IMP Advances
2017 – 2022

Capturing six years.
Six years of groundbreaking
research, six years of curiosity turned
into discovery. Molecular and cell biology.
Regeneration and development. Immunology
and cancer. IMP Advances invites you to dive into
six years of progress. Research requires inquisitive
looks, science thrives on vision. This is why we chose
the human eye as a symbol for exploration. But there is
more. Each lab's chapter showcases a scientist's iris.
The intricate patterns of the eye are unique to every
single person. A bow to the individual perspectives that
contribute to our scientific endeavours. This publication
aims to capture the essence of IMP research from 2017
to 2022. Cutting through the traditional boundaries
of print, it also draws in the digital space. Extend
your experience with QR codes for a celebration
of the minds behind the research. Explore,
switch between media. Eye up some
spectacular science.
Ready? Watch!

CONTENTS

INTERVIEW

Embracing progress – six years of discovery at the IMP

JAN-MICHAEL PETERS

MANAGING DIRECTOR / SCIENCE

HARALD ISEMANN

MANAGING DIRECTOR / FINANCE AND ADMINISTRATION

In the last six years, the IMP has experienced tremendous changes and rose to unexpected challenges: new Group Leaders, new research topics, and new technologies shaped this period, but also a pandemic and other global crises. Managing Directors Jan-Michael Peters (Science) and Harald Isemann (Finance and Administration) reflect on these years, and how they paved the ground for continuing the IMP's success story in the future.

What has the IMP achieved scientifically between 2017 and 2022?

Peters: We have been incredibly productive in making breakthrough discoveries and technological advances. IMP scientists published dozens of studies in high-calibre peer-reviewed journals each year. Advances ranged from sequencing the two largest vertebrate genomes known to science to revealing inner workings of genome folding, investigating the structure and functions of giant enzymes with cryo-electron microscopy, and pinpointing fundamental mechanisms of gene regulation, cancer evolution, and antibody production. One achievement left a lasting impression on me: the Covid-19 pandemic and related restrictions impacted the work of many, but the IMP largely resumed on-site operations within weeks of the first lockdown. Our campus was quick to fall back on its feet and establish an in-house testing pipeline.

I think this showed two important aspects of the community here: the research prowess and the agility to adapt through innovation.

As a result, IMP faculty has not only been successful in doing excellent research under challenging circumstances, but also in securing external funding and receiving prestigious awards. The IMP's success rate with ERC grants is exceptional. In 2020, for instance, the IMP ranked third among 172 European institutions in ERC success rate. Between 2017 and 2022, faculty received eleven ERC grants, and six more in 2023. Two IMP alumni were awarded a Breakthrough Prize, several faculty members were elected to the European Molecular Biology Organization and other prestigious societies, and many students and postdocs received awards for their accomplishments. Our success wouldn't be possible without Boehringer Ingelheim's continued support. For decades now, their contribution makes them the largest private funder of basic research in Austria year after year. This support has created a unique environment at the IMP that combines the curiosity-driven research questions of academia with the impact-seeking efficiency of a private entity.

In 2017, the IMP moved to a brand-new building. How has this impacted the institute?

Isemann: The new building was designed with three guiding principles in mind: enhanced communication, sustainability, and flexibility to meet future research needs,

and I think all these expectations were fully met. Time and again we hear alumni praising the collaborative spirit of the institute and its partners – I think the airy, open architecture of the building has further added to this mentality. Scientists with very different research questions get to interact and share their work on a daily basis, not only within the IMP, but also with the neighbouring Institute of Molecular Biotechnology, the Gregor Mendel Institute, and the Vienna BioCenter Core Facilities, all linked through a bridge and many shared facilities.

The building was designed for sustainability, with high insulation standards and energy-saving infrastructure. For instance, the air circulation system recovers about 70 percent of heat while filtering out pollutants, germs, and moisture. In recent years, we have implemented additional measures to make the institute more sustainable. We switched to a green electricity provider, saved energy by increasing the temperature of our ultra-low freezers, and reduced work-related flights by 40 percent.

New research groups and technologies have shown the adaptability of the building. For instance, our fish facility is seeing a significant expansion to enable the research of a new group working on the emergence of form and patterns during embryonic development using zebrafish as a model system. The necessary changes to the building might have been impossible in the old one.

How has the IMP's research portfolio changed since 2017?

Peters: Over the past few years, we've been consolidating three areas of research with new recruits and Group Leader promotions: molecular and cell biology, development and regeneration, and immunology and cancer. Anna Obenauf, Johannes Zuber, and Andrea Pauli were promoted to Senior Group Leader positions, strengthening the IMP's basic cancer research and developmental biology portfolio. We've also recruited new Group Leaders to investigate fundamental questions in developmental biology, immunology, and advances in microscopy and super resolution microscopy: Diana Pinheiro, Moritz Gaidt, Joris van der Veeken, Francisco Balzarotti, and David Haselbach. More recently, we were happy to offer David Haselbach a permanent position as Technology Platform Head in 2023, making the IMP a stronghold of the latest technological advances in cryo-electron microscopy.

The IMP also increased its efforts to boost its visibility, backed by a communications department since 2016. Did this yield?

Isemann: The IMP had active media relations since the 1990s, but especially when it comes to recruiting young talent, we felt that other research centres did more than us and we decided to follow. Building on a broad strategy, we relaunched our website in 2017, and made it the central stage for sharing discoveries and honours for our scientists. We also developed our channels: with the recruiting mission in mind, we mainly target a small niche of specialists in terms of their professional background, but these relatively few people are spread around the entire world. Social media have proved extremely valuable to reach them.

Digitisation has also extended to this publication. It is not a direct continuation of research reports that we used to do until 2016, but rather a chronicle of a six-year period. During this time, a lot of people have passed through the institute, and we wanted to include every single one of them. So we decided to split this publication into a print part and a digital part and to connect both sides through multiple links – thereby inviting readers of the printed part to apply their phones to the publication and continue reading it online.

Peters: But not everything that makes us visible in the scientific community is digital. Our excellent training programmes are in extremely high demand, such as the Vienna BioCenter Summer School, which appeals to thousands of students every year; we could increase the number and size of scientific conferences after moving to the new building; and initiatives such as our "rising investigator symposia" for promising junior scientists or the International Birnstiel Award for PhD students in molecular life sciences, which we present annually with the Max Birnstiel Foundation since 2019, have become trademark activities that often put the IMP on the map for young talent in the field.

Zooming out to the entire Vienna BioCenter, which highlights and changes between 2017 and 2022 stand out?

Isemann: A major addition to the Vienna BioCenter is the University of Vienna Biology Building, completed in 2021, which boosted our local community with hundreds of scientists from the university's Centre for Microbiology and Environmental Systems Science and the Faculty of Life

Sciences. This represents a huge extension of the research portfolios at the Vienna BioCenter and increased the number of staff on campus to more than 2800. I think we will all benefit from synergies between more diverse research topics.

Infrastructure, overall, has undergone significant transformations. We have successfully restructured our shared service facilities with IMBA and GMI, establishing a novel infrastructure department and enhanced the collaboration with the campus-wide core facilities. VBCF received a ten-year funding extension from the Science Ministry and the City until 2030, worth 60 million Euro.

These strengthened structures enable us to provide even more effective support to our research groups.

We've also established a new High Performance Computing Platform, CLIP, in collaboration with institutes of the Austrian Academy of Sciences and other partners. This supercomputer cluster was born out of collective expertise, and it accelerates computing speed for 'in silico' methods like DNA sequencing and theoretical modelling. Now, scientists across various fields have easy access to this one-of-a-kind resource, irrespective of their lab location. CLIP's impact spans from life sciences to geology, physics, mathematics, and aerospace research. It's the second largest installation of its kind in the country.

The Scientific Training Unit, shared with IMBA, GMI, and Max Perutz Labs has also expanded its activities, with a growing team and a brand-new, funded postdoctoral programme called VIP2. The Vienna BioCenter Summer School continued in its successful tracks. We attracted very talented students from across the world, and by the end of 2022, we started implementing a new branch of the Summer School, 'Talents for Future', with the goal of attracting students from low- to middle-income countries who previously had no opportunities to work or study abroad.

Generally, the IMP and partner institutes have ramped up efforts to improve the work culture on campus for everybody. A volunteer group for equity, diversity, and inclusion formed among staff and students and organised several events to raise awareness about these topics. The IMP, IMBA, and GMI have also developed an action plan with regards to equity, diversity, and inclusion, with the goal to have all employees and students thrive and feel supported. We have implemented measures to support the mental fitness of all colleagues, for example through in-house coaching. And the Training Unit has developed a leadership training program for principal investigators and department heads, which I think will make our campus a role model for other scientific organisations.

Which challenges have emerged from this period and how do they shape our future?

Peters: Research in molecular life sciences is inevitably linked to technological developments and we strive to stay at the forefront of this ever-changing portfolio. The emergence of artificial intelligence as an everyday tool for research is changing the way we do our work: AlphaFold, which predicts the structure of proteins from their sequence with high accuracy, is just one of many tools that has transformed our research. I can imagine AI will continue to empower innovative research at the IMP and form a new cornerstone of the Vienna BioCenter.

Developments in microscopy have also greatly impacted our work. We can now visualise individual molecules in different conformations and in action at unprecedented scales, for instance with MINFLUX super resolution microscopy or the new Krios G4 microscope set up on campus between 2021 and 2022, and shared with Boehringer Ingelheim.

One of the strengths of the IMP and the general mentality of our employees is the desire to be part of the change and to lead the way into the future of research. We will continue to push the boundaries of knowledge, tackling fundamental mechanistic questions by using our ever-growing toolbox and our most important quality: curiosity.

HIGHLIGHTS

The IMP's scientific achievements are frequently highlighted through publications, awards, grants, and honours. Each of these accomplishments is underscored in specific chapters within this brochure and online.

2017

MARCH The opening ceremony of the new IMP building takes place, attended by the Austrian President Alexander van der Bellen, the CEO of Boehringer Ingelheim Hubertus von Baumbach, and 2020 Nobel laureate Emmanuelle Charpentier.

MAY Manuel Zimmer is selected as one of the 41 HHMI-Wellcome International Research Scholars.

JUNE Elly Tanaka is elected to EMBO membership.

SEPTEMBER Elly Tanaka is presented with the Ernst Schering Prize.

David Haselbach starts his work as an IMP Fellow, investigating macromolecular machines with cryo-electron microscopy.

OCTOBER The new IMP building is celebrated with an opening conference drawing current members and alumni of the institute, Scientific Advisory Board members, and friends of the house.

DECEMBER IMP Emeritus Director Kim Nasmyth is awarded the Breakthrough Prize in Life Sciences, for his work on chromosome segregation partly done at the IMP.

2018

APRIL Clemens Plaschka establishes his lab at the IMP, using cryo-electron microscopy and biochemical methods to study messenger RNA maturation and export.

MAY Manuel Zimmer is elected to EMBO membership.

OCTOBER Manuel Zimmer becomes a professor at the University of Vienna.

NOVEMBER Angelika Amon, one of the first PhD students at the IMP, is awarded the Breakthrough Prize in Life Sciences for her discovery of the consequences of aneuploidy on cell physiology and tumour development made at the MIT.

DECEMBER Elly Tanaka is honoured by the Erwin Schrödinger Award of the Austrian Academy of Sciences.

2019

APRIL Anna Obenauf is elected as a member of the Austrian Academy of Sciences.

The Vienna International Postdoctoral Program (VIP2) is launched. It offers three years of funding for postdoctoral researchers with a background in biology, chemistry, physics, medicine, engineering, computer science, and bioinformatics.

DECEMBER The International Birnstiel Award for Doctoral Research in the Molecular Life Sciences sees its first award ceremony, acknowledging outstanding talent in molecular life sciences. This first call generated 134 nominations from many of the world's leading research institutions.

2020

JANUARY Francisco Balzarotti establishes his lab at the IMP, aiming to develop and apply novel super-resolution microscopy techniques in a high-throughput manner for entire cells and tissue in living conditions.

Johannes Zuber, who has joined the IMP as a Group Leader, is promoted to Senior Group Leader and continues his research on molecular mechanisms of cancer development.

FEBRUARY

The Federation of European Biochemical Societies (FEBS) announces Elly Tanaka as the recipient of the FEBS | Women in Science Award 2020.

MARCH

The Vienna Covid-19 Diagnostics Initiative (VCDI) is formed by an alliance of Vienna BioCenter scientists to develop new and improve existing testing protocols for SARS-CoV-2 assays. This allows the IMP to be back in operation within weeks of the beginning of the first lockdown.

SEPTEMBER

Anna Obenauf joins the project 'Deconstructing the evolution of metastasis' (Evomet), supported by the European Commission.

Meinrad Busslinger is awarded the Preis der Stadt Wien (Prize of the City of Vienna) in the field of mathematics, informatics, science, and technology.

2021

APRIL

Erwin Wagner (Medical University of Vienna), former IMP Senior Group Leader and Deputy Director, is elected to the US' National Academy of Sciences (NAS).

JUNE

Andrea Pauli is elected to EMBO membership.

Luisa Cochella moves to the Johns Hopkins University School of Medicine and continues her research as an Assistant Professor.

JULY

Joris van der Veeken establishes his lab at the IMP, where he aims to study molecular mechanisms underlying the differentiation and function of T cells.

SEPTEMBER

David Keays moves to LMU in Munich and takes up a role as professor and chair of Organismal and Developmental Neurobiology.

2022

FEBRUARY

Moritz Gaidt establishes his lab at the IMP, investigating innate immunology and the molecular mechanisms of inflammation.

The European Association for Cancer Research selects Anna Obenauf's publication on cross-resistance to be one of the ten most impactful cancer publications of the year.

MARCH

The IMP finishes installing a Krios G4 cryo-electron microscope, shared between the IMP and Boehringer Ingelheim. Its inauguration is celebrated with a keynote lecture by cryo-electron microscopy pioneer and Nobel laureate Richard Henderson.

MAY

Elly Tanaka becomes a full member of the Austrian Academy of Sciences. IMP alumnus Erwin Wagner is made honorary member.

JULY

Johannes Zuber is elected to EMBO membership.

Anna Obenauf's research in metastasis and cancer drug resistance is recognised with the AAAS Martin and Rose Wachtel Cancer Research Award.

SEPTEMBER

Wulf Haubensak leaves the IMP and joins the Medical University of Vienna as a professor.

OCTOBER

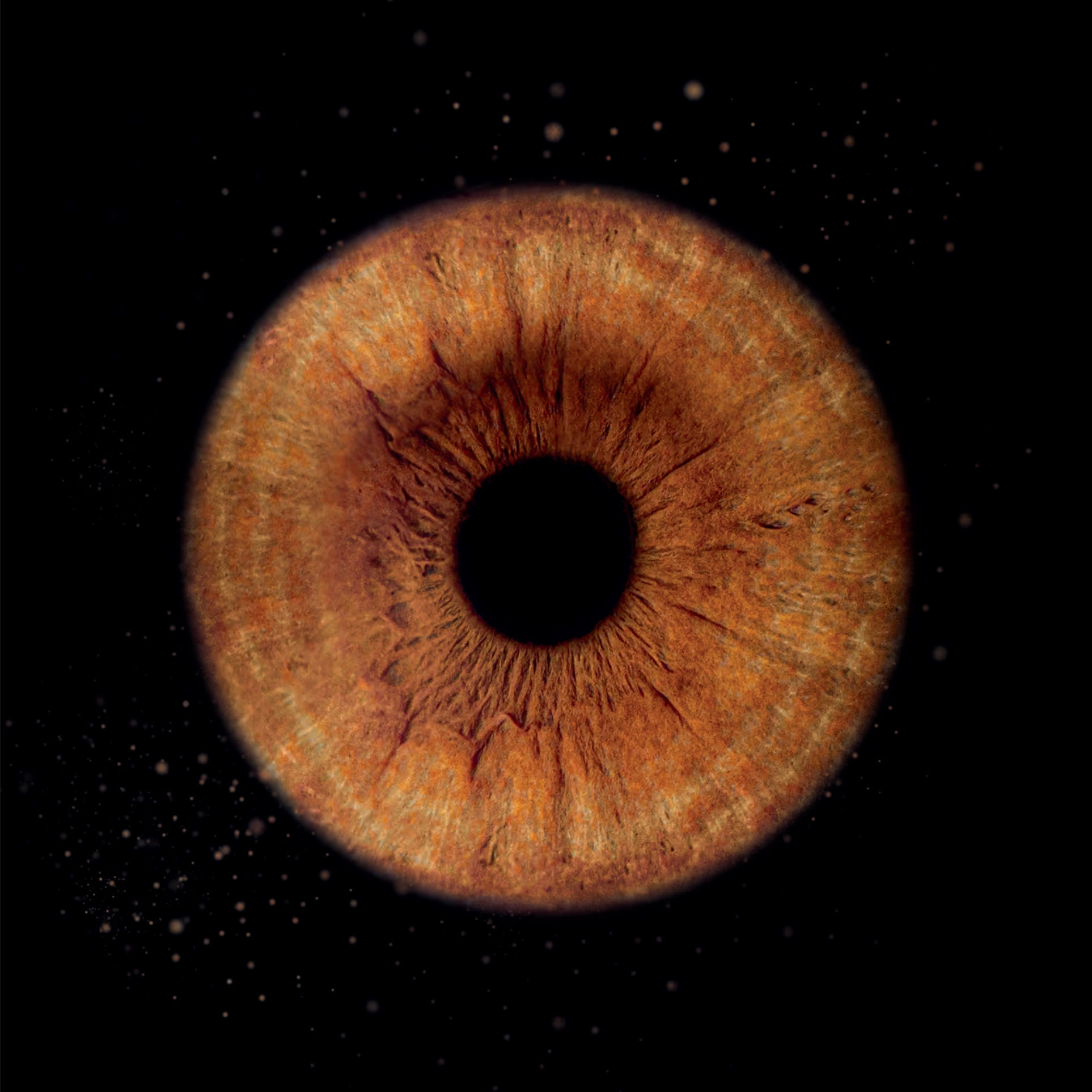
Anna Obenauf and Andrea Pauli are promoted to Senior Group Leaders.

Diana Pinheiro establishes her lab at the IMP to investigate the biophysical mechanisms responsible for the development of embryonic patterns and forms.

Research

FRANCISCO BALZAROTTI

How can we advance super-resolution microscopy using a multidisciplinary approach?



BALZAROTTI LAB 2020–2022

How can we advance super-resolution microscopy using a multidisciplinary approach?

How can light-based methods provide information on biological processes? What are the limits to the information we can extract? How deep and fast can we look? How does extreme resolution answer concrete questions? Recent developments in novel optical methods reveal that these questions remain unexplored or incomplete. By blending expertise from various fields such as optics, electronics, statistics, chemistry, and biology, the Balzarotti lab is pushing the boundaries of light microscopy, striving to solve transverse methodological challenges and profoundly influence life sciences along the way.

Fluorescence microscopy is an invaluable tool for exploring the structure and function of biological processes. It provides high specificity and contrast for the observation of cellular components such as DNA, RNA, proteins, or lipids tagged with fluorescent molecules in a minimally invasive fashion, even allowing live studies. The spatial resolution of classical fluorescence microscopy is limited to hundreds of nanometres due to the diffraction of light; however, resolutions of up to the tens of nanometres were unlocked with the development of 'super-resolution' methodologies, granting its developers the 2014 Nobel Prize in Chemistry.

Precise localisation with MINFLUX nanoscopy

Despite this revolution, attaining precisions in the single-nanometre range or time resolutions below the millisecond remained elusive. To overcome these limitations, Francisco Balzarotti developed a novel optical method during his postdoctoral work: maximally informative luminescence excitation, or 'MINFLUX' (1). This methodology merges elements of information theory with established tools in single-molecule studies, such as induced sparsity/blinking, and beam-shape engineering. MINFLUX is a single-molecule localisation strategy that uses multiple sequential measurements to determine its exact location. These measurements are performed with a beam of light that has a specific shape and contains intensity zero. Compared to classic alternatives, it utilises ten-fold fewer photons. This efficiency can be harvested towards increasing the temporal resolution in tracking (2) or spatial resolution in imaging (3).

A novel scanning technology

To provide 3D isotropic performance using a single objective lens, MINFLUX requires fast axial beam scanning, a stringent requirement that current technologies fail to satisfy. Previously, an electro-optical lens was used to this end, but it had significant drawbacks including degradation, aberrations, temperature sensitivity, and high cost, limiting microscope performance and lifespan. The Balzarotti lab

FRANCISCO BALZAROTTI

PHD:
UNIVERSITY OF BUENOS AIRES, ARGENTINA (2012)

POSTDOCTORAL RESEARCH:
MAX PLANCK INSTITUTE FOR BIOPHYSICAL CHEMISTRY, GERMANY

GROUP LEADER:
IMP, VIENNA (2020)

GROUP MEMBERS:

has developed an alternative technology that eliminates these limitations and is long-lived, at a fraction of the cost. This system utilises interferometric principles to convert electric field phase modulations into axial displacements.

Multi-emitter localisation: single-molecule tracking on steroids

Current state-of-the-art fluorescence resonance energy transfer (FRET) experiments retrieve conformational changes with limitations on the number of distances that can be probed (normally one) and how far away fluorescent probes can be. This limits their use when studying large macromolecular complexes with multiple degrees of freedom. The Balzarotti lab is tackling this limitation by developing a multi-emitter MINFLUX tracking scheme that incorporates fluorescence lifetime, spectra, and a pixelated detector. This will enable the tracking of up to four molecules simultaneously in 3D with a resolution of less than three nanometres and less than five milliseconds *in vitro* assays. This development has the potential to greatly impact fields that rely on information about conformational changes in macromolecular complexes.

Super-resolution techniques via DNA multiplexing

Labelling strategies and fluorescent probes directly affect completeness and resolution in super-resolution microscopy. DNA-PAINT is a labelling strategy that overcomes the scarcity of single-molecule emission and enables multiplexing capabilities. Targets are labelled with short DNA strands (7 nt–9 nt), which transiently hybridise (50 ms–300 ms) with complementary strands (imagers) with a fluorophore, giving rise to a blinking behaviour.

The Balzarotti lab has expanded MINFLUX so it is tailored to a recent fluorogenic DNA-PAINT variant for multiplexing the observation of 5–15 targets, without the need for fluidic exchanges. By systematically designing target and imager strands following binary coding, it is possible to use a few distinct fluorescent molecules (N) to distinguish multiple targets (2^N–1). With the resolution power of MINFLUX and advanced detection technologies, this system is expected to have broad applications and be the starting point for genome tracing at the nanoscale.

Outlook

In conclusion, MINFLUX is a powerful tool for single-molecule studies that is gaining popularity among research groups worldwide. The Balzarotti lab is dedicated to being at the forefront of technology development and, with the resources available at the IMP and the Vienna BioCenter, the lab has access to unmatched tools for observing single-molecule phenomena. With powerful studies on topics such as protein conformation dynamics, protein complex assembly and stoichiometry, and transport and motility, the lab looks forward to further advancing its understanding in these areas through light microscopy tools.

SELECTED PUBLICATIONS

1.
Balzarotti, F., Eilers, Y., Gwosch, K. C., Gynnå, A. H., Westphal, V., Stefani, F. D., Elf, J. and Hell, S. W. (2017). ***Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes***. *Science,* 355, 606–612. doi: 10.1126/science.aak9913.

2.
Eilers, Y., Ta, H., Gwosch, K. C., Balzarotti, F. and Hell, S. W. (2018). ***MINFLUX monitors rapid molecular jumps with superior spatiotemporal resolution***. *Proceedings of the National Academy of Sciences of the United States of America,* 115(24), 6117–6122. doi: 10.1073/pnas.1801672115.

3.
Gwosch, K. C., Pape, J. K., Balzarotti, F., Hoess, P., Ellenberg, J. and Ries, J. (2020). ***MINFLUX nanoscopy delivers 3D multicolor nanometer resolution in cells***. *Nature Methods,* 1–8. doi: 10.1038/s41592-019-0688-0.

MEINRAD BUSSLINGER

How do transcription factors control B cell development in health and disease?

BUSSLINGER LAB 2017–2022

How do transcription factors control B cell development in health and disease?

B cell immunity provides protection against infections through the generation and secretion of high-affinity antibodies that recognise an almost unlimited diversity of pathogens. This enormous adaptive potential of B cells is generated through V(D)J recombination of the immunoglobulin heavy-chain (*Igh*) and light-chain (*Igk* and *Igl*) genes during early B cell development. On the dark side, B cell tumours are frequently generated by misguided recombination or mutation of critical transcription factor genes. The focus of the Busslinger lab has been to investigate how transcription factors contribute to immunoglobulin gene recombination, B cell development, and disease.

