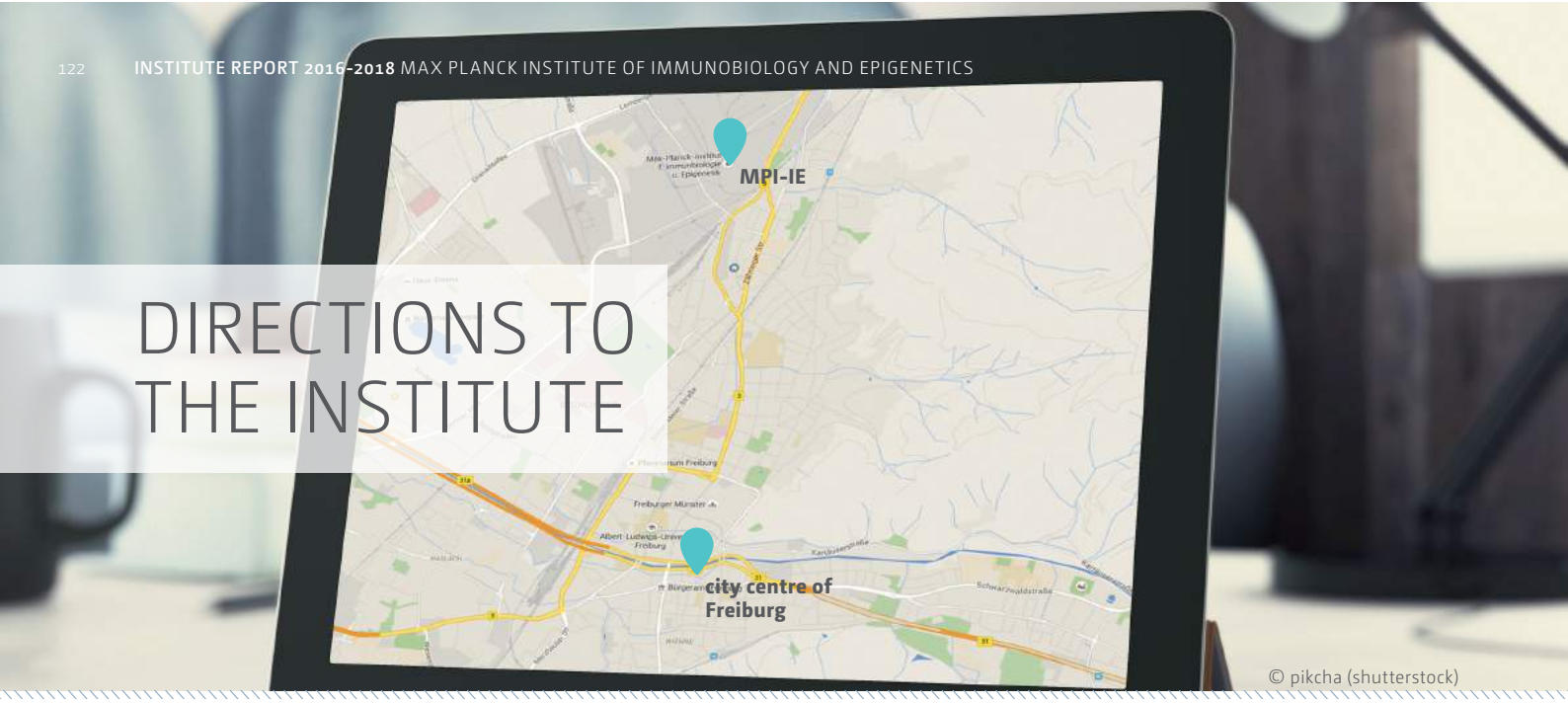
Genetic conflicts shape meiosis, centromeres and species

15. November 2018

Martin Guilliams & Charlotte Scott
VIB Center for Inflammation Research (IRC), Ghent University, Belgium

Resident and Recruited Macrophages in Health and Disease

Douglas Green (Memphis, USA) visited the MPI-IE in February 2017



DIRECTIONS TO THE INSTITUTE

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Web: www.ie-freiburg.mpg.de

By public transport (from main train station)

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

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

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

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

By car

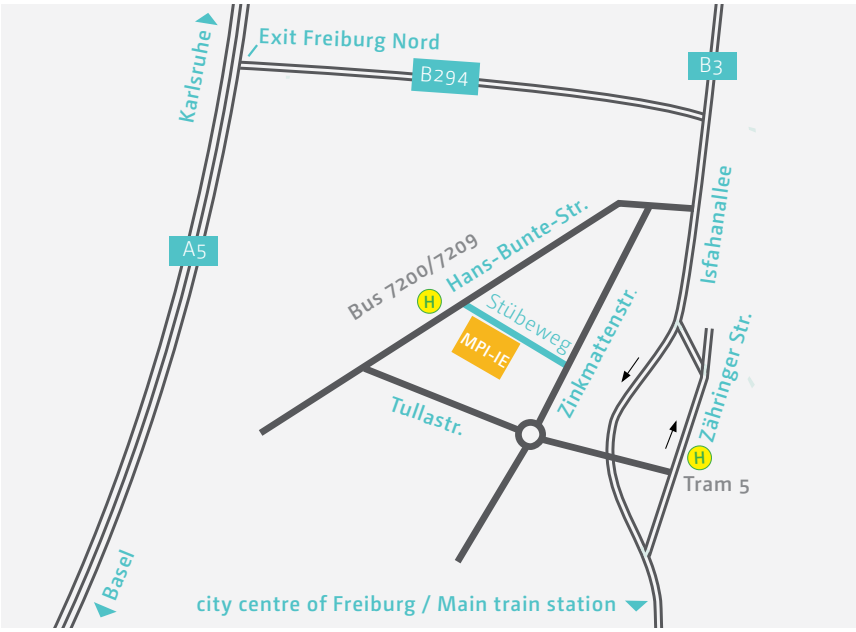
If you arrive via freeway A5, Karlsruhe to Basel, take the exit for "Freiburg Nord". Keep in the left lane and take highway B294 towards Freiburg. After approximately 3.5km highway B294 runs into highway B3 towards Freiburg. Take the exit for "Industriegebiet Nord" and keep in the left lane. Turn left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

By airplane

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

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

Map



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