Generating a broad antibody repertoire

The 195 V_H genes of the *Igh* locus are spread over a 2.44-megabase region. To undergo V(D)J recombination in pro-B cells, they need to come in close contact with the DJ_H-rearranged gene segment. The transcription factor Pax5 mediates the contraction of the Igh locus for V_H-to-DJ_H recombination to take place.

In collaboration with the Peters lab, the Busslinger lab recently demonstrated that *Igh* locus contraction is caused by extended chromatin loop extrusion across the entire *Igh* locus, which facilitates the alignment of V_H genes with the DJ_H-rearranged gene segment prior to recombination. Loop extrusion is facilitated by the protein complex cohesin. Pax5 mediates this process by down-regulating the expression of the cohesin-release factor Wapl by binding to and repressing the *Wapl* promoter (1). Reduced Wapl expression causes global alterations of the chromosome architecture. This indicates that the potential to generate a broad antibody repertoire by recombining all V_H genes entails structural changes of the entire genome in pro-B cells.

Transcriptional regulation of B cell development and function

The Busslinger lab discovered 23 years ago that Pax5 acts as the B-lineage commitment factor at the onset of B cell development. Subsequent studies revealed that Pax5 also suppresses tumour formation in the B cell lineage and is required for maintaining the identity of mature B cells. More recently, the lab demonstrated that all mature B cells depend on Pax5: it promotes PI3K signalling – a pathway that enhances B cell survival and proliferation – via downregulation of a repressor of this pathway, called PTEN (2).

By investigating transcription factors involved in the differentiation of antibody-secreting plasma cells, the Busslinger lab found that the transcription factor Blimp1

MEINRAD BUSSLINGER

PHD:
UNIVERSITY OF ZURICH, SWITZERLAND (1980)

POSTDOCTORAL RESEARCH:
MRC, LONDON, UK

JUNIOR GROUP LEADER:
UNIVERSITY OF ZURICH
(1983–1987)

SENIOR GROUP LEADER:
IMP, VIENNA (1987)

DIRECTOR OF ACADEMIC AFFAIRS: IMP, VIENNA
(2007)

SCIENTIFIC DEPUTY DIRECTOR: IMP, VIENNA
(2013)

GROUP MEMBERS:

represses the B cell gene expression program in plasma cells and activates immunoglobulin expression and secretion. Moreover, the lab discovered that the proteins E2A and E2-2 are also essential regulators of plasma cell differentiation: they induce Blimp1 expression and activate *Igh* and *Igk* enhancers. Finally, the transcription factor Bhlhe41 was shown to control the development, self-renewal, and specific B cell receptor repertoire of the innate-like B-1a cells, which provide a first line of defence against pathogens (3).

Transcriptional control of B cell diseases

The transcription factor Ikaros is essential for the initiation of B cell development. Recently, the Busslinger lab demonstrated that conditional inactivation of Ikaros in mature B cells results in systemic autoimmunity (4). Detailed analysis revealed that a deficiency in Ikaros leads to loss of the tolerance of B cells to self-antigens and to hyperreactive Toll-like receptor signalling. These results suggest that Ikaros acts as a guardian to prevent autoimmunity.

Consistent with these findings, heterozygous *IKAROS* mutations were identified in patients with autoimmunity. While *PAX5* was identified as a tumour suppressor gene in one-third of all human acute B lymphoblastic leukaemia (B-ALL) cases, a smaller B-ALL subset carries *PAX5* translocations involving a variety of partner genes. This results in novel chimeric PAX5 transcription factors that may promote the development of tumours.

By generating mouse models for the *PAX5-ETV6* and *PAX5-JAK2* translocations, the Busslinger lab showed that the PAX5-ETV6 protein supports B-ALL development, together with loss of the tumour suppressor gene *Cdkna2a/b*. In contrast, the *PAX5-JAK2* translocation acts as a dual-hit mutation that promotes aggressive B-ALL via nuclear activation of the transcription factor STAT5.

PAX5 mutations and autism spectrum disorder

The *Pax5* gene is also expressed at the midbrain-hindbrain boundary in the mouse embryo, which is an organising centre that induces the development of the midbrain and cerebellum. At the Erasmus Medical Center in Rotterdam, scientists identified a patient with hypogammaglobulinemia and autism spectrum disorder (ASD) who carries biallelic PAX5 mutations.

In collaboration with researchers at the Erasmus Medical Center, the Busslinger lab generated and analysed a patient-specific *Pax5* mutant mouse. The mouse model had an early B cell developmental block – explaining the patient's hypogammaglobulinemia – and exhibited autistic-like behaviours. Pax5 deficiency furthermore caused extensive hypoplasia of the substantia nigra and ventral tegmental area due to the loss of inhibitory GABAergic neurons, thus affecting these two midbrain hubs that control motor function and reward processing, respectively (5). These findings identified a causal role for *PAX5* mutations in the generation of a monogenic form of ASD, which is consistent with the reported association of heterozygous *PAX5* mutations in a few ASD individuals.

SELECTED PUBLICATIONS

1.
Hill, L., Ebert, A., Jaritz, M., Wutz, G., Nagasaka, K., Tagoh, H., Kostanova-Poliakova, D., Schindler, K., Sun, Q., Bönelt, P., Fischer, M., Peters, J. M., and Busslinger, M. (2020). ***Wapl repression by Pax5 promotes V gene recombination by Igh loop extrusion.*** *Nature,* 584, 142-147. doi: 10.1038/s41586-020-2454-y. Epub 2020 Jul 1.

2.
Calderón, L., Schindler, K., Malin, S.G., Schebesta, A., Sun, Q., Schwickert, T., Alberti, C., Fischer, M., Jaritz, M., Tagoh, H., Ebert, A., Minnich, M., Liston, A., Cochella, L., and Busslinger, M. (2021). ***Pax5 regulates B cell immunity by promoting PI3K signaling via PTEN down-regulation.*** *Science Immunology,* 6, eabg 5003. doi: 10.1126/sciimmunol.abg5003

3.
Kreslavsky, T., Vilagos, B., Tagoh, H., Kostanova Poliakova, D., Schwickert, T., Wöhner, M., Jaritz, M., Weiss, S., Taneja, R., Rossner, M.J., and Busslinger, M. (2017). ***Essential role for the transcription factor Bhlhe41 in regulating the development, self-renewal and BCR repertoire of B-1a cells.*** *Nature Immunology,* 18, 442–455. doi: 10.1038/ni.3694. Epub 2017 Feb 27.

4.
Schwickert, T.A., Tagoh, H., Schindler, K., Fischer, M., Jaritz, M., and Busslinger, M. (2019). ***Ikaros prevents autoimmunity by controlling anergy and Toll-like receptor signaling in B cells.*** *Nature Immunology,* 20, 1517–1529. doi: 10.1038/s41590-019-0490-2. Epub 2019 Oct 7.

5.
Kaiser, F.M.P., Gruenbacher, S., Roa Oyaga, M., Nio, E., Jaritz, M., Sun, Q., van der Zwaag, W., Kreidl, E., Zopf, L.M., Dalm, V.A.S.H., Pel, J., Gaiser, C., van der Vliet, R., Wahl, L., Rietman, A., Hill, L., Leca, I., Driessen, G., Laffeber, C., Brooks, A., Katsikis, P.D., Lebbink, J.H.G., Tachibana, K., van der Burg, M., De Zeeuw, C.I., Aleksandra Badura, A., and Busslinger, M. (2022). ***Biallelic PAX5 mutations cause hypogammaglobulinemia, sensorimotor deficits and autism spectrum disorder.*** *Journal of Experimental Medicine,* 219, e20220498. doi: 10.1084/jem.20220498. Epub 2022 Aug 10.

TIM CLAUSEN

Can we use the cellular protein degradation system to eliminate disease targets?

CLAUSEN LAB 2017–2022

Can we use the cellular protein degradation system to eliminate disease targets?

All cells use a series of quality-control checkpoints to maintain the functionality of their proteins. To understand the mechanistic details of these cellular safeguards, the Clausen lab employs an integrative structural and cell biology approach. The lab aims to develop strategies against misbehaving proteins connected with neurodegenerative diseases, myopathic disorders, cancer, and ageing. After deciphering key differences in the protein degradation pathways between bacterial pathogens and their hosts, the Clausen lab pioneered small-molecule degraders, called BacPROTACs, as new antibiotics, to reprogramme the bacterial degradation system.

Targeted protein degradation in bacteria

In bacteria, the protein modification phospho-arginine (pArg) serves as a ubiquitin-like degradation signal, targeting aberrant proteins to the ClpCP protease. The Clausen lab discovered this pathway and determined the structure and mechanism of the arginine kinase McsB that carries out the degradation labelling (1). The lab also uncovered the mechanism keeping the pArg labeller under control: proteotoxic stress activates McsB kinase, transforming an inactive 'caged' oligomer into active 'open' forms with elevated kinase activity. This data showed that the degradation labelling in bacteria is as carefully controlled as in eukaryotes. Moreover, the identification of distinct pArg reader domains among protein quality-control (PQC) factors points to a remarkably complex signalling network, challenging simplistic views of bacterial protein phosphorylation.

Having elucidated key differences to eukaryotic degradation pathways, the lab set out to develop antibiotics, selectively disturbing the bacterial system. Arguably, the most exciting field in medicinal chemistry comprises 'molecular degraders', enabling specific elimination of neo-substrates. Despite its pharmaceutical promise, degrader approaches are so far limited to eukaryotes. To overcome this, the Clausen lab developed 'BacPROTACs' active in bacteria (2). The bi-functional molecules tether substrates to the bacterial proteasome, ClpCP, inducing their degradation. In addition to pioneering a platform for antibioticdevelopment, the lab used BacPROTACs to characterise super-assemblies of ClpCP complexes, revealing a new compartmentalisation principle within the bacterial PQC system.

The ubiquitin-proteasome system in health and disease

Ubiquitin signalling is critical to mark specific client proteins for proteasomal degradation. The central factors in this process are E3 ubiquitin ligases, a diverse protein family

TIM CLAUSEN

PHD:
TECHNICAL UNIVERSITY MUNICH, GERMANY
(1997)

POSTDOCTORAL RESEARCH:
MPI OF BIOCHEMISTRY, MARTINSRIED, GERMANY

GROUP LEADER:
MPI OF BIOCHEMISTRY, MARTINSRIED, GERMANY
(1999)

GROUP LEADER:
IMP, VIENNA (2002)

SENIOR GROUP LEADER:
IMP, VIENNA (2009)

GROUP MEMBERS:

with more than 1,000 members. How E3 enzymes select the right substrate at the right time is a matter of intense research. The Clausen lab studies giant ubiquitin ligases implicated in innate immunity and cancer. Aside from their biological impact, the studied factors are intricate molecular machines that have extraordinary molecular complexity and operate by nonconventional mechanisms:

i. RNF213 is a puzzling E3 giant of 600 kilodaltons connected with lipid metabolism, hypoxia, and innate immunity. The lab determined its cryoEM structure and addressed the functional connection of its ATPase and E3 modules (3). Strikingly, ubiquitination activity is regulated by ATP binding, enabling RNF213 to act as a metabolic sensor in the cell. Using activity-based probes, the lab visualised its unique ubiquitin-transfer mechanism in molecular detail. The boosted yet promiscuous reactivity is used to target non-protein substrates, a distinct E3 function critical for cell-autonomous defence: RNF213 is able to mark bacterial lipids, inducing autophagic clearance of microbial invaders.

ii. HUWE1 is a quality-control E3 ligase targeting orphan proteins that may result from proteome imbalances. Structural and functional data revealed the coupling of E3 activity and substrate binding, and delineated a multi-site docking platform for targeting diverse substrates (4).

iii. BIRC6 is an E2/E3 hybrid enzyme of 1.1 megadaltons that targets apoptotic proteases (HtrA2, caspases) as well as the autophagy factor Atg8 for proteasomal degradation. New cryoEM data provides the first mechanistic insights into how BIRC6 coordinates autophagy and apoptosis pathways upon mitochondrial damage.

Myosin proteostasis

UNC-45 is a chaperone channelling muscle myosin in protein folding and degradation pathways. Reconstitution of complexes with irreversibly damaged and folding competent myosin and structural data of a minimal chaperone-substrate complex revealed how UNC-45 functions as a hub in myosin quality control (5). Remarkably, site-specific mutations disrupting the identified UNC-45:myosin interface are connected with severe myopathies, unveiling a novel point of intervention. A better understanding of the mechanisms of myosin proteostasis will help address protein misfolding defects underlying skeletal and cardiac muscle diseases.

Outlook

Future studies aim to further explore the 'dark' side of ubiquitin signalling, linked to non-conventional E3 enzymes and the ubiquitination of non-protein substrates. Aside from exciting mechanistic insights, these studies are highly relevant to better understand the cellular defence against bacterial and viral invaders. Expected findings will guide the development of tailored degrader technologies, combatting pathogens at the site of infection.

SELECTED PUBLICATIONS

1.
Suskiewicz, M. J., Hajdusits, B., Beveridge, R., Heuck, A., Vu, L. D., Kurzbauer, R., Hauer, K., Thoeny, V., Rumpel, K., Mechtler, K., Meinhart, A., and Clausen, T. (2019). ***Structure of McsB, a protein kinase for regulated arginine phosphorylation.*** *Nature Chemical Biology,* 15(5), 510–518. doi: 10.1038/s41589-019-0265-y. Epub 2019 Apr 8.

2.
Morreale, F. E., Kleine, S., Leodolter, J., Junker, S., Hoi, D. M., Ovchinnikov, S., Okun, A., Kley, J., Kurzbauer, R., Junk, L., Guha, S., Podlesainski, D., Kazmaier, U., Boehmelt, G., Weinstabl, H., Rumpel, K., Schmiedel, V. M., Hartl, M., Haselbach, D., Meinhart, A., Kaiser, M., and Clausen, T. (2022). ***BacPROTACs mediate targeted protein degradation in bacteria.*** *Cell*, 185(13), 2338–2353.e18. doi: 10.1016/j.cell.2022.05.009. Epub 2022 Jun 3.

3.
Ahel, J., Lehner, A., Vogel, A., Schleiffer, A., Meinhart, A., Haselbach, D., and Clausen, T. (2020). ***Moyamoya disease factor RNF213 is a giant E3 ligase with a dynein-like core and a distinct ubiquitin-transfer mechanism.*** *eLife,* 9, e56185. doi: 10.7554/eLife.56185.

4.
Grabarczyk, D. B., Petrova, O. A., Deszcz, L., Kurzbauer, R., Murphy, P., Ahel, J., Vogel, A., Gogova, R., Faas, V., Kordic, D., Schleiffer, A., Meinhart, A., Imre, R., Lehner, A., Neuhold, J., Bader, G., Stolt-Bergner, P., Böttcher, J., Wolkerstorfer, B., Fischer, G., Grishkovskaya, I., Haselbach, D., Kessler, D., and Clausen, T. (2021). ***HUWE1 employs a giant substrate-binding ring to feed and regulate its HECT E3 domain.*** *Nature Chemical Biology*, 17(10), 1084–1092. doi: 10.1038/s41589-021-00831-5. Epub 2021 Jul 22.

5.
Hellerschmied, D., Lehner, A., Franicevic, N., Arnese, R., Johnson, C., Vogel, A., Meinhart, A., Kurzbauer, R., Deszcz, L., Gazda, L., Geeves, M., and Clausen, T. (2019). ***Molecular features of the UNC-45 chaperone critical for binding and folding muscle myosin.*** *Nature Communications,* 10(1), 4781. doi: 10.1038/s41467-019-12667-8.

LUISA COCHELLA

What are the transcriptional and post-transcriptional mechanisms behind cell diversification?

COCHELLA LAB 2017–2021

What are the transcriptional and post-transcriptional mechanisms behind cell diversification?

The evolution of multicellular life forms occurred hand in hand with cell type specialisation. But while this functional segregation enabled astounding animal complexity, extreme specialisations often leave cells vulnerable to genetic or environmental variations. For example, the physiology of some neurons or muscle cells makes them particularly susceptible to mutations in broadly expressed genes. Thus, understanding how cells diversify and what makes them unique is important for understanding animal physiology in health and disease. Research in the Cochella lab focuses on the gene-regulatory mechanisms that underlie this cell diversification.

Roles of microRNAs in animal development

Repression is a recurrent mechanism for imposing spatial and temporal boundaries on biological processes. MicroRNAs form a class of post-transcriptional repressors, which play essential roles in development. The Cochella lab has used *C. elegans* to study the contribution of miRNAs to shaping cellular diversity, providing three new concepts in the following areas:

i. miRNA profiling

The Cochella lab, together with researchers from the Ameres lab at the Max Perutz Labs in Vienna, developed a novel approach to sequencing mature miRNAs. Their 'mime-seq' technique sequences miRNAs from individual cell types within a complex cell mix, without cell sorting or biochemical purification (1). It relies on a plant enzyme, HEN1, that methylates plant miRNAs. As animal miRNAs are not normally methylated, cell-specific expression of HEN1 provides a way to label miRNAs in cells of interest. Mime-seq is robust, specific, and sensitive, and applies to *C. elegans* and *Drosophila*. The Cochella lab uses mime-seq and a deconvolution strategy to generate an atlas of miRNA expression for the complete *C. elegans* nervous system. Also, together with the Ameres and Busslinger labs, they have expanded the use of mime-seq to mice.

ii. miRNA functions at the organismal level

By extensively profiling miRNAs using reporters and mime-seq, the Cochella lab revealed a natural classification of miRNAs in embryogenesis. A minority of miRNAs is broadly expressed in the early embryo, while the majority is expressed in one or a few cell types with onset in late embryos. The lab found that two conserved, early, and broadly expressed miRNA families (miR-35 and -51) are sufficient for early embryonic development in *C. elegans*, in the absence of all other miRNAs (2). In contrast, most other miRNAs play roles during terminal differentiation or function of specialised cells.

LUISA COCHELLA

PHD:
JOHNS HOPKINS SCHOOL OF MEDICINE, BALTIMORE, USA (2006)

POSTDOCTORAL RESEARCH:
COLUMBIA UNIVERSITY, NEW YORK, USA

GROUP LEADER:
IMP, VIENNA (2013)

GROUP MEMBERS:

iii. miRNA functions at the molecular and cellular levels

The Cochella lab has studied two miRNAs with high cell-type specificity. The lab discovered that miR-791 is present exclusively in the CO2-sensing neurons of *C. elegans* and is required for their function (Drexel *et al.*, 2016). The Cochella lab also studied miR-1, a conserved muscle miRNA that is necessary for muscle development and function. They identified the functionally relevant targets for each miRNA (3). Both act by repressing otherwise ubiquitously transcribed genes, revealing that miRNAs support the function of specialised cells by carving out the specificity of typically considered housekeeping genes.

A novel transcriptional strategy for neuronal diversity

Transcription factors (TFs) play crucial roles in the expression of different terminal gene sets, typically acting in a combinatorial manner. Such use of TFs is a widespread strategy for specifying distinct cell types.

Most cases of combinatorial activity represent spatial intersections, in which two or more TFs are co-expressed and jointly required to activate the transcription of a given locus. However, while studying the development of two sensory neurons in *C. elegans*, the Cochella lab discovered a novel type of combinatorial activity that they termed temporal intersection (Cochella & Hobert, 2012).

The lab recently dissected the molecular mechanism behind this (4) and showed that transient binding of an early TF leaves a 'memory' of activation that can then be boosted by a later-acting TF. This relies on changes in chromatin states and TF binding abilities that the lab is now unravelling using newly tailored means of manipulation and molecular readouts. Because cells are specified through progenitors that go through different transcriptional states, it is possible to have a vast number of TF combinations over time. This may contribute to diversifying cell types during development. In addition, this mechanism has important implications for current efforts to generate specific cell types *in vitro*.

COVID-19 genome sequencing

At the outset of the COVID-19 pandemic, several groups at the Vienna BioCenter initiated efforts to improve diagnostic capacity at the national and international levels. The Cochella lab teamed up with Ulrich Elling from the Institute of Molecular Biotechnology and Alexander Stark from the IMP to develop SARSeq, a high throughput sequencing-based method for SARS-CoV-2 detection (5) and variant identification (Özkan *et al.*, 2021). This method was used to monitor COVID-19 variants for the Austrian Government, a project that was co-led by Luisa Cochella until the end of June 2021.

Outlook

In July 2021, the Cochella lab moved to the Department of Molecular Biology and Genetics at the Johns Hopkins School of Medicine.

SELECTED PUBLICATIONS

1.
Alberti, C., Manzenreither, R. A., Sowemimo, I., Burkard, T. R., Wang, J., Mahofsky, K., Ameres, S. L., and Cochella, L. (2018). ***Cell-type specific sequencing of microRNAs from complex animal tissues.*** *Nature Methods,* 15(4), 283–289. doi: 10.1038/nmeth.4610. Epub 2018 Feb 26.

2.
Dexheimer, P. J., Wang, J., and Cochella, L. (2020). ***Two microRNAs are sufficient for embryonic patterning in C. elegans.*** *Current Biology,* 30(24), 5058–5065. doi: 10.1016/j.cub.2020.09.066. Epub 2020 Oct 29.

3.
Gutiérrez-Pérez, P., Santillán, E. M., Lendl, T., Wang, J., Schrempf, A., Steinacker, T. L., Asparuhova, M., Brandstetter, M., Haselbach, D., and Cochella, L. (2021). ***miR-1 sustains muscle physiology by controlling V-ATPase complex assembly.*** *Science Advances,* 7(42), eabh1434. doi: 10.1126/sciadv.abh1434. Epub 2021 Oct 15.

4.
Charest, J., Daniele, T., Wang, J., Bykov, A., Mandlbauer, A., Asparuhova, M., Röhsner, J., Gutiérrez-Pérez, P., and Cochella, L. (2020). ***Combinatorial action of temporally segregated transcription factors.*** *Developmental Cell,* 55(4), 483–499. doi: 10.1016/j.devcel.2020.09.002. Epub 2020 Sep 30.

5.
Yelagandula, R., Bykov, A., Vogt, A., Heinen, R., Özkan, E., Strobl, M. M., Baar, J. C., Uzunova, K., Hajdusits, B., Kordic, D., Suljic, E., Kurtovic-Kozaric, A., Izetbegovic, S., Schaeffer, J., Hufnagl, P., Zoufaly, A., Seitz, T., VCDI, Födinger, M., Allerberger, F., Stark, A., Cochella, L., and Elling, U. (2021). ***Multiplexed detection of SARS-CoV-2 and other respiratory infections in high throughput by SARSeq.*** *Nature Communications,* 12(1), 3132. doi: 10.1038/s41467-021-22664-5

MORITZ GAIDT

How does the innate immune system recognise pathogens and induce inflammation?

GAIDT LAB 2022

How does the innate immune system recognise pathogens and induce inflammation?

All organisms must defend themselves against pathogens. They use the innate immune system to detect foreign substances, induce inflammation, and fight intruders. While inflammation is critical to protecting against infections, activating this immune response in the absence of pathogens can cause disease. Thus, the decision to induce inflammation must be made with precision and fidelity. The Gaidt lab aims to understand the highly regulated molecular events of pathogen recognition and subsequent inflammation in human innate immune cells.

Generating a broad antibody repertoire

The primary mode of pathogen recognition by innate immune systems relies on detecting microbe-specific molecules by immune receptors. Sensed molecules are microbe-specific, so identifying them provides a means to detect 'non-self'. Bacterial lipopolysaccharide is one of these non-self molecules detected by our immune system. Moritz Gaidt and his co-authors have discovered one way that lipopolysaccharide recognition induces inflammation and have genetically characterised an alternative, highly pro-inflammatory immune pathway in human immune cells (1).

Another pathogen-derived molecule that is sensed by the immune system is microbial DNA. While self-DNA is stored in the nucleus and mitochondria, microbial DNA is often found in the cytosol – the replication compartment of many pathogens. The immune system specifically senses DNA in the cytosol to detect microbial DNA and not endogenous self-DNA. As part of his doctoral work, Gaidt has discovered how recognition of cytosolic DNA activates immunity by triggering a highly inflammatory immune pathway called the inflammasome, which culminates in inflammatory cell death and activates inflammatory cytokines (2).

Detecting virulent intruders through 'self-guarding'

While detecting non-self is a powerful tool of the immune system to identify microbes, this strategy cannot determine if the microbe is a pathogen or a harmless commensal organism. To detect virulent pathogens and adjust the strength of inflammation to the threat level, the immune system recognises pathogenic activities of intruders. Specifically, the immune system senses the activities of so-called virulence factors that pathogens use to inhibit immune defences. The 'guard hypothesis' proposes that the immune system monitors, or guards, host pathways ('guardees') that are prone to be inhibited by virulence factors.

MORITZ GAIDT

PHD:
UNIVERSITY OF BONN, GERMANY (2017)

POSTDOC:
UNIVERSITY OF CALIFORNIA BERKELEY, UNITED STATES

GROUP LEADER:
IMP, VIENNA (2022)

Guardee disruption activates receptors, or 'guards', that induce inflammation. During his postdoc, Gaidt described a novel mammalian immune pathway that recognises the virulence-associated activity of viruses (3). It involves the bi-functional protein MORC3.

MORC3's primary function directly restricts the replication of viruses. Pathogenic viruses employ virulence factors that degrade MORC3 to restore viral transcription and replication. However, MORC3 has a secondary role in repressing a DNA element that Gaidt and his colleagues called MRE (MORC3-regulated element). On loss of MORC3, the MRE potently activates the immune system to drive inflammation and immunity. MORC3's bifunctionality forces the pathogen to 'pick its poison': either the pathogen is restricted by its primary anti-viral activity or, if the pathogen interferes with this activity, a secondary inflammatory response is unleashed. Importantly, there is no dedicated guard protein involved in the MORC3-MRE pathway. Instead, the loss of MORC3's secondary function triggers inflammation. Gaidt and his colleagues have coined the term 'self-guarding' to describe this connection.

Outlook

Opened in 2022, the Gaidt lab aims to understand the highly regulated molecular events of pathogen recognition and subsequent induction of inflammation in human innate immune cells. They hypothesise that human immune cells have a broad ability to sense the activities of virulent pathogens. Firstly, they plan to use functional genomics and forward genetics to identify sensed pathogenic activities and genetically define their sensing pathways. Secondly, they hope to design therapeutic approaches inhibiting or activating inflammatory pathways in diseases of misregulated inflammation, including cancer.

GROUP MEMBERS:

SELECTED PUBLICATIONS

1.
Gaidt, M. M., Ebert, T. S., Chauhan, D., Schmidt, T., Schmid-Burgk, J. L., Rapino, F., Robertson, A. A., Cooper, M. A., Graf, T., and Hornung, V. (2016). ***Human monocytes engage an alternative inflammasome pathway. Immunity***, 44(4), 833–846. doi: 10.1016/j.immuni.2016.01.012. Epub 2016 Mar 29.

2.
Gaidt, M. M., Ebert, T. S., Chauhan, D., Ramshorn, K., Pinci, F., Zuber, S., O'Duill, F., Schmid-Burgk, J. L., Hoss, F., Buhmann, R., Wittmann, G., Latz, E., Subklewe, M., and Hornung, V. (2017). ***The DNA inflammasome in human myeloid cells is initiated by a STING-cell death program upstream of NLRP3.*** *Cell*, 171(5), 1110–1124. doi: 10.1016/j.cell.2017.09.039. Epub 2017 Oct 12.

3.
Gaidt, M. M., Morrow, A., Fairgrieve, M. R., Karr, J. P., Yosef, N., and Vance, R. E. (2021). ***Self-guarding of MORC3 enables virulence factor-triggered immunity.*** *Nature*, 600(7887), 138–142. doi: 10.1038/s41586-021-04054-5. Epub 2021 Nov 10.

DAVID HASELBACH

How are molecular machines designed and produced?

HASELBACH LAB 2017–2022

How are molecular machines designed and produced?

Molecular machines harness thermal energy from the environment to produce mechanical and chemical work. Which design principles enable these machines to perform their complex tasks, however, remains elusive. To understand these principles, the Haselbach lab uses structural and biophysical methods to analyse the naturally occurring specialisation, heterogeneity, and disease states of such machines. The lab's research focuses on highly essential and adaptable machines involved in protein homeostasis.

Cellular homeostasis depends on an equilibrium of protein synthesis and degradation. By fine-tuning individual rates within these processes, cells precisely control the level of each protein species. The ubiquitin-proteasome system (UPS) is at the centre of targeted protein degradation and the proteasome is the leading actor.

The proteasome is an astonishingly complex machine with a broad spectrum of protein substrates, ranging from small peptides to multimeric complexes. However, it must treat each substrate differently to shape the proteome and adapt to cellular states and environmental conditions. Consequently, proteasomes are highly modular. These adaptations come in all possible flavours, such as different complex compositions, post-translational modifications, or differential subcellular localisation. While many of these adaptations can have significant consequences on proteasomal activity and functionality, researchers have a limited understanding of how these adaptations are achieved and their effects. In turn, understanding how the proteasome is modulated will reveal its working mechanics.

Cryo-electron microscopy as a biophysical tool

Modern cryo-electron microscopy (cryo-EM) enables researchers to study how molecular machines such as the proteasome work precisely. The power of cryo-EM goes beyond the production of static structures as it can reveal the architecture of a whole ensemble of structures from one sample. This enables the Haselbach lab to understand the complex mechanics of a molecule, as well as infer thermodynamic and kinetic constants from such data in parallel. To strengthen these capabilities, the Haselbach lab is developing methods for improved sample preparation (1) and image processing. The next step forward is time-resolved sample preparation, which will allow the lab to follow the conformational dynamics of a machine with time.

DAVID HASELBACH

PHD:
MAX-PLANCK-INSTITUTE FOR BIOPHYSICAL CHEMISTRY, GÖTTINGEN, GERMANY (2014)

POSTDOCTORAL RESEARCH:
MAX-PLANCK-INSTITUTE FOR BIOPHYSICAL CHEMISTRY, GÖTTINGEN, GERMANY

FELLOW:
IMP, VIENNA (2017)

GROUP LEADER:
IMP, VIENNA (2020)

TECHNOLOGY PLATFORM HEAD: IMP, VIENNA (2023)

GROUP MEMBERS:

The UPS in the nucleus

The most critical modulation of the UPS is the localisation of its components. The majority of proteasomes are contained in the nucleus of mammalian cells. This is presumably for quick transcription factor turnover and, thus, a quick response to cellular cues. However, it is unclear how the proteasomal complex enters the nucleus intact. Recently, the Haselbach lab, in collaboration with the Zuber lab at the IMP, characterised an essential protein, AKIRIN2, as key for the postmitotic nuclear import of proteasomes (2). The structure of the AKIRIN2/proteasome revealed that AKIRIN2 inhibits proteasome function during transport. In addition, several E3 ligases are exclusively localised in the nucleus to ensure rapid turnover of nuclear proteins such as transcription factors. One of these is the HECT-E3 ligase UBR5. The Haselbach lab revealed that UBR5 forms a large tetrameric ring and acts as a ubiquitin chain elongator (3). It shortens the lifetime of many important nuclear regulators such as Myc.

Ribosome heterogeneity and biogenesis

An alternative to regulating protein homeostasis is the regulation of the ribosome. Ribosomal heterogeneity through different assembly states or alternative subunits has already been established in the translation field. In collaboration with the Pauli lab at the IMP, the Haselbach lab structurally analysed specialised ribosomal complexes seen, for example, in unfertilised eggs (4). These ribosomes are held dormant by binding a set of factors, essentially blocking several functionally important positions in the ribosome. Additionally, the lab is trying to understand the molecular machines involved in ribosome biogenesis. In collaboration with the Bergler lab from the University of Graz, the lab revealed an entropic ratchet motor mechanism involved in the first cytoplasmic ribosomal maturation step (5).

Outlook

In the coming years, the technology platform seeks to understand molecular machine heterogeneity in an organismal context. The proteasome and the ribosome frequently change composition and localisation, especially in proteome remodelling, which occurs during cell differentiation. This remodelling is essential for immune responses or fertilisation processes. The technology platform will follow a holistic approach, including cryo-electron tomography and functional genetics, to explore how changes in molecular machine heterogeneity shape organisms.

SELECTED PUBLICATIONS

1.
Sonn-Segev, A., Belacic, K., Bodrug, T., Young, G., VanderLinden, R. T., Schulman, B. A., Schimpf, J., Friedrich, T., Dip, P. V., Schwartz, T. U., Bauer, B., Peters, J. M., Struwe, W. B., Benesch, J. L. P., Brown, N. G., Haselbach, D., and Kukura, P. (2020). ***Quantifying the heterogeneity of macromolecular machines by mass photometry.*** *Nature Communications*, 11(1), 1772. doi: 10.1038/s41467-020-15642-w.

2.
de Almeida, M., Hinterndorfer, M., Brunner, H., Grishkovskaya, I., Singh, K., Schleiffer, A., Jude, J., Deswal, S., Kalis, R., Vunjak, M., Lendl, T., Imre, R., Roitinger, E., Neumann, T., Kandolf, S., Schutzbier, M., Mechtler, K., Versteeg, G. A., Haselbach, D., and Zuber, J. (2021). ***AKIRIN2 controls the nuclear import of proteasomes in vertebrates.*** *Nature*, 599(7885), 491–496. doi: 10.1038/s41586-021-04035-8. Epub 2021 Oct 28.

3.
Hodakova, Z., Grishkovskaya, I., Brunner, H. L., Bolhuis, D. L., Belacic, K., Schleiffer, A., Kotisch, H., Brown, N. G., and Haselbach, D. (2022) ***Cryo-EM structure of the chain-elongating E3 Ligase UBR5.*** *bioRxiv*, doi: 10.1101/2022.11.03.515015.

4.
Leesch, K. F., Lorenzo-Orts, L., Pribitzer, C., Grishkovskaya, I., Matzinger, M., Roitinger, E., Belacic, K., Kandolf,S ., Lin, T. Y., Mechtler, K., Meinhart, A., Haselbach, D., and Pauli, A. (2023) ***A molecular network of conserved factors keeps ribosomes dormant in the egg.*** *Nature*, 613(7945), 712-720. doi: 10.1038/s41586-022-05623-y. Epub 2023 Jan 18.

5.
Prattes, M., Grishkovskaya, I., Hodirnau, V. V., Hetzmannseder, C., Zisser, G., Sailer, C., Kargas, V., Loibl, M., Gerhalter, M., Kofler, L., Warren, A. J., Stengel, F., Haselbach, D., and Bergler, H. (2022). ***Visualizing maturation factor extraction from the nascent ribosome by the AAA-ATPase Drg1.*** *Nature Structural & Molecular Biology*, 29(9), 942–953. doi: 10.1038/s41594-022-00832-5. Epub 2022 Sep 12.

WULF HAUBENSAK

What is the neural architecture behind emotions?

HAUBENSAK LAB 2017–2022

What is the neural architecture behind emotions?

From surviving in the wild to living in complex societies, brains use emotions to navigate environments. In the most basic form, this involves staying clear from threats (fears) while following up on opportunities (rewards). But if we consider the brain as an information-processing machine of interconnected circuits, the most enigmatic question is: how can these circuits generate such subjective and personal things as emotions?

For the Haubensak lab, this interesting question offers a robust neuroscientific entry point for extracting general principles of brain computations. Using live imaging and optogenetics, the lab has delineated the dedicated neural network at the heart of emotional behaviours. Moreover, the lab hopes to reveal insights into biomedical mechanisms related to health and disease, given the prevalence of affective disorders such as depression, anxiety, and autism.

Tracing emotions

Combining local calcium imaging with global fMRI and optogenetic behavioural profiling in mice, the Haubensak lab mapped the architecture and dynamics of a core affective network for key translationally important steps in affective processing. First, such a network must assign *affective value* to external stimuli, identify what is important (salience), and decide if it is good or bad (valence).

The lab discovered that a dedicated cortico-limbic loop solves this problem by assembling stimulus value from its salience and valence dimensions: salient events in the amygdala recruit interoceptive feedback from the insular cortex (an area representing bodily states), which then assigns a negative or positive valence to the stimulus and chooses the behavioural response (1). This hierarchical process integrates the colloquial 'gut feeling' in behavioural decisions. Dysfunction may underly emotional blindness in autism-related conditions.

Second, the affective network writes affective associations into *emotional memory*. Short-term stress releases neuropeptides that sensitise the amygdala circuits to threat signals and generalise passive behavioural responding, a pattern observed in patients with generalised anxiety and antagonised by benzodiazepines (2). Long-term Pavlovian associations recruit a dopaminergic circuit module that connects pain centres with fear circuitry and writes emotional experiences into amygdala networks (3), possibly underlying the excessive aversive association coexisting in post-traumatic stress disorder (PTSD) and chronic pain.

Third, the affective core network gatekeeps affective responses in *space and time*. For instance, fear behaviour is tightly regulated in space, most prominently visible in defensive responses dependent on predator proximity – a process the lab discovered is tightly controlled by fear gradients generated by cortical object/space systems with

WULF HAUBENSAK

PHD:
MPI FOR MOLECULAR CELL BIOLOGY AND GENETICS, DRESDEN, GERMANY (2003)

POSTDOCTORAL RESEARCH AND STAFF SCIENTIST:
CALTECH, USA

GROUP LEADER:
IMP, VIENNA (2011)

GROUP MEMBERS:

behavioural response control in the amygdala. Likewise, behavioural strategies often require temporal withholding of affective action, like impulse control in the marshmallow test. In collaboration with Boehringer Ingelheim's central nervous system disease research, the Haubensak lab identified several hot spots that control such impulsive actions.

From emotion circuits to affective traits

So far, the lab's research has aimed to explain how brains generate and assign the world with emotions, such as why animals and humans like one thing but might avoid another. However, and perhaps luckily so, individual brains interpret and react to the world differently. But what contributes to this diversity? To a large extent, the affective circuitry characterised above can be genetically programmed for certain behavioural biases, which manifest as behavioural traits (anxiety, social dominance). One attractive model is that most of the genetic variance accumulates along specific sites in neuronal networks, biasing local computations, which in turn underly the transition between behavioural phenotypes. Indeed, genetic variance for a given affective trait (e.g. anxiety, impulsivity, sociality) maps to specific subnetworks in the brain (4).

These variances are most prominent over evolutionary scales, shaping neurocognitive traits between species. To explore underlying patterns, the Haubensak lab mined ancient genomes and human brain data for insights into the evolution of the human mind (5). This allows the lab to impute a brain atlas of human cognitive traits over 60 million years of ancestry evolution *in silico*. These data predict a peak for accelerated neurogenetic selection for language and verbal communication in an early hominin ancestor (7.4–1.7 million years ago) and modern humans (0.8 million years ago – present) that separated us from archaic Denisovan and Neanderthal hominins. This initiative highlighted the power of computational data mining of public resources for insights not accessible by any other means. The lab is currently exploring peak periods of adaptative selection of socio-affective networks (26–19 million years ago, 7.4 million years ago, 0.8 million years ago – present) and their relation to human emotionality.

Outlook

In 2021, the Haubensak lab moved to the Medical University of Vienna. Here, the lab will explore how genetic variation influences circuit computation across populations and how this controls behavioural traits in health and in psychiatric diseases (stress disorders, addiction).

SELECTED PUBLICATIONS

1.
Kargl, D., Kaczanowska, J., Ulonska, S., Groessl, F., Piszczek, L., Lazovic, J., Buehler, K., and Haubensak, W. (2020). ***The amygdala instructs insular feedback for affective learning.*** *eLife,* 9, e60336, 1–36. doi: 10.7554/eLife.60336.

2.
Griessner, J., Pasieka, M., Böhm, V., Grössl, F., Kaczanowska, J., Pliota, P., Kargl, D., Werner, B., Kaouane, N., Strobelt, S., Kreitz, S., Hess, A., and Haubensak, W. (2021). ***Central amygdala circuit dynamics underlying the benzodiazepine anxiolytic effect.*** *Molecular Psychiatry,* 26(2), 534–544. doi: 10.1038/s41380-018-0310-3. Epub 2018 Nov 30.

3.
Groessl, F., Munsch, T., Meis, S., Griessner, J., Kaczanowska, J., Pliota, P., Kargl, D., Badurek, S., Kraitsy, K., Rassoulpour, A., Zuber, J., Lessmann, V., and Haubensak, W. (2018). ***Dorsal tegmental dopamine neurons gate associative learning of fear.*** *Nature Neuroscience,* 21(7), 952–962. doi: 10.1038/s41593-018-0174-5. Epub 2018 Jun 27.

4.
Ganglberger, F., Kaczanowska, J., Penninger, J. M., Hess, A., Bühler, K., and Haubensak, W. (2018). ***Predicting functional neuroanatomical maps from fusing brain networks with genetic information.*** *Neuroimage,* 170, 113–120. doi: 10.1016/j.neuroimage.2017.08.070. Epub 2017 Sep 4.

5.
Kaczanowska, J., Ganglberger, F., Chernomor, O., Kargl, D., Galik, B., Hess, A., Moodley, Y., von Haeseler, A., Bühler, K., and Haubensak, W. (2022). ***Molecular archaeology of human cognitive traits.*** *Cell reports,* 40(9). doi: 10.1016/j.celrep.2022.111287.

DAVID KEAYS

How do animals detect magnetic fields?

KEAYS LAB 2017–2022

How do animals detect magnetic fields?

Each year millions of animals undertake remarkable migratory journeys, across oceans and through hemispheres, guided by the Earth's magnetic field. While there is unequivocal behavioural evidence demonstrating the existence of the magnetic sense, it is the least understood of all sensory faculties. The biophysical, molecular, cellular, and neurological underpinnings of the sense remain unclear. The Keays lab has focused on this scientific mystery, exploiting state-of-the-art technologies to map the neuronal circuits that encode magnetic information and to identify the receptors that transduce this invisible force into a neural impulse.

The 19th century naturalist Camille Viguier was among the first to predict the existence of a magnetic sense. He postulated: "What could be the physical force everywhere present in the heights of the atmosphere as well as the depths of the oceans that could direct animals that migrate? In my opinion, there is only one ... and that is the Earth's magnetic field".

Viguier's argument was dismissed by his peers, and it was not until the 1960s with a series of elegant behavioural experiments on the European Robin that the data was clear – the magnetic sense does exist.

In the intervening years, the list of magnetosensitive species has expanded to include bacteria, prokaryotes, honeybees, cockroaches, newts, turtles, rodents, lobsters, fish, bats, migratory birds, and pigeons (1). The critical question is: how do they do it?

Not just two theories

The field of magnetoreception has been dominated by two theories: the magnetite theory, and the light-dependent hypothesis. The first theory predicts that magnetic fields are detected by mechanically sensitive channels that are coupled to the iron oxide magnetite, which is associated with the trigeminal nerve. The second theory proposes that incident light creates a pair of radical pairs in photosensitive molecules that are influenced by the local magnetic environment in the eye.

At its inception, the Keays lab explored the magnetite hypothesis, revealing that iron-rich cells in the pigeon beak are macrophages, not magnetosensitive neurons (2). Moreover, extensive elemental analysis failed to identify magnetite associated with other sensory structures (3, 4). Armed with compelling evidence that pigeons can still detect magnetic fields in the dark, excluding a light-based mechanism, the lab was compelled to consider an alternative possibility – electromagnetic induction.

DAVID KEAYS

PHD:
UNIVERSITY OF OXFORD, UK (2006)

POSTDOCTORAL RESEARCH:
WELLCOME TRUST TRAINING FELLOW, UNIVERSITY OF OXFORD, UK

IMP FELLOW, VIENNA (2008)

GROUP LEADER:
IMP, VIENNA (2013)

GROUP MEMBERS:

Electromagnetic induction and electroreceptors in the inner ear

Electricity and magnetism are intimately linked – a fact that humanity has harnessed to great effect to generate and use electricity. Wind turbines, coffee grinders, and wireless phone chargers all rely on electromagnetic induction. Put simply, a changing magnetic field in the vicinity of a conductive circuit generates a current.

The Keays lab has been exploring whether the fluid-filled semi-circular canals within the inner ear of birds could serve as an ancient conductive circuit, enabling animals to directly translate magnetic information into an electric impulse when coupled with head scanning. This theory gained traction following the lab's discovery that a highly sensitive electroreceptor (CaV1.3) – first described in sharks and skates – is present in the inner ear of birds (5). Both theoretical and physical modelling demonstrated that small currents induced by Earth-strength magnetic fields are within the physiological range of the CaV1.3 electroreceptor – evidence that it's not impossible.

Circuits for the magnetic sense

Complementing this work, the Keays lab has mapped the neuronal circuits that process information in the pigeon brain by combining magnetic stimulation assays with whole-brain clearing. By quantifying the expression of C-FOS, a surrogate marker for neuronal activity, the lab identified a minimal neuronal circuit in the pigeon brain, which processes magnetic information. Relying on the vestibular nuclei as an entry point, the lab further demonstrated that this circuit is dependent on the activation of CaV1.3 in the pigeon inner ear. Blocking CaV1.3 with the potent snake toxin calciseptine inhibits magnetically induced neuronal activation.

Outlook

If correct, this work would provide a molecular candidate and a biophysical explanation for the magnetic sense in pigeons. The implications are far-reaching – what other species might employ this mechanism? How sensitive is it? What exactly is the animal detecting? Might other molecules be involved? How does an animal incorporate this information into spatial maps? And are there magnetic place cells? The mystery is far from over and the Keays lab continue their investigation, since 2021 at the Ludwig Maximilian University of Munich.

SELECTED PUBLICATIONS

1.
Nordmann, G. C., Hochstoeger, T., and Keays, D. A. (2017). ***Magnetoreception-A sense without a receptor.*** *PLoS Biology,* 15(10), e2003234. doi: 10.1371/journal.pbio.2003234. Epub 2017 Oct 23.

2.
Treiber, C. D., Salzer, M. C., Riegler, J., Edelman, N., Sugar, C., Breuss, M., Pichler, P., Cadiou, H., Saunders, M., Lythgoe, M., Shaw, J., and Keays, D. A. (2012). ***Clusters of iron-rich cells in the upper beak of pigeons are macrophages not magnetosensitive neurons.*** *Nature,* 484(7394), 367-370. doi: 10.1038/nature11046.

3.
Edelman, N. B., Fritz, T., Nimpf, S., Pichler, P., Lauwers, M., Hickman, R. W., Papadaki-Anastasopoulou, A., Ushakova, L., Heuser, T., Resch, G. P., Saunders, M., Shaw, J. A., and Keays, D. A. (2015). ***No evidence for intracellular magnetite in putative vertebrate magnetoreceptors identified by magnetic screening.*** *Proceedings of the National Academy of Sciences of the United States of America,* 112(1), 262–267. doi:10.1073/pnas.1407915112. Epub 2014 Dec 22.

4.
Malkemper, E. P., Kagerbauer, D., Ushakova, L., Nimpf, S., Pichler, P., Treiber, C. D., de Jonge, M., Shaw, J., and Keays, D. A. (2019). ***No evidence for a magnetite-based magnetoreceptor in the lagena of pigeons.*** *Current Biology,* 29(1), R14–R15. doi: 10.1016/j.cub.2018.11.032.

5.
Nimpf, S., Nordmann, G. C., Kagerbauer, D., Malkemper, E. P., Landler, L., Papadaki-Anastasopoulou, A., Ushakova, L., Wenninger-Weinzierl, A., Novatchkova, M., Vincent, P., Lendl, T., Colombini, M., Mason, M. J., and Keays, D. A. (2019). ***A Putative Mechanism for Magnetoreception by Electromagnetic Induction in the Pigeon Inner Ear.*** *Current Biology,* 29 (23), 4052–4059.e4. doi: 10.1016/j.cub.2019.09.048. Epub 2019 Nov 14.

ANNA OBENAUF

How can combination therapies treat metastatic cancer?

OBENAUF LAB 2017–2022

How can combination therapies treat metastatic cancer?

Targeted therapies and immunotherapies have transformed the clinical care of patients with metastatic cancer in recent years. Tailored, mechanism-based treatments inhibit oncogenic signalling pathways that are critical to tumour growth, while immunotherapies unleash a host response to destroy cancer cells. By combining these different therapies, a cure for many cancers appears to be within reach. The aim of the Obenauf lab is to guide the development of rational combination therapies in the clinic and provide insights into the molecular mechanisms underlying therapy resistance and immune evasion.

A key approach in the Obenauf lab is to exploit the evolution of tumours as they are shaped by selective bottlenecks under therapy and during cancer progression and metastasis. In this process, pre-existing minority clones are selected or clones acquire new features and then expand. Understanding tumour evolution can reveal molecular drivers of metastasis and therapy resistance, for example, by comparing selected clones with their parental populations. Molecular features that dictate clonal behaviour, such as genetic and non-genetic features in cancer cells or different tumour microenvironments (TMEs) can be linked to cell fates, and new vulnerabilities identified. Understanding how therapies affect tumour evolution and influence the response to subsequent therapies can also inform rational combination therapies.

Insights into tumour evolution

A major challenge for understanding tumour evolution is the lack of experimental strategies for identifying and functionally characterising the same clonal population at different stages along its evolutionary trajectory. To address this, the Obenauf lab developed a functional lineage tracing tool termed CaTCH (CRISPRa tracing of clones in heterogeneous cell populations) (1). CaTCH combines precise mapping of the lineage history of millions of cells with the ability to isolate any given clone alive from a complex population. It also serves as a time machine, as it can return to the founder population and isolate clones before or during exposure to the evolutionary bottleneck.

The Obenauf lab used CaTCH to generate resistance to targeted cancer therapies (RAFi/MEKi) *in vivo* and found that resistance was often an acquired trait. The lab also showed that mutations conferring drug resistance are frequently acquired during targeted therapy treatment, challenging the notion that these mutations generally exist before therapy. CaTCH has been quickly adopted by many labs worldwide to address important questions in basic and translational research.

ANNA OBENAUF

PHD:
MEDICAL UNIVERSITY OF GRAZ, AUSTRIA (2010)

POSTDOCTORAL RESEARCH:
MEMORIAL SLOAN KETTERING CANCER CENTER (MSKCC), USA

GROUP LEADER:
IMP, VIENNA (2016)

SENIOR GROUP LEADER:
IMP, VIENNA (2022)

GROUP MEMBERS:

Understanding the crosstalk between cancer cells and the immune system

Resistance to therapies is a major problem, thus targeted and immunotherapies are frequently given sequentially. But how targeted therapies change the tumour and whether it affects immunotherapy responses was previously unknown. The Obenauf lab uncovered that acquired resistance to targeted therapy confers cross-resistance to immunotherapy, despite the entirely different mode of action of these treatments (2). The lab showed that cross-resistance to immunotherapy is mediated via an immune-evasive TME, which is directly instructed by the targeted therapy-resistant cancer cells and characterised by impaired maturation and low abundance of CD103+ dendritic cells. Moreover, cross-resistance is instructed by the reactivated and rewired mitogen-activated protein kinase (MAPK) pathway.

This finding represents a new concept in therapy resistance. It implies that cancer cells can use the same MAPK pathway, which is initially important for tumour initiation and progression, and alter its transcriptional output to establish a completely different immune phenotype. The biological insights from this work and the strategies the Obenauf lab identified to overcome cross-resistance had immediate implications for melanoma patients.

Targeting rare skin cancers

To extend the concepts and experimental approaches from studies in melanoma to other malignancies, the Obenauf lab established a line of research on rare skin cancers. They discovered a new therapeutic target in Merkel cell carcinoma, an aggressive skin cancer (3), and identified a new oncogenic virus, human papillomavirus type 42 (HPV42), as the driver of digital papillary adenocarcinoma (4), adding a new member to the short list of tumorigenic viruses in humans. The lab discovered that all oncogenic HPVs directly evoke a germ cell-like state, which has diagnostic implications and represents an exciting immunotherapeutic vulnerability for all HPV-driven tumours.

Outlook

With an arsenal of therapeutic approaches available, cancer research is entering an exciting new era. Individual treatments are serving as building blocks for combination therapies to accomplish what monotherapies often cannot: durable tumour control in most patients with metastatic cancer. The number of possible combinations (simultaneous or sequential) will require a comprehensive and mechanistic understanding of how cancer cells and the TME change under different therapeutic challenges, and how this might influence the outcome of subsequent therapies. The long-term mission of the Obenauf lab is to understand the causes of resistance to targeted therapies and immunotherapies, and to identify new vulnerabilities.

SELECTED PUBLICATIONS

1.
Umkehrer, C., Holstein, F., Formenti, L., Jude, J., Froussios, K., Neumann, T., Cronin, S. M., Haas, L., Lipp, J. J., Burkard, T. R., Fellner, M., Wiesner, T., Zuber, J., and Obenauf, A. C. (2021). ***Isolating live cell clones from barcoded populations using CRISPRa-inducible reporters.*** *Nature Biotechnology,* 39(2), 174–178. doi: 10.1038/s41587-020-0614-0. Epub 2020 Jul 27.

2.
Haas, L., Elewaut, A., Gerard, C. L., Umkehrer, C., Leiendecker, L., Pedersen, M., Krecioch, I., Hoffmann, D., Novatchkova, M., Kuttke, M., Neumann, T., da Silva, I. P., Witthock, H., Cuendet, M. A., Carotta, S., Harrington, K. J., Zuber, J., Scolyer, R. A., Long, G. V., Wilmott, J. S., Michielin, O., Vanharanta, S., Wiesner, T., and Obenauf, A. C. (2021). ***Acquired resistance to anti-MAPK targeted therapy confers an immune-evasive tumor microenvironment and cross-resistance to immunotherapy in melanoma.*** *Nature Cancer,* 2(7), 693–708. doi: /10.1038/s43018-021-00221-9. Epub 2021 Jul 15.

3.
Leiendecker, L., Jung, P. S., Krecioch, I., Neumann, T., Schleiffer, A., Mechtler, K., Wiesner, T., and Obenauf, A. C. (2020). ***LSD1 inhibition induces differentiation and cell death in Merkel cell carcinoma.*** *EMBO Molecular Medicine*, 12(11), e12525. doi: 10.15252/emmm.202012525. Epub 2020 Oct 7.

4.
Leiendecker, L., Neumann, T., Jung, P. S., Cronin, S. M., Steinacker, T. L., Schleiffer, A., Schutzbier, M., Mechtler, K., Kervarrec, T., Laurent, E., Bachiri, K., Coyaud, E., Murali, R., Busam, K. J., Itzinger-Monshi, B., Kirnbauer, R., Cerroni, L., Calonje, E., Rütten, A., Stubenrauch, F., Griewank, K. G., Wiesner, T., and Obenauf, A. C. (2023). ***Human papillomavirus 42 drives digital papillary adenocarcinoma and elicits a germ cell-like program conserved in HPV-positive cancers.*** *Cancer Discovery,* 13(1), 70–84. doi: 10.1158/2159-8290.CD-22-0489. Epub 2023 Jan 9.

ANDREA PAULI

What mechanisms drive the egg-to-embryo transition?

PAULI LAB 2017–2022

What mechanisms drive the egg-to-embryo transition?

Fertilisation marks the beginning of life for any sexually reproducing organism. It also initiates the transformation of the dormant egg cell into distinct embryonic cells and ultimately into a fully patterned organism. During the past years, the Pauli lab has gained mechanistic insights into the egg-to-embryo transition, one of the most dramatic yet remarkably mysterious developmental transitions.

By focusing on three main areas, namely fertilisation, egg dormancy, and cell migration during gastrulation, the Pauli lab identified essential fertilisation factors that mediate sperm-egg interaction in fish and in mammals. The lab also discovered a conserved molecular mechanism that stabilises and represses ribosomes in fish and frog eggs. Finally, the Pauli lab showed that a self-generated signalling gradient is the guidance mechanism for mesodermal cells during gastrulation.

New insights into the mechanism of fertilisation

A challenge of fertilisation is that sperm-egg fusion must be highly efficient and restricted to conspecific gametes. Mechanisms that limit fertilisation to conspecific gametes have been mostly described in externally fertilising marine species lacking premating choice, in which species-specificity factors are of particular importance for the perpetuation of species. However, none of the previously identified essential vertebrate sperm-egg interaction proteins (e.g. CD9, IZUMO1, and JUNO) are known to restrict fertilisation in a species-specific manner.

The Pauli lab's discovery of the short, unannotated Ly6/uPAR-type protein Bouncer as an essential matchmaker between sperm and egg in zebrafish and medaka therefore was a milestone in the field of reproductive biology (1). The lab showed that egg-membrane-localised Bouncer is not only essential for sperm entry into the egg in fish but is also sufficient to switch the species-specificity of fertilisation between zebrafish and medaka, two fish species that diverged more than 150 million years ago.

The discovery of Bouncer has established fertilisation as a new research direction in the Pauli lab (2), which led to identifying and investigating several other vertebrate fertility factors, namely murine SPACA4 (3), zebrafish Dcst1, Dcst2, and Spaca6. These findings provide important steps towards the future investigation of the mechanism of fertilisation.

Gene regulatory mechanisms during the egg-to-embryo transition

The eggs of many organisms store large amounts of maternal components required for embryogenesis. However, eggs are in a 'dormant' state as many processes, including translation, are repressed. To address how dormancy is

ANDREA PAULI

PHD:
OXFORD UNIVERSITY, UK
(2009)

POSTDOCTORAL RESEARCH:
HARVARD UNIVERSITY,
USA

GROUP LEADER:
IMP, VIENNA (2015)

SENIOR GROUP LEADER:
IMP, VIENNA
(2022)

GROUP MEMBERS:

controlled and how eggs store maternal ribosomes, the Pauli lab used a combination of cryo-electron microscopy structural analyses (in collaboration with David Haselbach at the IMP), *in vivo* functional assays, and *in vitro* studies to uncover a conserved, previously unknown dormant egg ribosome state that preserves ribosomes and prevents premature translational activation. Egg ribosomes in fish and frogs are blocked at four functionally important sites by conserved factors that form two modules: a Habp4-eEF2 module that stabilises ribosomes, and a Dap1b/Dapl1-eIF5a module that represses translation. Dap1b/Dapl1 is a newly discovered translational inhibitor that stably inserts into the polypeptide exit tunnel and is sufficient to reconstitute the dormant egg ribosome state *in vitro* in mammalian translation extracts (4).

In addition, the Pauli lab used genome-wide assays combined with computational analyses and functional experiments to gain new insights into gene regulatory mechanisms during embryogenesis. Moreover, harnessing the species-specificity of Bouncer, the Pauli lab established reciprocal zebrafish-medaka hybrids as a new, powerful tool to dissect parental-specific effects during embryogenesis.

A self-generated Toddler gradient directs cell migration during gastrulation

The sculpting of germ layers during gastrulation relies on the coordinated migration of progenitor cells, yet the cues controlling these long-range directed movements remain largely unknown. By combining *in vivo* experiments and mathematical modelling (in collaboration with Edouard Hannezo at the Institute of Science and Technology Austria), the lab's research on the small, secreted protein Toddler/ELABELA/Apela uncovered a single receptor-based self-generated signal gradient as the enigmatic guidance mechanism that steers the directional migration of mesoderm during gastrulation (5).

The Pauli lab found that the self-generated Toddler gradient does not rely on a localised source of Toddler, but instead on a localised sink constituted by Toddler receptor (Aplnr) expressing mesodermal cells. The receptor acts as a scavenger receptor for Toddler to convert the uniformly expressed signal into a graded guidance cue, and as a chemokine receptor that senses this cue.

Outlook

The Pauli lab aims to unravel new concepts and molecular principles governing the egg-to-embryo transition by focusing on the underlying mechanism of vertebrate sperm-egg fusion and the control of egg and early embryo dormancy.

SELECTED PUBLICATIONS

1.
Herberg, S., Gert, K. R., Schleiffer, A., and Pauli, A. (2018). ***The Ly6/uPAR protein Bouncer is necessary and sufficient for species-specific fertilization.*** *Science,* 361, 1029–1033. doi: 10.1126/science.aat7113. Epub 2018 Sep 7.

2.
Deneke, VE., Pauli, A. (2021). ***The Fertilization Enigma: How Sperm and Egg Fuse.*** *Annu Rev Cell Dev Biol,* 37, 391-414. doi: 10.1146/annurev-cellbio-120219-021751. Epub 2021 Oct 6.

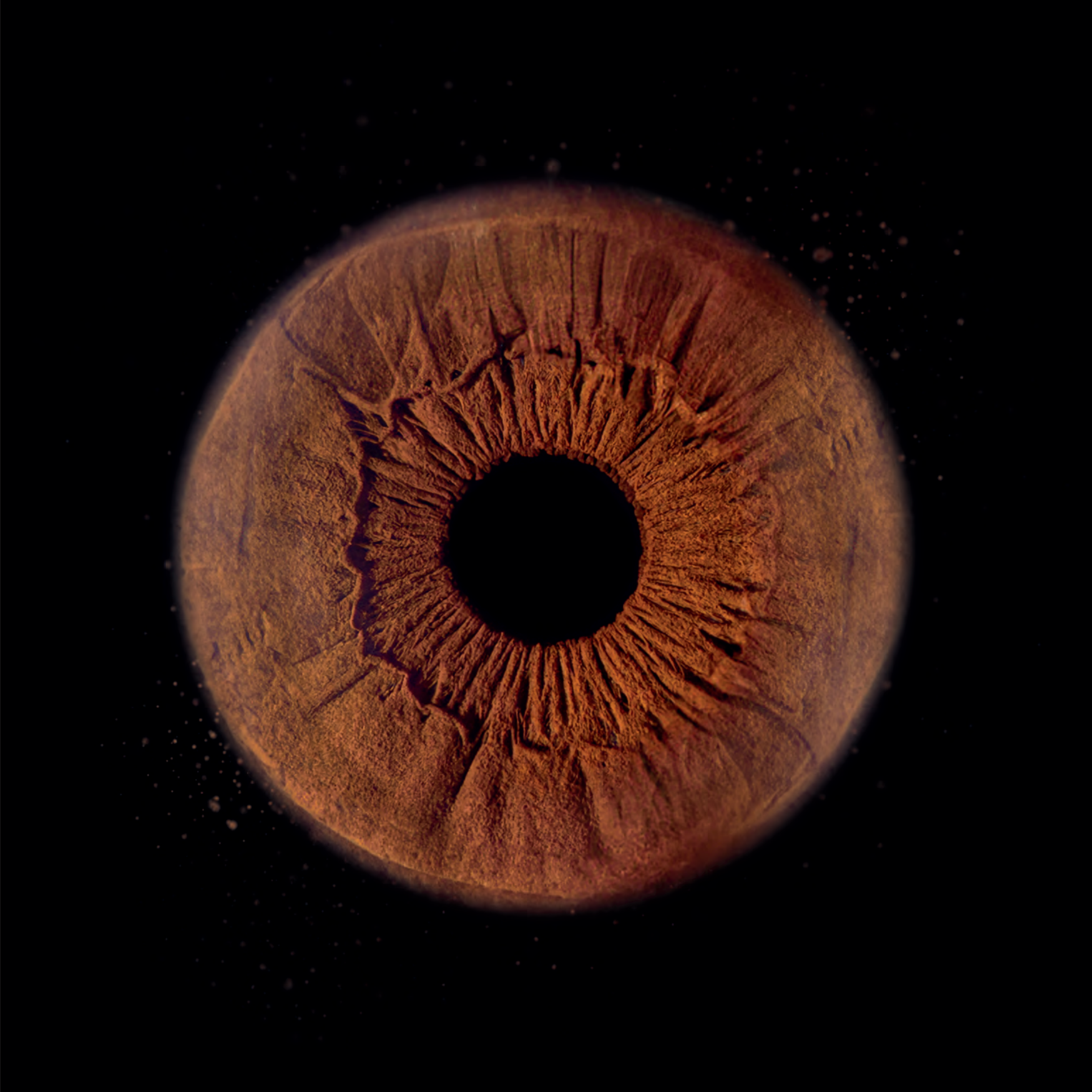
3.
Fujihara, Y., Herberg, S., Blaha, A., Panser, K., Kobayashi, K., Larasati, T., Novatchkova, M., Theussl, HC., Olszanska, O., Ikawa, M., and Pauli, A. (2021). ***The conserved fertility factor SPACA4/Bouncer has divergent modes of action in vertebrate fertilization.*** *Proc Natl Acad Sci U S A,* 118 (39), e2108777118. doi: 10.1073/pnas.2108777118. Epub 2021 Sep 28.

4.
Leesch, F., Lorenzo-Orts, L., Pribitzer, C., Grishkovskaya, I., Roehsner, J., Chugunova, A., Matzinger, M., Roitinger, E., Belačić, K., Kandolf, S., Lin, T.-Y., Mechtler, K., Meinhart, A., Haselbach, D., Pauli, A (2023). ***A molecular network of conserved factors keeps ribosomes dormant in the egg.*** *Nature,* 613 (7945), 712-720. doi: 10.1038/s41586-022-05623-y. Epub 2023 Jan 18.

5.
Stock, J., Kazmar, T., Schlumm, F., Hannezo, E., and Pauli, A. (2022). ***A self-generated Toddler gradient guides mesodermal cell migration.*** *Science Advances,* 8(37), eadd2488. doi: 10.1126/sciadv.add2488. Epub 2022 Sep 14.

RUSHAD PAVRI

What are the molecular mechanisms behind somatic hypermutation in B cells?



PAVRI LAB 2017–2022

What are the molecular mechanisms behind somatic hypermutation in B cells?

Our immune system can generate a vast antibody repertoire to fight infections and fend off pathogens. This diversification occurs in highly proliferating B cells and consists of two processes: somatic hypermutation (SHM) and class switch recombination (CSR). The Pavri lab is providing insights into the transcriptional and co-transcriptional mechanisms that underly these processes, with a particular focus on the role of 3D chromatin architecture and translocation biogenesis.

Antibody maturation is triggered by activation-induced cytidine deaminase (AID), which introduces mutations in the immunoglobulin (Ig) genes at the level of the antigen-binding variable (V) regions. AID occasionally targets other genes too, such as the oncogenes *MYC* and *BCL6*.

CSR and SHM require the activity of the distal 3' immunoglobulin super-enhancer, which regulates the transcription of the immunoglobulin genes, as well as the recruitment of AID. CSR also needs the juxtaposition of transcribed *Igh* switch (S) sequences (S-S synapsis) that are spread across 50–200 kilobases.

Transcription of the *Igh* super-enhancer is necessary for enhancer-promoter contact during CSR

The Pavri lab showed that transcription at the *Igh* super-enhancer, mediated by the elongation factor Spt5, is necessary for the enhancer to contact immunoglobulin promoters. The lab found that in Spt5-depleted cells, the enhancer chromatin was accessible and acetylated but in a disengaged state, resulting in severely decreased *Igh* gene expression. This defect in gene expression could be partially rescued by restoring transcription at the enhancer with a CRISPR-activation approach, which could also restore enhancer-promoter contact.

More generally, this work demonstrated that enhancer transcription directly contributes to enhancer function (1). By examining the global transcriptional changes in Spt5-depleted cells, both in B cells and fibroblasts, the Pavri lab found that the catalytic ability of RNA polymerase II complexes within genes decreased by ~12–15 kilobases from promoters, resulting in the decreased expression of long genes. This feature constituted a new regulatory step in transcription elongation mediated by Spt5 (2).

TAD boundaries and multiway chromatin interactions

The Pavri lab used the Tri-C assay to uncover multiway interactions on single *Igh* alleles during CSR in murine B cells.

RUSHAD PAVRI

PHD:
UNIVERSITY OF MEDICINE AND DENTISTRY OF NEW JERSEY, USA (2006)

POSTDOCTORAL RESEARCH:
ROCKEFELLER UNIVERSITY, NEW YORK, USA

GROUP LEADER:
IMP, VIENNA (2013)

This showed that gene activation by the super-enhancer topologically uncoupled from S-S synapsis, in contrast to the prevailing model.

Tri-C also revealed the presence of a topological barrier at transcribed S regions resembling a topologically associating domain (TAD) boundary. These, and additional analyses, led to a revised model where transcriptional activity at S regions creates a *de novo* TAD boundary that impedes cohesin-containing loop extrusion complexes, resulting in S-S synapsis at the TAD boundary interface (3). This study also revealed a physiological role for transcription-dependent TAD boundaries, which have been frequently observed, but whose biological relevance has been unclear.

Mechanisms underlying SHM patterns

AID exhibits differential mutagenesis of its target motifs. Features of the nascent transcriptional landscape are thought to contribute to SHM activity, but direct supportive evidence is lacking, especially because V region nascent transcriptional landscapes are uncharacterised.

The Pavri lab addressed this question using high-resolution comparative analyses of nascent transcription and mutation in different V regions and non-Ig SHM target loci. Surprisingly, transcriptional patterns were not predictive of SHM patterns. It appears, therefore, that although transcription is necessary for AID targeting, the actual rules of mutagenesis are likely to be sequence-intrinsic and regulated by unknown mechanisms (4).

DNA replication timing and AID-dependent chromosomal translocations

Previous work from the Pavri lab had shown that CSR required the activity of the MCM complex, the replicative helicase, and the firing of origins of replication in Igh S regions. The lab has now reported that DNA replication timing directly regulates lymphomagenic Myc translocations during antibody maturation in B cells downstream of double-strand breaks and independently of the breaks' frequency.

Depletion of MCM complexes alters replication origin activity, decreases translocations, and deregulates global replication timing. Ablating a single origin at Myc causes an early-to-late replication timing switch, loss of translocations, and reduced proximity with Igh, its major translocation partner. The Pavri lab reversed these phenotypes by restoring early replication timing and showed that disrupting early replication timing also reduced tumorigenic *AF4-MLL1* translocations in human leukemic cells. Thus, the lab discovered that replication timing constitutes a general mechanism in translocation biogenesis linking double-strand break formation to their ligation (5).

Outlook

In the future, the Pavri lab will address how locus architecture regulates SHM and will continue to investigate the rules governing SHM patterns in V regions. A CRISPR screen has identified new SHM factors, and functionally characterising these will be a major research focus. The lab will also dissect the *Myc* origin of replication, which appears to work as a novel early replication enhancer element, and will investigate whether similar elements and mechanisms regulate other tumorigenic translocations in different cell types.

GROUP MEMBERS:

SELECTED PUBLICATIONS

1.
Fitz, J., Neumann, T., Steininger, M., Wiedemann, E. M., Garcia, A. C., Athanasiadis, A., Schoeberl, U. E., and Pavri, R. (2020). ***Spt5-mediated enhancer transcription directly couples enhancer activation with physical promoter interaction.*** *Nature Genetics*, 52(5), 505–515. doi: 10.1038/s41588-020-0605-6. Epub 2020 Apr 6.

2.
Fitz, J., Neumann, T., and Pavri, R. (2018). ***Regulation of RNA polymerase II processivity by Spt5 is restricted to a narrow window during elongation.*** *The EMBO Journal*, 37(8), e97965. doi: 10.15252/embj.201797965. Epub 2018 Mar 7.

3.
Costea, J., Schoeberl, U. E., Malzl, D., Von der Linde, M., Fitz, J., Makharova, M., Goloborodko, A., and Pavri, R. (2023). ***A de novo transcription-dependent TAD boundary underpins critical multiway interactions during antibody class switch recombination.*** *Molecular Cell*, 83(5), 681-697. doi: 10.1016/j.molcel.2023.01.014. Epub 2023 Feb 2.

4.
Schoeberl, U. E., Fitz, J., Froussios, K., Valieris, R., Makharova, M., Ourailidis, I., Bauer, B., Neumann, T., Wiedemann, E. M., Steininger, M., Garcia, A. C., Mastrovito, M., Mouquet, H., Da Silva, I. T., and Pavri, R. (2022). ***Somatic hypermutation spectra are independent of the local transcriptional and epigenetic landscape.*** *bioRxiv*. doi: 10.1101/2022.05.21.492925.

5.
Peycheva, M., Neumann, T., Malzl, D., Nazarova, M., Schoeberl, U. E., and Pavri, R. (2022). ***DNA replication timing directly regulates the frequency of oncogenic chromosomal translocations.*** *Science*, 377, eabj5502. doi: 10.1126/science.abj5502. Epub 2022 Sep 16.

JAN-MICHAEL PETERS

How does cohesin fold the genome by loop extrusion?

PETERS LAB 2017–2022

How does cohesin fold the genome by loop extrusion?

DNA is a remarkable molecule. It carries the instructions for virtually all biological processes, from dictating the simplest of chemical reactions to deciding our personalities. These data-packed molecules can reach lengths of almost a meter, so to fit inside the microscopic nucleus of a cell, DNA exists in a highly folded state. The Peters lab discovered that this DNA folding process is far from random and is an active and regulated process mediated by cohesin. The DNA loops formed by cohesin have important roles in chromosome assembly, gene regulation, and recombination, and the extrusion process that cohesin uses to generate these looping structures might be as old as DNA genomes themselves.

The IMP is well known for its 1997 co-discovery of cohesin, a milestone recognised by the 2018 Life Sciences Breakthrough Prize awarded to Kim Nasmyth. Nasmyth identified cohesin in yeast for its ability to connect replicated 'sister' DNA molecules so they can segregate during cell division. But in 2008 the Peters lab, working on cohesin in human cells, realised that this sister chromatid cohesion might only be one of cohesin's functions.

Based on their finding that human cohesin co-localises with CTCF, a DNA binding protein linked to chromatin looping, the Peters lab speculated that cohesin might also fold DNA into loops. In the past six years, the Peters lab has tested this hypothesis. These experiments led to the discovery that cohesin organises the genome by extruding DNA into loops and that CTCF regulates this process by forming barriers to loop extrusion.

Cohesin folds DNA by loop extrusion

Genomic DNA is organised into loops that occur predominantly inside topologically-associating domains (TADs). The Peters lab discovered that while cohesin is essential for chromatin looping, CTCF is dispensable. However, the lab found that CTCF is required for generating TAD boundaries (1) and for accumulating cohesin at these sites (2).

Scientists had previously proposed that cohesin folds DNA by reeling it into loops, but this loop extrusion hypothesis remained controversial and unsupported by experimental evidence. By using biochemical reconstitution and single-molecule imaging, the Peters lab showed for the first time that cohesin can extrude DNA into loops (3).

These experiments revealed that loop extrusion depends on cohesin's ATPase activity and a protein called NIPBL. It does not, however, require DNA to be trapped inside a ring structure that is formed by cohesin. Researchers previously proposed that cohesin was a passive linker that mediated

JAN-MICHAEL PETERS

PHD:
UNIVERSITY HEIDELBERG, GERMANY (1991)

POSTDOCTORAL RESEARCH:
DKFZ HEIDELBERG, GERMANY AND HARVARD MEDICAL SCHOOL, USA

GROUP LEADER:
IMP, VIENNA (1996)

SENIOR GROUP LEADER:
IMP, VIENNA (2002)

SCIENTIFIC DEPUTY DIRECTOR:
IMP, VIENNA (2011)

SCIENTIFIC DIRECTOR:
IMP, VIENNA (2013)

GROUP MEMBERS:

cohesion by this DNA entrapment, so these findings from the Peters lab were unexpected. It was also believed that NIPBL was essential for loading cohesin onto DNA but not thereafter. Instead, the results of the Peters lab indicate that NIPBL functions in addition – or possibly instead – as a processivity factor for loop extrusion and that cohesin performs this process as an active 'motor' protein without entrapping DNA.

Roles of cohesin-mediated loop extrusion in gene regulation, recombination, and disease

Collaborations with the Spivakov and Busslinger labs at the IMP revealed important functions of cohesin-mediated loop extrusion. These studies showed that long-range enhancer-promoter interactions require cohesin and that cohesin-mediated loop extrusion is essential for V gene recombination to generate a broad repertoire of B-cell receptors and antibodies.

Cornelia de Lange Syndrome (CdLS) is a developmental disease associated with NIPBL and cohesin mutations. The Peters lab discovered that several of these reduce loop extrusion, suggesting that defects in this process contribute to CdLS (Panarotto *et al.*, 2022). Since CdLS is characterised by gene regulation defects, it is possible that CdLS mutations reduce enhancer-promoter interactions during human development.

First insights into the mechanism of loop extrusion

The Peters lab identified DNA binding sites and large-scale conformational changes in cohesin-NIPBL that are required for loop extrusion and analysed how these are coordinated with cohesin's ATP binding-hydrolysis cycle. These results suggested how cohesin translocates DNA and thus revealed the first mechanistic principles of loop extrusion (4).

To understand how CTCF establishes TAD boundaries, the Peters lab collaborated with the Dekker lab at the Delft University of Technology to reconstitute interactions of single CTCF and cohesin molecules on DNA *in vitro* (5). These experiments revealed that CTCF's ability to block loop extrusion depends on DNA tension and that CTCF regulates cohesin's loop extrusion activity by changing its direction and inducing loop shrinkage. This data indicates that CTCF is not, as previously assumed, simply a barrier to cohesin-mediated loop extrusion but is an active regulator of this process, where the permeability of TAD boundaries can be modulated by DNA tension.

Outlook

Cohesin's loop extrusion activity is now considered to be its original function in evolutionary terms. This is because 'structural maintenance of chromosomes' (SMC) complexes related to cohesin exist in all kingdoms of life and fold genomic DNA in both eubacteria and archaebacteria. In the coming years, the Peters lab will analyse the mechanisms, regulation and cellular functions of this fundamental process and address how defects in loop extrusion contribute to human diseases associated with cohesin mutations.

SELECTED PUBLICATIONS

1.
Busslinger, G.A., Stocsits, R.R.,van der Lelij, P., Axelsson, E., Tedeschi, A., Galjart, N. and Peters, J.-M. (2017). ***Cohesin is positioned in mammalian genomes by transcription, CTCF and Wapl.*** *Nature,* 544, 503-507. doi: 10.1038/nature22063. Epub 2017 Apr 19.

2.
Wutz, G., Várnai, C., Nagasaka, K., Cisneros, D.A., Stocsits, R.R., Tang, W., Schoenfelder, S., Jessberger, G., Muhar, M., Hossain, M.J., Walther, N., Koch, B., Kueblbeck, M., Ellenberg, J., Zuber, J., Fraser, P. and Peters, J.-M. (2017). ***Topologically associating domains and chromatin loops depend on cohesin and are regulated by CTCF, WAPL, and PDS5 proteins.*** *EMBO Journal,* 36(24):3573-3599. doi: 10.15252/embj.201798004. Epub 2017 Dec 7.

3.
Davidson, I.F., Bauer, B., Goetz, D., Tang, W., Wutz, G. and Peters, J.-M. (2019). ***DNA loop extrusion by human cohesin.*** *Science,* 366, 1388-1345. doi: 10.1126/science.aaz3418. Epub 2019 Nov 21.

4.
Bauer, B., Davidson, I.F., Canena, D., Wutz, G., Tang, W., Litos, G., Horn, S., Hinterdorfer, P. and Peters, J.-M. (2021). ***Cohesin mediates DNA loop extrusion by a 'swing and clamp' mechanism.*** *Cell,* 184, 5448-5464. doi: 10.1016/j cell.2021.09.016. Epub 2021 Oct 7.

5.
Davidson, I.F., Barth, R., Zaczek, M., van der Torre, J., Tang, W., Nagasaka, K., Janissen, R., Kerssemakers, J., Wutz, G., Dekker, C. and Peters, J.-M. (2022). ***CTCF is a DNA-tension-dependent barrier to cohesin-mediated DNA loop extrusion.*** *bioRxiv.* doi: 10.1101/2022.09.08.507093.

DIANA PINHEIRO

How do embryos form patterns and shapes during development?

PINHEIRO LAB 2022

How do embryos form patterns and shapes during development?

How embryos acquire their shape from a single fertilised egg is one of life sciences' most fascinating and fundamental processes. It involves both a progressive increase in cell type diversity (patterning), and the physical moulding of these newly formed progenitors into a functional shape (morphogenesis). Despite the intrinsic challenges of transmitting and integrating information across scales, from molecules to cells to tissues and back, developmental programs are remarkably robust and mostly self-organised – akin to building a skyscraper without an architect or blueprint. Understanding how biological systems achieve this incredible feat of multiscale organisation will yield new insights into regenerative processes, engineering of organoid technologies or cancer biology, and will reveal the fundamental design principles of life.

Morphogens (e.g. BMP, Wnt, Nodal, or FGF) – a term coined by Alan Turing in his seminal 1952 theory on morphogenetic patterning – are secreted molecules shown to play highly conserved roles in tissue patterning in various developmental contexts. In the past decade, research has also brought significant advances to our understanding of the mechanical forces and cellular behaviours (e.g., active migration, junctional rearrangement, division, extrusion, and shape changes) that shape developing tissues (1). Building on this body of work, the Pinheiro lab is tackling the next frontier, namely how the distinct signalling inputs that regulate tissue patterning are integrated, in space and time, with the mechanical cues organising morphogenesis.

Recent synthetic approaches have suggested a key role for morphogen signalling in controlling both patterning and morphogenetic programs. Accordingly, in the presence of a minimal set of molecular cues, pluripotent stem cells self-assemble into embryonic or organ-like structures. During her postdoctoral work, Pinheiro established, in collaboration with a PhD student, a novel zebrafish-derived gastruloid system (2). These gastruloids recapitulate the patterning and morphogenesis of intact embryos up to a remarkable degree, in a signalling-dependent manner. While this supports a crucial role for morphogens in coordinating the acquisition of pattern and form, how this coupling is mechanistically achieved remains an open question.

Identifying a mechanical role for morphogen signals during gastrulation

Gastrulation is an excellent model to address this fundamental question since it is the first major morphogenetic event of embryogenesis, during which the germ layers (ectoderm, mesoderm, and endoderm) are both specified and reshaped (3). Pinheiro's recent work identified a **key mechanical role** for Nodal signals, well-established morphogens determining mesendoderm

DIANA PINHEIRO

PHD:
INSTITUTE CURIE, FRANCE
(2016)

POSTDOCTORAL RESEARCH:
ISTA – INSTITUTE OF SCIENCE AND TECHNOLOGY, AUSTRIA

GROUP LEADER:
IMP VIENNA (2022)

patterning, in orchestrating the tissue's internalisation movements (4). On the one hand, graded Nodal signalling specifies a small fraction of highly protrusive leader cells needed to drive tissue-scale internalisation. On the other hand, Nodal signalling enforces a code of heterotypic, or preferential, adhesion coupling leaders to their immediate followers. Integrating this dual mechanical role of Nodal signalling into minimal active-particle simulations quantitatively predicts both physiological and experimentally perturbed gastrulation movements. This work provided a conceptual framework for how a morphogen gradient can couple germ layer patterning and morphogenesis during gastrulation, and highlights the need to understand the mechanisms by which Nodal, and more broadly, morphogen signalling can simultaneously encode such diversity of biological functions.

Outlook

The Pinheiro lab is interested in the idea that the complex temporal dynamics displayed by single (and multiple) morphogen gradients are key to understand their ability to simultaneously encode the diversity of cell fates and mechanical properties. Some key questions in the lab include:

i) How do cells integrate dynamic and combinatorial morphogen signalling into well-defined patterns of mechanical behaviours, such as cell migration or adhesion?
ii) Can we quantify how precise this signal integration is? Could emergent mechanical properties of tissue help buffer against developmental noise?
iii) How do mechanical forces and signalling cooperate to give rise to dynamic morphogen gradients? Are there feedbacks?

To gain insights into the molecular and biophysical mechanisms linking morphogen signalling, tissue patterning, and morphogenesis, the Pinheiro lab focuses on vertebrate gastrulation in zebrafish embryos and human embryonic stem cell colonies, previously shown to self-organise into the three germ layers upon geometric confinement and BMP stimulation. The lab has chosen a highly interdisciplinary approach, employing a combination of genetic, cell biological, biochemical, optogenetic, and biophysical tools.

GROUP MEMBERS:

SELECTED PUBLICATIONS

1.
Pinheiro, D., and Bellaïche, Y. (2018). ***Mechanical force-driven adherens junction remodeling and epithelial dynamics.*** *Developmental Cell*, 47(1), 3–19. doi: 10.1016/j.devcel.2018.09.014.

2.
Schauer, A., Pinheiro, D., Hauschild, R., and Heisenberg, C. P. (2020). ***Zebrafish embryonic explants undergo genetically encoded self-assembly.*** *eLife*, 9. doi: 10.7554/eLife.55190.

3.
Pinheiro, D., and Heisenberg, C. P. (2020). ***Zebrafish gastrulation: Putting fate in motion.*** *Current Topics in Developmental Biology,* 136, 343–375. doi: 10.1016/bs.ctdb.2019.10.009. Epub 2019 Dec 27.

4.
Pinheiro, D., Kardos, R., Hannezo, É. and Heisenberg, C. P. (2022). ***Morphogen gradient orchestrates pattern-preserving tissue morphogenesis via motility-driven unjamming.*** *Nature Physics*, 18, 1482–1493. doi: 10.1038/s41567-022-01787-6.

CLEMENS PLASCHKA

Which molecular mechanism drive human mRNA packaging and export?

PLASCHKA LAB 2018–2022

Which molecular mechanism drive human mRNA packaging and export?

Our genetic information is stored in DNA inside the cell nucleus and used to make proteins in the cell cytoplasm. Messenger RNA (mRNA) transfers this information from DNA to protein across these two cellular compartments, a process that involves multiple molecular machines. Together, these machines ensure that only correctly made and mature mRNA moves to the cytoplasm. However, individual mature mRNAs are very different, and it remains unclear how a key machine, the transcription-and-export complex (TREX), identifies and distinguishes diverse mRNAs from their immature precursors. Over the past four years, the Plaschka lab has discovered how TREX recognises and organises mRNA in three dimensions, leading to new models for how human mRNA is made and regulated.

During nuclear mRNA maturation, the nascent precursor mRNA is matured through capping, splicing, and cleavage and polyadenylation. At each successful step, a series of unique proteins bind to the mature mRNA to form a mature ribonucleoprotein complex (mRNP). TREX then recognises specific mRNP-bound proteins, called mRNA maturation marks, and enables the global mRNA export factor to be loaded onto the mRNP. This licenses the mRNP for nuclear export (1).

The correct maturation of mRNA and mRNA export from the nucleus are key steps of eukaryotic gene expression. However, the mechanisms and dynamics of how mRNAs are matured, recognised, and licensed for export are poorly understood. In 2018, the Plaschka lab set out to address these questions in the human system, using an integrative structural biology approach coupled with *in vitro* and *in vivo* assays.

Structure of the core TREX complex

At the heart of recognising and licensing an mRNP for nuclear export is the multi-protein complex TREX, which is conserved from yeast to humans. As a first step towards understanding the structural basis of TREX activities, the Plaschka lab determined the cryo-electron microscopy (cryo-EM) structure of the 28-subunit human TREX subcomplex THO-UAP56 (2). By combining the new THO-UAP56 cryo-EM structure with *in vitro* biochemical data and endogenous purifications, the lab proposed that multivalent interactions between TREX, the mRNA export factor, mRNA proteins, and mRNA may ensure the specificity and efficiency of export licensing.

CLEMENS PLASCHKA

PHD:
LMU MUNICH, GERMANY (2015)

POSTDOCTORAL RESEARCH:
MAX PLANCK INSTITUTE FOR BIOPHYSICAL CHEMISTRY, GÖTTINGEN, GERMANY (2015)
AND MRC LABORATORY OF MOLECULAR BIOLOGY, CAMBRIDGE, UNITED KINGDOM (2016)

GROUP LEADER:
IMP, VIENNA (2018)

GROUP MEMBERS:

Nuclear mRNA recognition and packaging by TREX

The isolated THO-UAP56 structure gave the Plaschka lab an entry point to study how TREX recognises an mRNP. The lab combined biochemistry with structural analyses of reconstituted and endogenous TREX-mRNP complexes to demonstrate how human mRNAs can be recognised and packaged by the complete TREX complex at macroscopic and microscopic mRNP scales (3).

At the macroscopic scale, the lab observed that native mRNPs – part of TREX-mRNPs – form compact globules. The mRNP globules in turn are coated on their surface by one or more 32-subunit TREX complexes. Thus, TREX may spatially confine mRNPs during their maturation and export factor loading, and protect the mRNP from the emergence of harmful RNA-DNA hybrids, called R-loops. Moreover, biochemical data and an additional cryo-EM structure of endogenous TREX-mRNP contacts revealed that TREX-mRNP-binding is achieved through multivalent interactions between the TREX subunits UAP56 and ALYREF with mRNA and mRNA-bound exon junction complexes (EJC), a marker of spliced mRNPs. This could explain how TREX-mediated packaging remains specific towards mRNAs and how packaging can generally scale with mRNA length, as longer mRNAs tend to contain more introns, and hence EJCs.

At the microscopic scale, the Plaschka lab revealed how the TREX subunit ALYREF directly recognises and multimerises EJCs, providing insights into mRNP packaging. These results are based on *in vitro* data and a cryo-EM structure of a reconstituted TREX-EJC-RNA complex. The lab's combined data established a close link between mRNP recognition and packaging and helped to explain diverse TREX activities in mRNA biogenesis via a single mechanism, the chaperoning of nascent mRNAs. Further, the three-dimensional organisation of human mRNPs as 'globules' has broad implications for mRNA biogenesis and its transport through the nucleus and nuclear pore complex.

Outlook

In the coming years, the Plaschka lab aims to mechanistically understand the final steps of nuclear mRNA biogenesis. Specifically, the lab hopes to understand the biophysical rules for mRNP packaging and how an mRNP is made competent for nuclear export after recognition and packaging by TREX.

SELECTED PUBLICATIONS

1.
Vorländer, M. K., Pacheco Fiallos, B., and Plaschka, C. (2022). ***Structural basis of mRNA maturation: Time to put it together.*** *Current Opinion in Structural Biology,* 75, 102431. doi: 0.1016/j.sbi.2022.102431. Epub 2022 Aug 2.

2.
Pühringer, T., Hohmann, U., Fin, L., Pacheco-Fiallos, B., Schellhaas, U., Brennecke, J., and Plaschka, C. (2020). ***Structure of the human core transcription-export complex reveals a hub for multivalent interactions.*** *eLife,* 9, e61503. doi: 10.7554/eLife.61503.

3.
Pacheco-Fiallos, B., Vorländer, M. K., Riabov-Bassat, D., Fin, L., O'Reilly, F. J., Ayala, F., Schellhaas, U., Rappsilber, J., and Plaschka, C. (2023) ***Nuclear mRNA recognition and packaging by the human transcription-export complex.*** *Nature,* in press.

ALEXANDER STARK

How do genomic DNA and cellular proteins mediate gene regulation?

STARK LAB 2017–2022

How do genomic DNA and cellular proteins mediate gene regulation?

Transcription – the process of copying genomic DNA into mRNA – is a fundamentally important process in life. It enables one genome to give rise to the many specialised cell types of our body, which differ in form and function. The Stark lab studies how gene-regulatory information is encoded in genomic regulatory elements and how cellular proteins are used to read and decode this information. Using an interdisciplinary approach with systematic genome-wide experiments, computational analyses, and deep learning, the lab identified an intricate code of specificity between different types of regulatory elements and proteins and, for the first time, designed synthetic regulatory enhancer elements *de novo*.

All multicellular life depends on differential gene expression. This expression is predominantly regulated at the transcriptional level by transcription factors (TFs) and cofactor proteins that engage with genomic gene-regulatory elements. The Stark lab has a long-standing interest in understanding the regulatory information in genomic enhancer and promoter elements and how the DNA sequences of these elements encode transcription activating and repressing cues. The lab also works to understand the proteins that mediate regulation and how all these components function together.

Between 2017 and 2022, the Stark lab pursued three main scientific goals:

i. understand and mechanistically explain regulatory cues and compatibilities in fly and human cells
ii. predict and design enhancers using computational deep learning approaches
iii. develop novel directions and projects exploring transcriptional repression and regulatory peptide modules

Regulatory compatibilities in transcription control

In 2015, the Stark lab uncovered initial evidence showing that not all enhancers or transcription-activating cofactor proteins can activate all promoters. Instead, they found that distinct compatibilities exist, both between enhancers and promoters (Zabidi *et al.*, 2015) and between cofactors and promoters (Stampfel *et al.*, 2015). In the years that followed, the Stark lab discovered that regulatory compatibilities are widespread between cofactors and promoters, functionally defining distinct promoter types that vary in sequence and chromatin (1).

The existence of these compatibilities implies that the mechanisms by which promoters recruit and activate RNA polymerase II must also differ between promoter types. Using DNA-affinity purification coupled with mass spectrometry, the Stark lab demonstrated that different types of *Drosophila* promoters recruit distinct sets of

ALEXANDER STARK

PHD:
EMBL HEIDELBERG,
GERMANY (2004)

POSTDOCTORAL RESEARCH:
EMBL HEIDELBERG,
GERMANY, MIT,
AND HARVARD, USA

GROUP LEADER:
IMP, VIENNA (2008)

SENIOR GROUP LEADER:
IMP, VIENNA (2015)

GROUP MEMBERS:

regulators. Rapid protein depletion and nascent transcription assays validated that different promoters indeed depend on different factors and suggest that mechanisms of initiation vary (Serebreni *et al.*, 2022). In addition, they found that different *Drosophila* promoter types depend on different chromatin remodelers, which create distinct chromatin structures (2).

Using rapid cofactor degradation and enhancer-activity screening, the Stark lab demonstrated that distinct human enhancer types exist, which vary in the cofactors they use. Unexpectedly, they discovered that P53-mediated transcription is insensitive to mediator depletion and that TATA-box promoters do not require Brd4 (3).

Transcription-regulatory proteins and their activating and repressing domains

Transcription factor and cofactor proteins mediate regulatory activities and have DNA-binding as well as transcription activating and repressing functions. The Stark lab developed a high-throughput method to identify peptides that mediate these activities (Arnold *et al.*, 2018) and screened a comprehensive peptide library for repressive protein domains (RDs) in fly cells. This screen revealed a comprehensive set of RDs, shared peptide motifs and the corepressor proteins recruited by these motifs (4).

Unexpectedly, they also found that well-known corepressor proteins were not able to promiscuously and dominantly repress all enhancers. Rather, they discovered that distinct corepressor–enhancer compatibilities exist and that certain enhancers are resistant to some corepressors but not others. The sensitivity and resistance of enhancers seem to be mediated by enhancer-bound transcription factor proteins and could be modulated by mutagenesis of the transcription factors' binding sites (Jacobs *et al.*, 2022).

Convolutional neural networks to model enhancer activities

A major aim in modern biology and a key goal of the Stark lab's work is the computational modelling of enhancers to allow the prediction of enhancer activities from the DNA sequence and the *de novo* design of enhancers. The Stark lab has established DeepSTARR, a convolution neural network model to predict enhancer activity and strength in the *Drosophila* S2 model cell line directly from the DNA sequence. This lead to the discovery of various important syntax features, which they validated and researched further (Reiter *et al.*, 2022), and enabled the design of synthetic enhancers with defined strengths (5). The modelling of enhancer activities and various chromatin properties across different cell types in flies and mice will remain a major goal of the lab.

Outlook

Over the next years, the Stark lab aims to determine the DNA-sequence rules of enhancers in various cellular contexts as well as how chromatin properties, enhancer – enhancer cooperativity, and transcription bursting parameters are sequence-encoded. They will also develop novel tools and approaches and work towards understanding repression and silencing.

SELECTED PUBLICATIONS

1.
Haberle, V., Arnold, C. D., Pagani, M., Rath, M., Schernhuber, K., and Stark, A. (2019). ***Transcriptional cofactors display specificity for distinct types of core promoters.*** *Nature,* 570(7759), 122–126. doi: 10.1038/s41586-019-1210-7. Epub 2019 May 15.

2.
Hendy, O., Serebreni, L., Bergauer, K., Muerdter, F., Huber, L., Nemčko, F., and Stark, A. (2022). ***Developmental and housekeeping transcriptional programs in Drosophila require distinct chromatin remodelers.*** *Molecular Cell*, 82(19), 3598–3612.e7. doi: 10.1016/j.molcel.2022.08.019. Epub 2022 Sep 15.

3.
Neumayr, C., Haberle, V., Serebreni, L., Karner, K., Hendy, O., Boija, A., Henninger, J. E., Li, C. H., Stejskal, K., Lin, G., Bergauer, K., Pagani, M., Rath, M., Mechtler, K., Arnold, C. D., and Stark, A. (2022). ***Differential cofactor dependencies define distinct types of human enhancers.*** *Nature*, 606(7913), 406–413. doi: 10.1038/s41586-022-04779-x. Epub 2022 Jun 1.

4.
Klaus, L., de Almeida, BP., Vlasova, A., Nemčko, F., Schleiffer, A., Bergauer, K., Hofbauer, L., Rath, M., Stark, A. (2022). ***Systematic identification and characterization of repressive domains in Drosophila transcription factors.*** *The EMBO Journal,* 42, e112100. doi: 10.15252/embj.2022112100. Epub 2022 Dec 22.

5.
de Almeida, B. P., Reiter, F., Pagani, M., and Stark, A. (2022). ***DeepSTARR predicts enhancer activity from DNA sequence and enables the de novo design of synthetic enhancers.*** *Nature Genetics,* 54(5), 613–624. doi: 10.1038/s41588-022-01048-5. Epub 2022 May 12.

ELLY TANAKA

What are the molecular mechanisms of regeneration?

TANAKA LAB 2017–2022

What are the molecular mechanisms of regeneration?

Salamanders have the extraordinary ability to regrow complete body parts, whether it is a lost limb or an injured spinal cord. The Tanaka lab uses this remarkably regenerative animal as a model to understand how successful regeneration can occur in vertebrates. In recent years, the lab has demonstrated that a blood protein signals the onset of cell proliferation during limb regeneration. The researchers also showed that fibroblasts – cells that in humans normally form scars – instead form stem cells in salamanders, which can regenerate new limbs. In the process of studying regeneration, the Tanaka lab has sequenced and decoded axolotl and lungfish genomes – the largest genomes fully sequenced so far.

Molecular genetics of regenerative tetrapods and their ancestors

The Tanaka lab uses a laboratory-bred species of salamander, the axolotl, to study regeneration at the molecular level. Capitalising on new developments in gene editing, the lab has elucidated the role of muscle stem cells in regeneration and development by deleting the axolotl Pax7 gene and tagging the Pax7 gene (1). This work went hand-in-hand with sequencing the giant axolotl genome in collaboration with Eugene Myers from the Max Planck Institute of Molecular Cell Biology and Genetics in Germany. The axolotl genome is ten times larger than the human genome, so this effort was made possible by implementing cutting-edge single-molecule long-read sequencing methods.

In collaboration with Axel Meyer from the University of Konstanz and Manfred Schartl from the University of Würzburg, the Tanaka lab also sequenced the genome of the lungfish, the closest living ancestor to four-legged animals. This sequencing marked a milestone in the decoding of genomic features related to the evolution of limbs and their regeneration.

Single-cell analysis of stem cell formation during regeneration

Limb regeneration involves the development of a stem cell zone. How these stem cells form is an important question. The Tanaka lab showed that a blood protein, bone morphogenetic protein 4/7, becomes activated by clotting enzymes and then signals to limb cells to proliferate. A key responding cell type is fibroblasts.

In collaboration with Barbara Treutlein at the ETH Zürich, the Tanaka lab performed transcriptome sequencing of thousands of single cells during regeneration (2). This work showed that by progressing through an inflammatory response, followed by degradation of extracellular matrix

ELLY TANAKA

PHD:
UCSF, USA (1993)

POSTDOC:
UCL, LONDON, UK

GROUP LEADER:
MPI-CBG, DRESDEN, GERMANY (1999)

PROFESSOR:
DFG-CRTD AT TU DRESDEN, GERMANY (2008)

DIRECTOR:
DFG-CRTD AT TU DRESDEN, GERMANY (2014)

SENIOR GROUP LEADER:
IMP VIENNA (2016)

GROUP MEMBERS:

proteins, axolotl fibroblasts lose their adult character and convert into stem cells, a process called dedifferentiation. This work was highlighted by the journal *Science* in its 2018 'Breakthrough of the Year' award.

Extending this single-cell work into the axolotl brain showed that a similar injury response is initiated during axolotl brain regeneration (3). The lab also extended their studies to a non-regenerative amphibian relative, the African clawed frog, *Xenopus laevis*, in which they showed that dedifferentiation does not occur because the fibroblasts themselves are incapable of turning into stem cells (4).

Stem cell approaches to blinding diseases

The Tanaka lab also uses human stem cells to develop approaches to cure eye diseases such as age-related macular degeneration (AMD). The lab developed a protocol to differentiate human stem cells into retinal pigment epithelium (RPE) in the cell culture dish, providing a model for human eye research. RPE cells normally clear toxic waste from the eye and this functionality could be reproduced in cultured cells. This waste-clearing function declines with age and is thought to be a cause of AMD. A proof-of-principle drug screen on the cultured RPE identified factors that can increase this waste absorbance (5).

Outlook

Fibroblast dedifferentiation is a key attribute and driver of successful regeneration that is missing in non-regenerative animals. In the coming years, the Tanaka lab will dissect the molecular machinery of dedifferentiation, what is missing in frogs and mammals, and how it may be instated in mammalian cells. Using the human RPE system, the lab will develop approaches to regenerate aged human RPE.

SELECTED PUBLICATIONS

1.
Nowoshilow, S., Schloissnig, S., Fei, J. F., Dahl, A., Pang, A. W. C., Pippel, M., Winkler, S., Hastie, A. R., Young, G., Roscito, J. G., Falcon, F., Knapp, D., Powell, S., Cruz, A., Cao, H., Habermann, B., Hiller, M., Tanaka, E. M., and Myers, E. W. (2018). ***The axolotl genome and the evolution of key tissue formation regulators.*** *Nature,* 554(7690), 50–55. doi: 10.1038/nature25458. Epub 2018 Jan 24. Erratum in: 2018. Nature, 559(7712).e2. doi: 10.1038/s41586-018-0141-z

2.
Gerber, T., Murawala, P., Knapp, D., Masselink, W., Schuez, M., Hermann, S., Gac-Santel, M., Nowoshilow, S., Kageyama, J., Khattak, S., Currie, J. D., Camp, J. G., Tanaka, E. M., and Treutlein, B. (2018). ***Single-cell analysis uncovers convergence of cell identities during axolotl limb regeneration.*** *Science,* 362(6413), eaaq0681. doi: 10.1126/science.aaq0681. Epub 2018 Sep 27.

3.
Lust, K., Maynard, A., Gomes, T., Fleck, J. S., Camp, J. G., Tanaka, E. M., and Treutlein, B. (2022). ***Single-cell analyses of axolotl telencephalon organization, neurogenesis, and regeneration.*** *Science,* 377(6610), eabp9262. doi: 10.1126/science.abp9262. Epub 2022 Sep 2.

4.
Lin, T. Y., Gerber, T., Taniguchi-Sugiura, Y., Murawala, P., Hermann, S., Grosser, L., Shibata, E., Treutlein, B., and Tanaka, E. M. (2021). ***Fibroblast dedifferentiation as a determinant of successful regeneration.*** *Developmental Cell*, 56(10), 1541–1551.e6. doi: 10.1016/j.devcel.2021.04.016.

5.
Schreiter, S., Vafia, K., Barsacchi, R., Tsang, S. H., Bickle, M., Ader, M., Karl, M. O., Tanaka, E. M., and Almedawar, S. (2020). ***A human retinal pigment epithelium-based screening platform reveals inducers of photoreceptor outer segments phagocytosis.*** *Stem Cell Reports*, 15(6), 1347–1361. doi: 10.1016/j.stemcr.2020.10.013. Epub 2020 Nov 25.

JORIS VAN DER VEEKEN

How are T cell activation states regulated at the transcriptional level?

VAN DER VEEKEN 2021–2022

How are T cell activation states regulated at the transcriptional level?

T cells are central cellular components of the vertebrate adaptive immune system. T cells can differentiate into effector cells with divergent properties tailored for protection against different types of pathogens and tumours. Naïve CD4 T cells can differentiate into Th1, Th2, Th17, and Tfh cells whereas naïve CD8 T cells give rise to cytotoxic T cells. A dedicated subset of immunosuppressive regulatory T cells enforces immunological tolerance and prevents autoimmunity. Additionally, T cells can transiently adapt their functionality to changing environmental conditions. The van der Veeken lab is interested in understanding the transcriptional regulation of T cell activation states in settings of infection, autoimmunity, and cancer.

The van der Veeken lab was established at the IMP in 2021. Joris van der Veeken previously worked as a PhD student and postdoctoral fellow in the lab of Alexander Rudensky at the Memorial Sloan Kettering Cancer Center in New York. There, his research focused on two distinct types of T cell responses: pro-inflammatory effector T cell responses to viral infections, and tolerogenic T cell responses to self and non-self antigens.

Natural genetic variation reveals key features of anti-viral T cell programming

In response to an acute viral infection, CD4 and CD8 T cells undergo rapid proliferative expansion and acquire pro-inflammatory and cytolytic effector functions that help clear the invading pathogen. T cell activation is associated with widespread transcriptional and epigenetic changes, induced by a large number of essential transcription factors (TFs). Redundancy, competition, and indirect effects make it difficult to dissect how these TFs affect T cell epigenetic identity.

To overcome this challenge, van der Veeken's work leveraged the millions of genetic polymorphisms present in laboratory (B6) and wild-derived inbred (CAST/EiJ) mouse strains (1, 2, 3). Using allele-specific readouts in B6/CAST F1 mice, his research defined the effects of cis-regulatory sequence variants on chromatin accessibility and TF binding. He found that Ets, Runx and TCF/LEF motif variants strongly affected chromatin accessibility in T cells, whereas most other TF-binding motifs had only minor effects. Representative TFs belonging to these three families occupied approximately 95 percent of the accessible genome in T cells and their activity could account for the majority of activation-induced chromatin accessibility changes.

These findings suggest that a small number of broadly active TFs dominantly shape chromatin accessibility in T cells.

JORIS VAN DER VEEKEN

PHD:
CORNELL UNIVERSITY, US
(2018)

POSTDOCTORAL RESEARCH:
MEMORIAL SLOAN
KETTERING CANCER
CENTER, US

GROUP LEADER:
IMP, VIENNA (2021)

GROUP MEMBERS:

This also implies that many of the previously identified TFs essential for anti-viral T cell responses carry out highly specific gene regulatory functions that affect only a limited number of genes.

Foxp3-dependent and -independent functions of regulatory T (Treg) cells

In parallel studies, van der Veeken focused on understanding the differentiation and function of Treg cells and their role in maintaining immune homeostasis. Treg cells are a subset of CD4 T cells that express the lineage-defining transcription factor (TF) Foxp3 and continuously suppress the activation of self-reactive T cells to prevent the onset of lethal multi-organ autoimmunity. By analysing Foxp3 'reporter-null' cells, van der Veeken found that Foxp3 regulates only a very small fraction of the regulatory elements to which it binds (4). However, many chromatin regions not bound by Foxp3 are differentially accessible in its absence. Van der Veeken found that Foxp3-dependent repression of a major chromatin remodelling TF, TCF1, could account for many of Foxp3's indirect gene regulatory functions. Thus, Foxp3 seems to control Treg cell identity in a largely indirect manner by controlling the activity of a few critical intermediaries, including TCF1.

In addition to suppressing self-reactivity, Treg cells also prevent harmful immune responses to innocuous foreign entities such as food and commensal microbes. These two types of tolerance are thought to rely on developmentally distinct Treg cell subsets. Treg cells arising in the thymus ('tTreg' cells) are thought to mediate tolerance to self, while Treg cells induced in the secondary lymphoid tissues (peripherally induced 'pTreg' cells) have been implicated in suppressing immune responses to commensal microbes and dietary antigens. To understand the transcriptional and functional differences between these two subsets, van der Veeken developed a genetic tracing strategy that enabled, for the first time, the unbiased identification and isolation of polyclonal pTreg cells based on their developmental history (5). This work revealed that in contrast to their thymically-derived counterparts, pTreg cells can differentiate in the absence of functional Foxp3 protein and even have Foxp3-independent suppressive functions.

Outlook

Ongoing work in the van der Veeken lab leverages chemical-genetic protein degradation to gain new insights into the molecular mechanisms underlying T cell activation and differentiation states. The lab has generated new genetic mouse models to define the gene regulatory functions of key TFs implicated in Treg cell development and T cell responses to chronic viral infection.

SELECTED PUBLICATIONS

1.
van der Veeken, J., Zhong, Y., Sharma, R., Mazutis, L., Dao, P., Pe'er, D., Leslie, C. S., and Rudensky, A. Y. (2019). ***Natural Genetic Variation Reveals Key Features of Epigenetic and Transcriptional Memory in Virus-Specific CD8 T Cells.*** *Immunity,* 50(5), 1202–1217.e7. doi: 10.1016/j.immuni.2019.03.031. Epub 2019 Apr 23.

2.
Pritykin, Y., van der Veeken, J., Pine, A. R., Zhong, Y., Sahin, M., Mazutis, L., Pe'er, D., Rudensky, A. Y., and Leslie, C. S. (2021). ***A unified atlas of CD8 T cell dysfunctional states in cancer and infection.*** *Molecular Cell,* 81(11), 2477–2493.e10. doi: 10.1016/j.molcel.2021.03.045. Epub 2021 Apr 22.

3.
Zhong, Y., Walker, S. K., Pritykin, Y., Leslie, C. S., Rudensky, A. Y., and van der Veeken, J. (2022). ***Hierarchical regulation of the resting and activated T cell epigenome by major transcription factor families.*** *Nature Immunology,* 23(1), 122–134. doi: 10.1038/s41590-021-01086-x. Epub 2021 Dec 22.

4.
van der Veeken, J., Glasner, A., Zhong, Y., Hu, W., Wang, Z. M., Bou-Puerto, R., Charbonnier, L. M., Chatila, T. A., Leslie, C. S., and Rudensky, A. Y. (2020). ***The Transcription Factor Foxp3 Shapes Regulatory T Cell Identity by Tuning the Activity of trans-Acting Intermediaries.*** *Immunity,* 53(5), 971–984.e5. doi: 10.1016/j.immuni.2020.10.010. Epub 2020 Nov 10.

5.
van der Veeken, J., Campbell, C., Pritykin, Y., Schizas, M., Verter, J., Hu, W., Wang, Z. M., Matheis, F., Mucida, D., Charbonnier, L. M., Chatila, T. A., and Rudensky, A. Y. (2022). ***Genetic tracing reveals transcription factor Foxp3-dependent and Foxp3-independent functionality of peripherally induced Treg cells.*** *Immunity,* 55(7), 1173–1184.e7. doi: 10.1016/j.immuni.2022.05.010. Epub 2022 Jun 13.

MANUEL ZIMMER

What can nematode worms tell us about brains and behaviour?

ZIMMER LAB 2017–2022

What can nematode worms tell us about brains and behaviour?

How do the nerve cells in our brains connect with each other to collectively generate perceptions, thoughts, and actions? In recent years, the Zimmer lab has made major advances to answer this question by taking a holistic approach using the small nematode worm, *C. elegans*. Through visualising the worm's brain activity in unprecedented detail, the lab revealed how the activity of entire neuronal networks relies on their architectural building blocks embodied in the animals' connectome. Moreover, the lab found that brain dynamics are highly organised, which corresponds to a hierarchical organisation of behaviour.

Over the past decade, the Zimmer lab has pioneered novel 3D imaging techniques in *C. elegans* to quantify the model organism's behaviour with single-cell resolution in real-time (1). The first studies showed that the brains of immobilised animals are still vigorously active, engaging almost half of all neurons. These activities appeared highly organised, and many neurons shared similar activity patterns, occurring in repeating cycles. Experts in dynamical systems theory call such phenomena network attractors.

The Zimmer lab investigated the functions of these dynamics and found they correspond to action commands and their assembly into an action sequence, during which the worms switch between behavioural states, such as forward crawling, backward crawling, and turning. Thus, the network attractors correlate with an ordered sequence of specific behaviours (2). This work led the lab to address three fundamental questions:

i. How do other, more complex behaviours fit into this big picture?
ii. Why is it important to represent behaviour at the level of the whole brain?
iii. How do coordinated neuronal dynamics arise as a function of network architecture?

Behavioural hierarchies and neuronal dynamics

Animal behaviour is hierarchically organised in a way that our ongoing actions represent sub-components of other longer-lasting behavioural programs and goals. Here, this action cycle would be at the upper level of a hierarchy controlling other subordinate movements. Any neuronal mechanism underlying this organisation of behaviour previously remained elusive, but the Zimmer lab found that these hierarchies correspond to a ranking of neuronal dynamics where network-wide shared activity patterns correspond to behaviours on top of the hierarchy, and diverse and faster activity patterns correspond to the lower levels of the hierarchy. Such a principle could be widely applicable in animals with larger brains and even richer behavioural repertoire (3).

MANUEL ZIMMER

PHD:
EMBL HEIDELBERG
AND MPI OF
NEUROBIOLOGY
MUNICH, GERMANY
(2003)

POSTDOCTORAL RESEARCH:
UCSF AND ROCKEFELLER
UNIVERSITY, USA

GROUP LEADER:
IMP, VIENNA (2010)

GROUP MEMBERS:

Behavioural representations and sensory circuits

A surprising result of the Zimmer lab's earlier study was that information about behaviour reaches sensory circuits, which generally were thought to be exclusively involved in perception. But why should such neurons be modulated by behaviour? To address this problem, the lab focused on a carbon dioxide (CO_2) sensing circuit. They observed that worms smell one another via the CO_2 expelled by their own metabolism. But how do *C. elegans* distinguish their own CO_2 from that of other sources when they crawl backwards into their own self-produced plume of CO_2?

The lab found that an interneuron participating in global brain dynamics releases a neuro-hormone, tyramine (TA), during backward locomotion. TA binds to a receptor on the principal CO_2 sensory neuron, termed BAG, to inhibit it. In conclusion, one of the functions of behaviour-dependent modulation of sensory neurons is to cancel out the perception induced by the self-motion of the animal to draw the focus of attention to what happens in the environment (4).

Solving the structure-function problem of neuroscience

Next, the Zimmer lab set out to establish a functional connectome from whole-brain imaging data to indicate how *C. elegans* neurons interact with each other under experimental conditions. The lab applied graph-theoretical approaches to the anatomical connectome to find which features of connectivity matched best with correlated neuronal dynamics. The surprising results suggest that the strength of the direct synaptic connections between neurons does not matter. However, the network neighbourhood that surrounds two neurons best predicted how strongly these neurons would interact.

The Zimmer lab found that a key architecture for establishing such similarities of connectivity was the hub-organisation – a core of rich club neurons that are densely connected with each other as well as with the periphery of the network. When experimentally interfering with such rich club neurons, brain-wide coordination of brain dynamics was largely disrupted and neurons exhibited independent, erratic activity patterns. This study, therefore, revealed the architectural features of a neuronal network that establish brain-wide coordination of brain dynamics. The Zimmer lab posits that these principles apply to the brains of larger animals as well (5).

Outlook

In 2021, the Zimmer lab moved to the University of Vienna. Their current research focuses on new technologies that enable whole-brain imaging in animals that can freely navigate their environments without any constraints. This will enable the lab to uncover how animals integrate their own behaviours with sensory perception to generate decisions. The Zimmer lab is now exploring similar principles in a new model organism, the fish *Danionelle cerebrum*, which is among the smallest vertebrates known to science.

SELECTED PUBLICATIONS

1.
Schrödel, T., Prevedel, R., Aumayr, K., Zimmer, M., and Vaziri, A. (2013). ***Brain-wide 3D imaging of neuronal activity in Caenorhabditis elegans with sculpted light.*** *Nature Methods*, 10(10), 1013–1020. doi: 10.1038/nmeth.2637. Epub 2013 Sep 8.

2.
Kato, S., Kaplan, H. S., Schrödel, T., Skora, S., Lindsay, T. H., Yemini, E., Lockery, S., and Zimmer, M. (2015). ***Global brain dynamics embed the motor command sequence of Caenorhabditis elegans.*** *Cell*, 163(3), 656–669. doi: 10.1016/j.cell.2015.09.034. Epub 2015 Oct 17.

3.
Kaplan, H. S., Salazar Thula, O., Khoss, N., and Zimmer, M. (2020). ***Nested neuronal dynamics orchestrate a behavioral hierarchy across timescales.*** *Neuron*, 105(3), 562–576.e9. doi: 10.1016/j.neuron.2019.10.037. Epub 2019 Nov 27.

4.
Riedl, J., Fieseler, C., and Zimmer, M. (2022). ***Tyraminergic corollary discharge filters reafferent perception in a chemosensory neuron.*** *Current Biology*, 32(14), 3048–3058.e6. doi: 10.1016/j.cub.2022.05.051. Epub 2022 Jun 10.

5.
Uzel, K., Kato, S., and Zimmer, M. (2022). ***A set of hub neurons and non-local connectivity features support global brain dynamics in C. elegans.*** *Current Biology*, 32(16), 3443–3459.e8. doi: 10.1016/j.cub.2022.06.039. Epub 2022 Jul 8.

JOHANNES ZUBER

How can we find and understand cancer dependencies?

ZUBER LAB 2017–2022

How can we find and understand cancer dependencies?

Cancer arises from complex and heterogeneous mutations in over 500 cancer-associated genes, most of which are not directly 'druggable'. While this complexity poses a daunting challenge for the development of rational cancer therapies, individual mutations converge at the functional level to deregulate a limited number of fundamental cellular processes. Functional-genetic tools such as CRISPR/Cas9, advanced RNAi, and degron technologies, together with methods for time-resolved molecular profiling and imaging, have transformed our ability to decipher common pathomechanisms and explore new therapeutic concepts prior to drug development. The Zuber lab develops and applies these tools in three complementary lines of research:

Deciphering the regulation and regulatory function of oncogenic transcription factors

To maintain their aberrant identity, cancer cells depend on transcription factors (TFs) that normally control developmental programs. While TFs have long been viewed as 'undruggable targets', pathways controlling their expression, regulatory function, or turnover could provide alternative entry points for drug development.

To characterise such pathways, the Zuber lab has pioneered methods to investigate the regulation and regulatory function of TFs in a time-resolved manner. By combining auxin-inducible protein degradation (AID) and SLAM-seq, the lab has established a simple and scalable method for defining direct TF target genes (1). A first collaborative study with the lab of Stefan Ameres at the Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA) clarified gene regulatory functions of two transcriptional master regulators in cancer, MYC, and BRD4, and led to the foundation of QUANTRO Therapeutics as a joint spin-off of the IMP and IMBA. In parallel work, the Zuber lab has identified and characterised the transcription factor ERG as the key transcriptional target of EVI1/MECOM, which defines a particularly aggressive form of acute myeloid leukaemia (2).

To decipher pathways controlling the expression and turnover of MYC and other oncogenic TFs, the lab has established time-resolved FACS-based CRISPR screens that enable the systematic identification of regulators independent of essential protein functions. Besides specific regulators, the first FACS-based regulator screens uncovered an evolutionary conserved protein, AKIRIN2, that turned out to be the long-sought factor mediating nuclear proteasome import in animals (3). Collaborative structural studies with the Haselbach lab at the IMP revealed that AKIRIN2 forms homodimers that tightly bind to 20S and 26S proteasome complexes to mediate their nuclear import.

JOHANNES ZUBER

MD:
HUMBOLDT-UNIVERSITY OF BERLIN, GERMANY (2003)

POSTDOCTORAL RESEARCH AND CLINICAL FELLOW:
COLD SPRING HARBOR LABORATORY, NY, USA

GROUP LEADER:
IMP, VIENNA (2011)

SENIOR GROUP LEADER:
IMP, VIENNA (2020)

GROUP MEMBERS:

During mitosis, proteasomes are excluded from condensing chromatin and re-imported into newly formed daughter nuclei in a highly dynamic, strictly AKIRIN2-dependent process. Cells undergoing mitosis in the absence of AKIRIN2 become devoid of nuclear proteasomes, causing rapid accumulation of MYC and other nuclear proteins. Thus, the interaction between AKIRIN2 and the 20S core proteasome may enable the development of small molecule inhibitors that selectively block the nuclear proteasome compartment in proliferating cells.

Exploring metabolic dependencies and rational combination therapies

CRISPR/Cas9- and RNAi-based negative selection screens have revolutionised the search for molecular targets and biomarkers towards the development of rational cancer therapies. To fully harness the potential of this approach, the Zuber lab has developed optimised RNAi and CRISPR/Cas9 reagents and screening workflows (4) and applies them to systematically probe cancer-specific dependencies and gene-drug interactions in leukaemia, pancreatic cancer, and colon cancer models. One major focus is the study of metabolic adaptations and dependencies in cancer, which are investigated in close collaboration with the lab of Wilhelm Palm at the German Cancer Research Centre (DKFZ). In the first screens, the team discovered an uncharacterised transmembrane protein (TMEM251, renamed to LYSET) that is selectively required when cancer cells feed on extracellular proteins (5). LYSET was subsequently characterised as a core component of the lysosomal enzyme trafficking pathway and a promising molecular entry point to target one of the most common metabolic adaptations in cancer.

Mechanisms and candidate targets in T cell-mediated anti-tumour immunity

Beyond cancer-cell intrinsic dependencies, the Zuber lab is investigating mechanisms and candidate targets in T cell-mediated anti-tumour immunity. CRISPR/Cas9-based screens in genetically engineered cancer models and tumour-specific CD8 T cells have uncovered signalling pathways and transcriptional regulators that constrain anti-tumour immune responses and might be exploitable for boosting the activity of endogenous or CAR-engineered tumour-specific T cells.

Outlook

In current and future work, the Zuber lab aims to apply innovative functional-genetic approaches to decipher regulatory pathways and identify molecular targets in transcriptional gene regulation, cancer cell metabolism, and immune evasion, and ultimately use them to develop combinatorial concepts that will be needed to advance targeted into curative therapies.

SELECTED PUBLICATIONS

1.
J.J., Herzog, V.A., Reichholf, B., Cisneros, D.A., Hoffmann, T., Schlapansky, M.F., Bhat, P., von Haeseler, A., Kocher, T., Obenauf, A.C., Popow, J., Ameres, S.L. and Zuber, J. (2018). ***SLAM-seq defines direct gene-regulatory functions of the BRD4-MYC axis.*** *Science,* 360, 800-805. doi: 10.1126/science.aao2793. Epub 2018 Apr 5.

2.
Schmoellerl, J., Barbosa, I.A.M., Minnich, M., Andersch, F., Smeenk, L., Havermans, M., Eder, T., Neumann, T., Jude, J., Fellner, M., Ebert, A., Steininger, M., Delwel, R., Grebien, F. and Zuber, J. (2022). ***EVI1 drives leukemogenesis through aberrant ERG activation.*** *Blood.* doi: 10.1182/blood.2022016592. Online ahead of print.

3.
De Almeida, M., Hinterndorfer, M., Brunner, H., Grishkovskaya, I., Singh, K., Schleiffer, A., Jude, J., Deswal, S., Kalis, R., Vunjak, M., Lendl, T., Imre, R., Roitinger, E., Neumann, T., Kandolf, S., Schutzbier, M., Mechtler, K., Versteeg, G.A., Haselbach, D. and Zuber, J. (2021). ***AKIRIN2 controls the nuclear import of proteasomes in vertebrates.*** *Nature,* 599, 491-496. doi: 10.1038/s41586-021-04035-8. Epub 2021 Oct 28.

4.
Michlits, G., Jude, J., Hinterndorfer, M., de Almeida, M., Vainorius, G., Hubmann, M., Neumann, T., Schleiffer, A., Burkard, T.R., Fellner, M., Gijsbertsen, M., Traunbauer, A., Zuber, J. and Elling, U. (2020). ***Multilayered VBC score predicts sgRNAs that efficiently generate loss-of-function alleles.*** *Nature Methods,* 17, 708-716. doi: 10.1038/s41592-020-0850-8. Epub 2020 Jun 8.

5.
Pechincha, C., Groessl, S., Kalis, R., de Almeida, M., Zanotti, A., Wittmann, M., Schneider, M., de Campos, R.P., Rieser, S., Brandstetter, M., Schleiffer, A., Muller-Decker, K., Helm, D., Jabs, S., Haselbach, D., Lemberg, M.K., Zuber, J. and Palm, W. (2022). ***Lysosomal enzyme trafficking factor LYSET enables nutritional usage of extracellular proteins.*** *Science,* 378, eabn5637. doi: 10.1126/science.abn5637. Epub 2022 Oct 7.

Glimpses

NEW BUILDING, NEW ERA

The IMP opens the doors to its new home.

In March 2017 the IMP celebrated the opening of its new building on the Vienna BioCenter campus. More than 30 years prior, the institute was the first research organisation to move into a former industrial site next to Vienna's old slaughterhouses, in which the Vienna BioCenter would later develop. Over time, it became increasingly difficult for the original building to accommodate the needs of a living institute. When a nearby site became available, the IMP secured investment of 52 million Euro from its sponsor Boehringer Ingelheim, and work on the new building was soon underway.

After less than two years of construction, the IMP welcomed its 280-strong staff to the new site, where they have now been located for several years. The IMP's new home combines extraordinary design with the functionality required of a modern research institute.

Bridging the architectural gap

The architecture of the building was underpinned by three guiding principles: enhanced communication, greater sustainability, and the flexibility to adapt to future needs. The layout of the new building fosters collaboration and the exchange of ideas, both for IMP staff and the wider Vienna BioCenter community. The neighbouring organisations of the Institute of Molecular Biotechnology (IMBA) and the Gregor Mendel Institute of Molecular Plant Biology (GMI) are now connected to the IMP building via a glass bridge, and the new lecture hall – the largest on campus, seating up to 280 people – is at the centre of many conferences and events.

The 15,000 square-metre building also closes the gap – in architectural terms – on other leading research institutes, placing the IMP firmly on the international map. The new design meets the diverse research demands of science, and strengthens the IMP's status as a key research establishment.

A flagship building

The prominent location of the new site, as well as its position as the flagship building on campus, symbolises the IMP as the heart and founding seed of the Vienna BioCenter. To celebrate the opening, over 200 guests attended a ceremony on 1 March 2017, including Austria's president Alexander van der Bellen, and many other dignitaries.

To officially open the building, Emmanuelle Charpentier – whose work at the Vienna BioCenter laid foundations for her discovery of the CRISPR-Cas9-System – cut a four-metre-long DNA double helix in lieu of a red ribbon. A rising curtain then revealed an audience of IMP colleagues, symbolically filling the new building with life – and curiosity.

The ceremony was followed by a 'scientific housewarming' in the form of a three-day conference in autumn. The conference comprised talks from speakers with close ties to the IMP, including two Nobel laureates: David Baltimore, who served as one of the IMP's first Scientific Advisory Board members in the 1980s, and Sir Paul Nurse, who joined the board in 2017. Talks covered topics such as immunology, epigenetics, and neurobiology.

This unique event combined academic excellence with the spirit of a family reunion, with IMP members from over three decades of research joining to mark this new era for the institute.

SUPPORTING SCIENCE FROM ALL ANGLES: SERVICES AND CORE FACILITIES

A key feature of the IMP is the amount and quality of support that its research groups enjoy: world-class facilities run by professional staff scientists and administrative support that allows scientists to focus on their research. And just like "all things IMP", the core facilities are constantly developed to stay at the forefront of technical advances.

Services available to IMP research groups cover a broad range of equipment and expertise. They are provided by facilities, often run jointly with our partnering institutes IMBA and GMI, or through the Vienna BioCenter Core Facilities (VBCF). In either case, the mission of any service facility is to put science first and to contribute their best to the IMP's research. Sometimes more than that: when Covid-19 disrupted operations in 2020, core facilities were re-aligned to support PCR testing, which eventually grew into a professional testing facility within the VBCF (see page 106).

Technology

To stay ahead of newest technologies, core facilities and their staff require continuing investment and development. This includes organisational changes. In the years leading up to 2022, the integration of core facilities between IMP, IMBA, and GMI was increased, now also backed by cross-institutional **project management**. By late 2022, facilities linked to infrastructure such as **IT**, **purchasing**, or **facility management** were grouped to operate for all three institutes plus the VBCF. Some services such as the VBCF's bioinformatics, preclinical imaging, or advanced microscopy ceased operations. On the other hand, many new tasks were taken up – and entire new facilities were established.

CLIP (Cloud Infrastructure Platform) is one of these new facilities, spearheaded by the **IT Department**. It provides fundamental high performance computing resources with low entry threshold. These resources comprise the HPC computer cluster itself, as well as support and training for its users. CLIP comprises approximately 200 computer nodes with 8000 CPU (central processing unit) cores and more than 30 terabyte working memory, as well as 120 CPU accelerators and approximately 250 terabyte shared flash storage. Unique for a facility at the Vienna BioCenter, CLIP is operated as a collaboration between the IMP, the Austrian Academy of Sciences (IMBA, GMI, HEPHY, SMI), the University of Vienna, and the Technical University of Vienna. In addition to this development, the IT Department led a flawless switch to home office during the Covid-19 pandemic, including the renewal of a Virtual Private Network (VPN). Since 2020,

the department has also implemented multiple cybersecurity features to protect IMP staff and their data, and energy-saving measures for the IT data centre.

Another recent investment highlight in IMP facilities is the **Krios G4**, opened by Nobel laureate Richard Henderson in March 2022. The Krios G4 is an advanced cryo-electron microscope with sophisticated optics. Its voltage of 300 kilovolts produces a high-energy electron beam that is in contact with the sample for an even shorter period of time than in older models, and thus causes less radiation damage. The device is housed in a room specially shielded from surrounding magnetic fields. The Krios G4 at the IMP is operated in collaboration with Boehringer Ingelheim.

The **Bioinformatics Facility** has implemented advances in high-throughput technologies, enabling molecular profiling across modalities and locations, and incorporated emerging technologies in data processing, such as artificial intelligence. The department is continuously adopting new data analysis techniques in close collaboration with the research groups.

Over the last six years, the **BioOptics Facility** has continued to pursue its mission to support research with flow cytometry and light microscopy imaging. Its microscopy portfolio has grown, including spectral and high-throughput instruments added to flow cytometry, light-sheet, super-resolution, and fluorescence life-time (FLIM) microscopy. The facility also supports rapid atomic force microscopy (AFM) and laser ablation. BioOptics keeps a strong focus on automation and workflow development integrating image analyses closely with microscopy. This involves new software and tools using artificial intelligence for both acquisition and analysis, as well as infrastructure for fast and reliable data transfer and powerful GPU (graphics processing unit) computation.

The **Molecular Biology Facility** developed and established strategies for the expression and production of proteins in high demand at the IMP (in particular DNA and RNA polymerases, ligases, growth and transcription factors, and recombinant antibodies), doubling its production since 2017. The facility offers recombinant protein production in *Escherichia coli* and *Pichia pastoris* and creates and maintains a rich collection of expression constructs and host strains. Since 2020, the facility produces supplements for tissue culture medium and developed an RNA beads isolation kit, which allowed sample preparation for 300,000 Covid-19 tests. The staff's experience with pathogen diagnosis was of crucial importance to develop the Covid-19 gargle test. To this day, these tests have been expanded to cover numerous pathogens and are routinely offered for human cells.

Formerly part of the Mass Spectrometry Facility, the **Peptide Synthesis Facility** is offering its services to the IMP, IMBA, and GMI since 2021. The facility produces peptides at various scales, ranging from nanomole scale SPOT synthesis to large scale with up to 250 micromoles. In addition to the natural 20 amino acids, the facility tailor-makes peptides with a variety of modifications, such as methylation, phosphorylation, or acetylation, as well as labelling the peptides with biotin or fluorophores. Staff in the Peptide Synthesis Facility also have expertise in purifying small (fluorophore) labelled molecules such as oligos and CoA.

A different kind of new is the **Proteomics Tech Hub**, formed at the end of 2022 as a spin-out of the Proteomics Facility. This unit conducts research and development in proteomics and serves as the anchor point of innovation in this field, while routine services remain with the **Proteomics Facility**. The Proteomics Tech Hub focuses on two major lines of research and technological innovation holding great relevance to the Vienna BioCenter community: single cell proteomics and crosslinking mass spectrometry. Single cell proteomics will allow to identify the determinants of specific phenotypes in single cells and will help unveil the secrets of cellular proteomic heterogeneity, transforming it from a source of noise to a source of discovery. Crosslinking mass spectrometry, on the other hand, is a crucial technology to decipher protein structures as well as protein-protein interactions. It provides invaluable clues to pinpoint mechanistic details of biological questions. More than 30 years of protein chemistry experience are twinned with a dedication to innovation and an uncompromising commitment to excellence. Leading expertise and equipment, with easy access for all scientists – maintaining the facilities' value as a top selling point for the IMP.

Research support

Publications sit at the core of academic research. The **Max Perutz Library** supports the IMP's mission to share all its discoveries with the scientific community and the public. The library services can be used by affiliates of the IMP, IMBA, and GMI. It maintains and develops literature collections and information services in support of present and future research as well as teaching needs of the institutes. A quiet reading room is available to all students and members of staff. The library provides and supports eLabJournal, a tool for keeping electronic laboratory notebooks. An introduction as well as advanced training is provided, either via hands-on or web-based modules. The library also offers support for administrative issues related to publishing, especially for questions regarding Open Access or publication fees.

Scientific publications also require effective visual communication. The **Graphics Department** has been boosting its services for scientists, increasingly embracing digital media, replacing printed versions of booklets, brochures, and conference programs, and setting benchmarks for efficiency and environmental friendliness. Video production has also been on the rise, from short interviews to animated content, bringing IMP research publications to life. Since 2017, the department has offered workshops on illustrations, poster design, image creation, and typographic design to researchers on campus. The future of the Graphics Department promises a progressive integration of artificial intelligence, enhancing efficiency and opening up new creative horizons.

Animal experiments continue to play a vital role in life sciences, as they provide the unique opportunity to study complex biological processes within a living organism. The **Comparative Medicine Facility** offers the most suitable animal models for the cutting-edge research questions asked at the IMP. Its mission is to advance research through the ethical care and use of laboratory animals. Research using nimal models requires the highest standards of animal welfare as responsibility of researchers and their institutions and as a fundamental prerequisite for reliable and robust research outcomes. In light of the "3R principles" of responsible animal research (replace, reduce, refine), the facility envisions several key initiatives: (1) expansion of the program for environmental enrichment of animal housing units, (2) augmented training programs for researchers and animal care staff for refinement of husbandry and experimental conditions, and (3) ongoing investment in advanced equipment and technology, including cryopreservation and rederivation efforts to reduce animal numbers.

The **Mechanical Engineering Centre** (formerly Workshop) provides technical solutions for scientific projects including design, development, and manufacturing of tailor-made prototypes and devices. To provide this service, the centre acquired a sandblasting machine, a laser cutting machine, a thermoforming machine, and a 3D printer. Besides the new circular table saw, a new CNC milling machine and multi-purpose precision lathe replaced 25-year-old machines. This expansion and modernisation of existing equipment enables the production of parts with greater flexibility, precision, and automation. The team is regularly trained to make optimal use of the available resources to provide scientists with the best possible support for individual projects.

Infrastructure and administration

The **Facility Management Department** cares for the IMP building, as well as the neighbouring building of IMBA and GMI. After the construction of the new IMP, the building's services and systems had to be started up, regulated, and adjusted for research operations. The architectural features of the building were optimised to save on energy (water, electricity, gas, and heating), and the department strives to push sustainability to the next level.

All structural changes and maintenance work aim to provide a safe and productive environment for uninterrupted research work. The department is also a crucial player in the organisation of conferences and other events.

Between 2017 and 2022, the department has taken on many projects, including a massive extension of the fish facility for the labs of Andrea Pauli and Diana Pinheiro, the installation of the Krios G4 in a tailor-made room, and the construction of an optics room for the lab of Francisco Balzarotti.

Throughout these infrastructural changes, the **Environment, Health, and Safety (EHS) Department** has been at the forefront of ensuring safety, legal compliance, and occupational health. Safety at the workplace is a top priority, evidenced by monthly safety tours, audits, and comprehensive safety instructions ensuring a secure work environment. Accident prevention and evaluation are integral, with guidelines established for various tasks, including chemical storage, personal protective equipment usage, and waste management. The department's proactive engagement with occupational health is reflected in ergonomic evaluations and consultations for each individual workplace, vaccination campaigns, lung and visual tests, or even a 24/7 Covid on-call service during the pandemic. Beyond traditional health measures, the department has

put a spotlight on wellbeing with several mental health initiatives and providing counselling resources on and off campus. The IMP strives to create a caring and supportive environment for young families, providing a safety lab and breastfeeding rooms. Furthermore, the department is addressing environmental sustainability, actively reducing the IMP's carbon footprint through collaborative efforts internally and with neighbouring companies.

Good science does not sprout on an empty stomach. The **Cafeteria**, shared between the IMP, IMBA, and GMI, provides up to a thousand hot, fresh lunches every day, in addition to a generous selection of salads. The cafeteria also covers ever-growing catering and event demand with multi-day catering up to evening buffets. During the Covid-19 pandemic, the cafeteria has resumed its activities a few weeks after the beginning of the first lockdown, providing staff with a feeling of normalcy. In recent years, the team has made further efforts to provide locally and sustainably sourced products, and to support Austrian companies. Re-usable containers have replaced single-use boxes and vegetarian days have increased, leaving only two meat days per week as of the end of 2022.

The **Human Resources and Grants Department** has processed a lot of changes between 2017 and 2022. The evolution of the IMP's organigram reflects this eventful period. Four research groups have left the institute, and six more have started, bringing new fields to the IMP's portfolio. Administrative departments have also been reorganised and realigned with individual research institutes. Staff numbers and diversity have seen an increase, with 268 employees in 2017 and 289 in 2022, with 33 nationalities represented in 2017 and 39 by the end of 2022.

In addition to Boehringer Ingelheim's generous core funding, IMP research groups have secured more than 44 million Euro in external funding during this period, including 14 million from 11 grants of the European Research Council.

The **Accounting Department** is responsible for various tasks related to financial management. One of the primary responsibilities is to collect and process financial records and prepare the annual financial statements as well as ensuring statutory compliance with accounting standards and tax regulations. Further, the department performs the accurate, timely billing, and correct settlement of payments together with the monthly financial reporting. All these functions together provide a solid financial data which reflects the results of all operations at the IMP and can be used in making business decisions.

The **Purchasing Department** is responsible for all purchasing activities for goods and services, operation of the storeroom, performing annual stocktaking, and numerous administrative tasks related to the core function of the department. In their daily work, they coordinate and cooperate with suppliers of goods and services, research groups, scientific services, and administrative departments, authorities, partner institutes, Boehringer Ingelheim, and auditing organisations.

MEMBERS:

INTERNATIONAL BIRNSTIEL AWARDS

Celebrating outstanding talent in molecular life sciences.

In 2019, the IMP presented the inaugural ceremony for the International Birnstiel Award for Doctoral Studies in the Molecular Life Sciences, a now annual event celebrating the research successes of up-and-coming scientists from across the world. Named in honour of the IMP's founding director and acclaimed molecular biologist Max Birnstiel, the awards are a flagship accolade for doctoral students in molecular life sciences.

"Max Birnstiel was not only a brilliant scientist, but also an excellent mentor for his students," recalls Meinrad Busslinger, IMP Deputy Director and trustee of the Max Birnstiel Foundation. "In this sense, to honour the excellent performance of PhD students with the Birnstiel Award is a part of Max Birnstiel's legacy and in the spirit of his philosophy."

The award targets doctoral students at an advanced stage of their PhD research who have contributed to an exceptional discovery in their field.

The selection committee comprises four IMP scientists who evaluate the nominees' research according to scientific quality, originality, and impact in molecular life sciences.

Celebrating successes

Each year, awardees are invited to a ceremony at the IMP to present their research and discuss their work with researchers from the IMP and the wider Vienna BioCenter community. Laureates are then presented with a trophy, a certificate, and a prize of 2,000 Euro.

For the first ceremony in 2019, Max Birnstiel's wife, Margaret Birnstiel, presented the three awards. A molecular biologist herself, Margaret Birnstiel was a long-standing friend and benefactor of the IMP, keeping close links with the institute until her death in 2021.

The inaugural awards received 130 nominations from institutions in Europe, the US, and Asia. Following this overwhelming success, the IMP increased the number of laureates from three to six for the 2020 awards. While the Covid-19 pandemic hindered plans for an on-site ceremony, awardees recorded their acceptance talks for viewing online instead.

In 2021, competition was even higher, with an almost global spread of nominees. "I have reviewed a lot of candidates in many different selection procedures," said one committee member at the decisive selection meeting. "But I have never seen such a line-up before."

The ceremony returned on-site at the IMP, with an opening address from Meinrad Busslinger. "It is in honour of Max Birnstiel that we named these awards," he said, "and it would please him immensely to witness the talent we have identified in his name."

Birnstiel Awardees 2019

EMILY BAYER
COLUMBIA UNIVERSITY

MOHAMED EL-BROLOSY
MAX PLANCK INSTITUTE FOR HEART AND LUNG RESEARCH

JUSTIN SILPE
PRINCETON UNIVERSITY

Birnstiel Awardees 2020

PATRICK CHITWOOD
MRC LABORATORY OF MOLECULAR BIOLOGY

ELIRAN KADOSH
HEBREW UNIVERSITY

FANGYU LIU
THE ROCKEFELLER UNIVERSITY

ANNA-KATHARINA PFITZNER
UNIVERSITY OF GENEVA

CHRISTOPHER REINKEMEIER
EUROPEAN MOLECULAR BIOLOGY LABORATORY

VAYU MAINI REKDAL
HARVARD UNIVERSITY

Birnstiel Awardees 2021

SANNE BOERSMA
HUBRECHT INSTITUTE

FANNY MATHEIS
THE ROCKEFELLER UNIVERSITY

ADI MILLMAN
WEIZMANN INSTITUTE OF SCIENCE

SANNE VAN NEERVEN
AMSTERDAM UNIVERSITY MEDICAL CENTERS AND ONCODE INSTITUTE

KRISTINA STAPORNWONGKUL
UNIVERSITY COLLEGE LONDON/THE FRANCIS CRICK INSTITUTE

GREGOR WEISS
ETH ZURICH

Birnstiel Awardees 2022

MARGARETE DIAZ CUADROS
HARVARD MEDICAL SCHOOL

CHRISTY HONG
UNIVERSITY MEDICAL CENTER GRONINGEN

IOANNIS SARROPOULOS
HEIDELBERG UNIVERSITY

MARTA SECZYNSKA
UNIVERSITY OF CAMBRIDGE

MICHAEL SKINNIDER
UNIVERSITY OF BRITISH COLUMBIA

ZHEXIN WANG
MAX PLANCK INSTITUTE OF MOLECULAR PHYSIOLOGY

SCIENTIFIC TRAINING

The Vienna BioCenter fosters the training of young scientists in a vibrant environment in one of the largest life science hubs in Europe. The institutes of the Vienna BioCenter, including the IMP, train students at the undergraduate level, the graduate level, and the post-graduate level.

Vienna BioCenter Summer School

The Vienna BioCenter Summer School was founded in 2010 and provides an excellent opportunity for approximately 30 undergraduate and Master's students per year to work side by side with leading researchers in the life sciences. The programme is highly competitive and funded by the Max Birnstiel Foundation. It celebrated its tenth anniversary with a symposium in summer 2019, bringing together 2019 participants with past cohorts for a series of scientific talk.

In 2020, IMP Group Leader David Keays moved his lab to Munich and stepped down as dean of the Summer School, replaced by IMP Senior Group Leader Andrea Pauli.

Collaborations with European universities

In the past years, the Scientific Training unit established partnerships with European universities, such as the University of Tübingen, Utrecht University, Freie Universität Berlin, and Paris Diderot University. The aim of these partnerships is to enable Master's students to receive high-quality training at the Vienna BioCenter, and in return to increase the campus' visibility abroad.

Vienna BioCenter PhD Program

The Vienna BioCenter PhD Program is a doctoral school of the University of Vienna and the Medical University of Vienna, in partnership with four research institutes: the IMP, the Institute of Molecular Biotechnology (IMBA) and the Gregor Mendel Institute (GMI), both of the Austrian Academy of Sciences, and Max Perutz Labs. The Program is highly collaborative and embedded in an exceptionally diverse and intersectoral framework. The Program's wide reach and international visibility allows for recruitment of top talent from across the world: the Program receives 1000 to 1600 inquiries per year, from which about 60 students are selected to join the labs on campus.

Participants are encouraged to take an active role in steering their projects and publishing their findings. In 2021, for example, about 30 peer-reviewed publications had a program participant as first author, 70 as co-author. In the past years, students have received competitive awards such as the Weintraub Award (2018, Annika Nichols; 2020, Anete Romanauska; 2022, Katarzyna Parys).

In 2020, the Vienna BioCenter PhD Program was merged with the Molecules of Life Program to establish the current doctoral school. The school is now home to about 250 PhD students and 70 faculty members who participate as supervisors.

Vienna BioCenter International Postdoc Program (VIP²)

The Vienna BioCenter International Postdoctoral Program (VIP²) is a postdoctoral fellowship program launched in April 2019. It is co-funded by the European Union's Horizon 2020 programme and supported by four research institutes on campus: the IMP, IMBA, GMI, and Max Perutz Labs.

The goal of VIP² is to act as a stepping stone for promising young scientists to launch their independent careers. VIP² operates under a two-mentor scheme, whereby fellows select a main academic mentor and a second mentor from academia, the biotechnology sector, or beyond. The program aims to give fellows the opportunity to establish a peer group, a network of colleagues to support their postdoc journey.

So far, 39 outstanding fellows were selected and joined 30 different labs on campus.

Vienna BioCenter Leadership Program

In 2022, the Vienna BioCenter Leadership Program was designed to strengthen mentorship and a sense of community amongst leaders on campus. It intends to provide inspiration, support, and concrete tools to continuously improve in our leadership roles. The first cohort will start the program in March 2023.

Transferrable skills and career development

A large portfolio of transferrable skills courses is offered to all trainees every year.

Between 2017 and 2022, the Scientific Training unit continued and extended their career development services. As part of the PhD student symposium, two half-days of workshops on different career-related topics were organised by a PhD student committee and very well attended. Additionally, the unit offered a variety of courses as well as personal coaching sessions and peer support groups.

In response to the COVID-19 pandemic, in 2020, the Scientific Training unit transferred all their training activities to virtual formats. They established a virtual selection procedure for their international programmes, which proved successful and was adopted for the long-term to reduce air-travel.

Scientific Training team

Scientific Training is a cross-campus initiative. The unit's responsibilities range from attracting new talent to the Vienna BioCenter to supporting researchers with opportunities for professional growth. Scientific Training empowers scientists to follow their curiosity and to thrive in their profession. The various programmes managed by Scientific Training are implemented by a diverse team.

BREAKTHROUGH PRIZE WINNERS

Life science awards for IMP alumni.

Scientists and alumni of the IMP regularly of the IMP regularly receive awards in recognition of their ground-breaking work and scientific talent. Among the most glamorous of scientific merits are the Breakthrough Prizes, of which two were awarded to IMP alumni in this reporting period: one to IMP Emeritus Director Kim Nasmyth in 2018, and another to his one-time student Angelika Amon in 2019.

The Breakthrough Prizes celebrate the achievements of the world's top researchers in life sciences, fundamental physics, and mathematics. Described as the 'Oscars of Science', recipients are honoured at a televised awards gala, and each receive three million US dollars, making it the largest monetary science prize worldwide. Up to five awards in the Life Sciences category are presented every year to honour transformative advances towards understanding living systems and extending human life.

Kim Nasmyth and chromosome segregation

Kim Nasmyth was awarded a Breakthrough Prize in Life Sciences in 2018 for his work on chromosome segregation, largely performed at the IMP. During his 18 years at the institute, he revealed the fundamental role of cohesin in separating duplicated chromosomes during cell division.

Nasmyth's work is not only key for understanding how the genome is passed from one cell generation to the next, but it also explores the functions of cohesin in DNA repair, genome architecture, and gene regulation. Scientists can now understand why cohesin defects can lead to chromosome anomalies and spontaneous abortions, or contribute to genetic diseases such as cancer.

Nasmyth joined the newly founded IMP in Vienna in 1987 as one of the institute's first three senior scientists. He served as Scientific Director of the IMP from 1997 to 2006. He then moved to the University of Oxford as the Whitley Professor of Biochemistry.

Angelika Amon and the consequences of aneuploidy

Angelika Amon received her Breakthrough Prize in Life Sciences in 2019. The award honoured her discovery of the consequences of abnormal chromosome numbers – aneuploidy – on cell physiology and tumour development.

At the time, Amon was working as the Kathleen and Curtis Marble Professor of Cancer Research at the Massachusetts Institute of Technology. There, her group was exploring the regulatory networks that control cell division during normal development and in disease. Amon's studies on yeast and mice showed that changes in chromosome number causes various cellular stresses. They also revealed how tumour cells overcome these stresses and exploit the selective benefits that come with aneuploidy. These findings are now helping to identify new targets for cancer therapies.

Amon joined the IMP in 1987 as one of its first students, just a few months after her supervisor Kim Nasmyth had established his lab. She worked with Nasmyth for her Master's thesis and PhD, laying the foundations for a successful research career. She lived and worked in the US from 1994, but accompanied the IMP as a member of the Scientific Advisory Board from 2009 to 2019. Amon always maintained strong relations with the institute up until her death in 2020.

SCIENTIFIC ADVISORY BOARD

The IMP strives for excellence in the molecular life sciences. To ensure that the institute maintains its world-class quality, an international panel of scientists, all distinguished leaders in their respective fields, reviews the institute annually.

The Scientific Advisory Board (SAB) provides invaluable feedback on current research lines, rising technologies, and the best academic practices. The IMP is grateful for this advice, and for the ties to other research institutions around the World that the board members carry.

CHAIRS

DIRK SCHÜBELER
CHAIR SINCE 2020, MEMBER SINCE 2014

FRIEDRICH MIESCHER
INSTITUTE FOR BIOMEDICAL RESEARCH BASEL (SWITZERLAND)

LESLIE VOSSHALL
CHAIR – 2013 TO 2020
ROCKEFELLER UNIVERSITY, NEW YORK (USA)

MEMBERS

ANGELIKA AMON
2009 TO 2019
MASSACHUSETTS INSTITUTE OF TECHNOLOGY (MIT), CAMBRIDGE (USA)

ADRIAN BIRD
SINCE 2020
UNIVERSITY OF EDINBURGH, EDINBURGH (UNITED KINGDOM)

HANS CLEVERS
2015 TO 2021
HUBRECHT INSTITUTE, UTRECHT (NETHERLANDS)

MICHAEL HÄUSSER
SINCE 2015
UNIVERSITY COLLEGE LONDON, LONDON (UNITED KINGDOM)

NORBERT KRAUT
SINCE 2011
BOEHRINGER INGELHEIM RCV GMBH, VIENNA (AUSTRIA)

RUTH LEHMANN
SINCE 2017
NYU LANGONE MEDICAL CENTER, NEW YORK (USA)

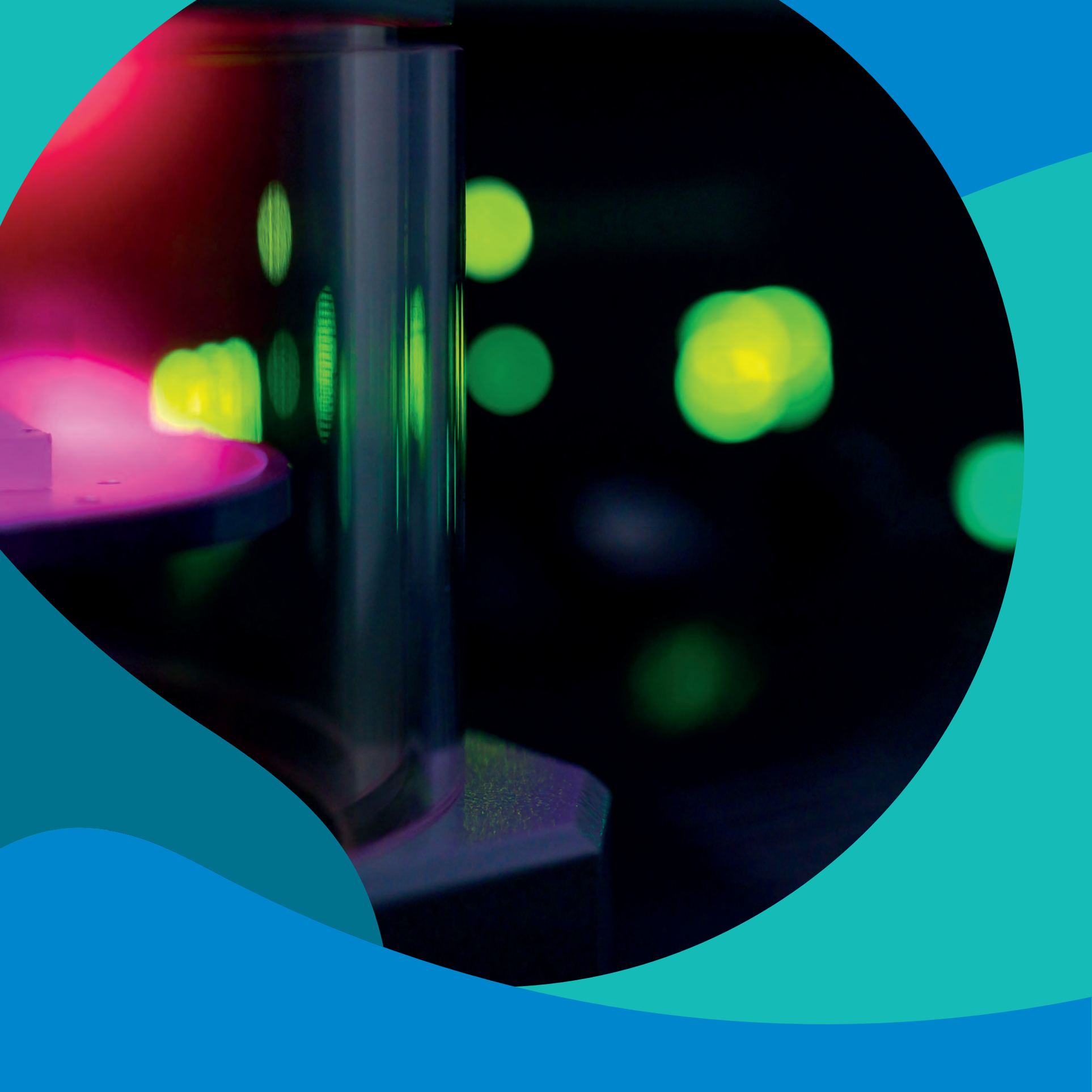
DAN LITTMAN
2009 TO 2017
NYU LANGONE MEDICAL CENTER, NEW YORK (USA)

RUSLAN MEDZHITOV
SINCE 2016
YALE SCHOOL OF MEDICINE/ HHMI, NEW HAVEN (USA)

EVA NOGALES
SINCE 2021
UNIVERSITY OF CALIFORNIA, BERKELEY (USA)

PAUL NURSE
2017 TO 2019
THE FRANCIS CRICK INSTITUTE, LONDON (UNITED KINGDOM)

TOM RAPOPORT
2011 TO 2021
HARVARD MEDICAL SCHOOL, BOSTON (USA)



PANDEMIC RESEARCH PIVOTS

Finding solutions for Covid-19: Vienna BioCenter collaborations.

When SARS-CoV-2 began to spread rapidly across Europe in spring 2020, researchers at the IMP and Vienna BioCenter were quick to respond. Several scientists became advisors to the Austrian government, and many realigned their research efforts to assist with the changing challenges posed by the pandemic. As with the rest of the world, there was a desperate need for diagnostic tests in Austria. Testimony to the spirit of collaboration at the Vienna BioCenter, IMP scientists began developing new testing techniques with colleagues from partner institutes on campus and beyond. Their procedures, which went on to be used in schools, care homes, and companies across Austria, would prove instrumental in monitoring the spread of the virus.

Gold standard RT-qPCR tests

From the outset, one of the biggest challenges was testing for Covid-19 in large groups, particularly in retirement and nursing homes. Shortly after the World Health Organization declared a pandemic on 11 March, Johannes Zuber and his team at the IMP started researching sampling methods to monitor such groups. Alongside collaborators, the team created a protocol using RT-qPCR analysis on gargle samples. Test results were available within hours.

Starting in April 2020, employees at the Vienna BioCenter were using the method to test for Covid-19 typically twice a week, and the protocol formed the basis for extensive monitoring in Austrian schools. By mid-October, a pilot project monitoring staff in nursing homes in Vienna and Lower Austria was underway.

Rapid diagnostics with RT-LAMP

While PCR tests were an effective technique for well-equipped laboratories, there was demand for diagnostic tests that could be used in simpler settings. In response, a cross-institutional team from the Vienna BioCenter set out to develop a detection method as a fast and inexpensive alternative.

Lead by Andrea Pauli from the IMP and Julius Brennecke from IMBA, the team created a new method based on the established technique RT-LAMP. By refining the method and optimising the protocol, the group increased both the sensitivity and robustness of the test. As a result, even small laboratories had access to this cheaper alternative to PCR testing, while still achieving results of comparable specificity.

NGS for mutant monitoring

With more diagnostic tests being developed during the first lockdown in Austria, scientists at the Vienna BioCenter were determined to find a way to scale up testing. Luisa Cochella and Ulrich Elling, from the IMP and IMBA respectively, did so with the creation of their SARSeq technique. The test procedure combined the sensitivity of PCR with the high throughput of NGS to enable mass sequencing of viral RNA. It could simultaneously process up to 36,000 samples in less than 48 hours. The procedure ran in parallel to existing diagnostics, and worked with any type of sample: saliva, gargle, or nasal swabs. As well as detecting SARS-CoV-2, SARSeq could also be applied to other respiratory pathogens such as the flu virus and common cold rhinoviruses. The method was used to monitor mutations of Covid-19 variants and could become a crucial tool in preventing diseases from spreading in the future.

VIENNA BIOCENTER: SHINING ITS LIGHT FURTHER AND FURTHER

Excellent research, education, and entrepreneurship in the life sciences: these are the three pillars of the Vienna BioCenter. Seeded by the establishment of the IMP and now among Europe's most dynamic life science hubs, the Vienna BioCenter added more to its success story in recent years.

More than 140 research groups across four institutes and a university, 2650 staff members from 80 countries, nearly 40 biotech companies. Taking toll by the end of 2022 shows how far the Vienna BioCenter has come and how strong it is going. Significant milestones were reached since 2017.

By early 2017, the IMP had moved to Campus-Vienna-BioCenter 1. In front of 200 guests and backed by all IMP colleagues, biochemist Emmanuelle Charpentier and Austria's president Alexander Van der Bellen opened the new IMP building in March 2017.

Lecture hall capacity had long been the bottle neck for scientific conferences. The new IMP lecture hall seats 280 people; and taken together with seminar rooms, foyer, and the ÖAW building's atrium, it equipped the Vienna BioCenter with the necessary means to host events at a new scale. From 2018, EMBO Young Investigator Meetings, Keystone Conferences, and other prestigious scientific events drew crowds of previously unseen magnitude. More people than ever before could experience the Vienna BioCenter spirit first-hand.

The Vienna BioCenter further built its reputation through increased efforts to raise visibility. By 2018, it endorsed a new logo and brand, used by joint initiatives such as the Vienna BioCenter Core Facilities (VBCF) and all training programmes, including summer school and PhD program. While this helped with projecting a united image into the world of science, the next boost to the campus was already in the making: the University of Vienna had started the construction of a new building.

When Covid-19 hit in early 2020, the scientists of the Vienna BioCenter quickly jumped into action. Different initiatives – most importantly to test colleagues and thus allow them to return to the labs - helped to mitigate the local impact of the pandemic. It also fostered a sense of community and pride among colleagues (see page 107).

The summer of 2020 saw a big step for the entrepreneurship pillar of the campus: after three years of work, VBC6 (which had previously hosted the IMP) was opened for different users, including the Vienna StartUp Labs. Since then, 60 lab places and 50 office workstations allow start-ups to use first-class facilities with easy and flexible access. Boehringer Ingelheim supports the young biotech scene with its annually presented Innovation Prize.

Not the only prize which caused joy at the Vienna BioCenter that year: in autumn of 2020, a Nobel Prize for Chemistry was awarded to Emmanuelle Charpentier and Jennifer Doudna for their ground-breaking discoveries on the CRISPR/Cas9 system. Charpentier was a principal investigator at the Max Perutz Labs from 2002 to 2009, and some of her discoveries from this time contributed to developing the CRISPR/Cas9 technology.

The University Biology Building was completed in 2021, and the Vienna BioCenter gained 500 new colleagues and over 3000 more students. The building is home to most of the faculty of life sciences, and CeMESS, a centre for microbiology. A year later, the University of Applied Sciences left the campus when it moved its local branch to a central facility. Nevertheless, by 2022 the Vienna BioCenter had an unprecedented size - and determination to expand further. It will continue to build on its position as one of Europe's top locations for biomedical science, studies, and business.

LIFE AND LEGACY OF THREE EARLY INNOVATORS

Paying tribute to three former IMP scientists.

The IMP community was deeply saddened by the loss in recent years of three former IMP scientists: Angelika Amon, Denise Barlow, and Andreas Weith. These trailblazing researchers all joined the IMP in its infancy and remained close to the institute throughout their lives. Here, we pay tribute to their remarkable scientific careers and legacies.

Angelika Amon (1967 to 2020)

Angelika Amon built a reputation as one of the world's leading geneticists of her time. From early on in her career, her research was central to the development of the field of chromosomal biology.

In 1987, Amon joined the IMP as one of the institute's first students. She was curious, bold, and eager to learn. Amon worked with Kim Nasmyth, initially for her Master's thesis and subsequently her PhD.

Following her graduation in 1993, Amon embarked on a remarkable scientific journey. Alongside her numerous publications came copious awards, including the esteemed Breakthrough Prize in Life Sciences, which she received in 2019.

As the Kathleen and Curtis Marble Professor in Cancer Research at the Massachusetts Institute of Technology, Amon continued to pursue her research with great passion despite her battle with cancer. She maintained close ties to the IMP and served on its Scientific Advisory Board from 2009 to 2019.

Denise P. Barlow (1950 to 2017)

Denise Barlow was a leading pioneer of epigenetics. She made outstanding contributions to the study of genomic imprinting over the course of her career.

Barlow joined the newly opened institute in 1988. Just a few years later, she made her first major discovery when she identified the mammalian imprinted gene IGF2R, the insulin-like growth factor type 2 receptor.

After eight successful years at the IMP, Barlow left Vienna to take up Group Leader positions at several prestigious research institutes. She continued to make important discoveries regarding long-coding regulatory RNAs and later returned to Vienna to join the Center for Molecular Medicine. There, she continued her epigenetic research as a Principal Investigator until her retirement in 2015.

Andreas Weith (1953 to 2019)

An assiduous scientist with great technical skills, a deep thinker and considerate colleague – these are just some of the ways that IMP staff and alumni remember Andreas Weith's work ethic and personality.

Weith joined the IMP as a Group Leader in 1990. He quickly assembled a small laboratory comprising three students and a technician and set out to study genes associated with tumorigenesis. Although sceptical about a life in Vienna, he would later describe this time as the best years of his scientific career.

When his term at the IMP ended in 1997, Weith set up a dedicated genomics research group within Boehringer Ingelheim. His group provided state-of-the-art functional genomics expertise, supporting the discovery of new concepts and biomarkers for many of the pharmaceutical company's therapeutic areas.

AWARDS AND HONOURS

If an IMP scientist is awarded a prize, competitive grant, or other honour, the entire institute takes pride in this achievement and joins in celebrating the merits of hard-working colleagues. Academic honours also provide testimony for the high scientific standard that the IMP is committed to.

2017

MEINRAD BUSSLINGER
ERC Advanced Grant

ELLY TANAKA
ERC Advanced Grant

JAN-MICHAEL PETERS
Adjunct Professor, Medical University of Vienna

JOHANNES ZUBER
Adjunct Professor, Medical University of Vienna

TIM CLAUSEN
Adjunct Professor, Medical University of Vienna

ALEXANDER STARK
Adjunct Professor, Medical University of Vienna

MANUEL ZIMMER
HHMI Wellcome International Research Scholar

ELLY TANAKA
Elected EMBO Member

ANDREA PAULI
START Grant by the Austrian Government

ELLY TANAKA
Female Scientist Award by German Stem Cell Network

ANNA OBENAUF
ERC Starting Grant

ELLY TANAKA
Ernst Schering Prize

MATTHIAS MUHAR (ZUBER LAB)
Mattias Lauwers Award

HARRIS KAPLAN (ZIMMER LAB)
Conference Award by Austrian Neuroscience Association

JOHANNES GRIESSNER (HAUBENSAK LAB)
Conference Award by Austrian Neuroscience Association

MUHAMMED MAMDUH BIN AHMAD ZABIDI (STARK LAB)
Vienna BioCenter PhD Award

LUISA COCHELLA
Elected EMBO Young Investigator

MAREIKE ROTH (ZUBER LAB)
Kirsten P. Rabitsch Award
Vienna BioCenter PhD Award

MARCIN SUSKIEWICZ (CLAUSEN LAB)
Ubiquitin Prize 2017

SIMON NIMPF (KEAYS LAB)
DOC Fellowship, Austrian Academy of Sciences

SARAH HERBERG (PAULI LAB)
DOC Fellowship, Austrian Academy of Sciences

2018

MEINRAD BUSSLINGER
FWF stand-alone grant

ELLY TANAKA
WWTF grant, Life Sciences Call 2017 Chemical Biology

TIM CLAUSEN
WWTF grant, Life Sciences Call 2017 Chemical Biology

MARA ANDRIONE (ZIMMER LAB)
Marie Skłodowska-Curie Individual Fellowship

THOMAS CUSHION (KEAYS LAB)
Marie Skłodowska-Curie Individual Fellowship

AKANE KAWAGUCHI (TANAKA LAB)
JSPS Overseas Research fellowship

WOUTER MASSELINK (TANAKA LAB)
LISE MEITNER FELLOWSHIP
KATHARINA LUST (TANAKA LAB)
HFSP Long-term Fellowship

JAN-MICHAEL PETERS
HFSP Project Grant

ANNIKA NICHOLS (ZIMMER LAB)
Harold M. Weintraub Graduate Student Award

MANUEL ZIMMER
Full Member, European Molecular Biology Organization (EMBO)

ANDREA PAULI
Whitman Center Fellowship, MBL, Woods Hole, Massachusetts

CLEMENS PLASCHKA
Walther Flemming Award, German Society for Cell Biology

COSMAS ARNOLD (STARK LAB)
ÖGMBT Life Science Research Award Austria in Basic Science

MATTHIAS MUHAR (ZUBER LAB)
ÖGMBT Life Science Research Award Austria in Applied Research

ANNIKA NICHOLS (ALUMNA ZIMMER LAB)
Kirsten Peter Rabitsch Award

SARAH HERBERG (PAULI LAB)
Mattias Lauwers Award

ANNIKA NICHOLS
(ALUMNA ZIMMER LAB)
Vienna BioCenter PhD Award

ANDREA PAULI
Elected EMBO Young Investigator

DAVID KEAYS
ERC Consolidator Grant

FELIX HOLSTEIN (OBENAUF LAB)
„Würdigungspreis“ of the Austrian Federal Government

LEONID SEREBRENI (STARK GROUP)
DOC Fellowship of the Austrian Academy of Sciences

ELLY TANAKA
Erwin Schrödinger Award of the Austrian Academy of Sciences

ANNIKA NICHOLS
(ALUMNA ZIMMER LAB)
Award of Excellence of the Austrian Federal Government

ANASTASIA POLIKARPOVA
(TANAKA LAB)
Best Abstract Award 2018 by the Ludwig Boltzmann Society

RUSHAD PAVRI
FWF stand-alone Grant

2019

ROBYN SCHENK (BUSSLINGER LAB)
Marie Skłodowska-Curie Individual Fellowship

JELLE JACOBS (STARK LAB)
Marie Skłodowska-Curie Individual Fellowship

FILIP NEMCKO (STARK LAB)
Boehringer Ingelheim Fonds (BIF) Fellowship

LEO OTSUKI (TANAKA LAB)
HFSP Post-doctoral Fellowship

ANNA OBENAUF
Election to the Austrian Academy of Sciences (ÖAW)

LUISA COCHELLA
FWF Stand-alone Grant

LUKAS LANDLER (KEAYS LAB)
FWF Stand-alone Grant

THERESA PINTER (BUSSLINGER LAB)
Boehringer Ingelheim Fonds (BIF) Fellowship

FRANCISCO BALZAROTTI
ERC Starting Grant

JOHANNES ZUBER
ERC Proof of Concept Grant

HARRIS KAPLAN (ZIMMER LAB)
ÖGMBT PhD Award

KRISTA GERT (PAULI LAB)
DOC Fellowship, Austrian Academy of Sciences

ALEXANDER PHILLIPS (KEAYS LAB)
DOC Fellowship, Austrian Academy of Sciences

CHIARA ALBERTI (COCHELLA LAB)
Vienna BioCenter PhD Award

SARAH HERBERG (PAULI LAB)
Vienna BioCenter PhD Award

HARRIS KAPLAN (ZIMMER LAB)
Vienna BioCenter PhD Award

MATTHIAS MUHAR (ZUBER LAB)
Vienna BioCenter PhD Award

LORENA HOFBAUER (STARK LAB)
Boehringer Ingelheim Fonds (BIF) Fellowship

ANASTASIA CHUGUNOVA (PAULI LAB)
VIP2 Programme

DIEGO RODRIGUEZ TERRONES
(TANAKA LAB)
VIP2 Programme

ITAMAR LEV (ZIMMER LAB)
VIP2 Programme

LAURA LORENZO ORTS (PAULI LAB)
EMBO Postdoc Fellowship

ULRICH HOHMANN
(PLASCHKA/BRENNECKE LAB)
EMBO Postdoc Fellowship

VINCENT LOUBIÈRE (STARK LAB)
EMBO Postdoc Fellowship

ALEXANDER STARK
FWF Stand-alone Grant

JAN-MICHAEL PETERS (COORDINATOR)
& DAVID HASELBACH
WWTF Grant

2020

MARIA INÊS LEÇA (KEAYS LAB)
Vienna BioCenter PhD Award

PAULA GUTIÉRREZ PÉREZ
(COCHELLA LAB)
Mattias Lauwers Award

MATTHIAS VORLÄNDER
(PLASCHKA LAB)
EMBO Long-Term Fellowship

MEINRAD BUSSLINGER
Preis der Stadt Wien

CLEMENS PLASCHKA
ERC Starting Grant

ELLY TANAKA
DACH Grant (FWF/DFG)

ANASTASIA POLIKARPOVA
(TANAKA LAB)
FWF Hertha Firnberg Fellowship

ELAD BASSAT (TANAKA LAB)
EMBO Postdoc Fellowship

LONI KLAUS (STARK LAB)
DOC Fellowship, Austrian Academy
of Sciences

OLIVER HENDY (STARK LAB)
DOC Fellowship, Austrian Academy
of Sciences

ANDREAS BLAHA (PAULI LAB)
Boehringer Ingelheim Fonds Fellowship

LAURA LORENZO ORTS
(PAULI LAB)
EMBO Postdoc Fellowship

VINCENT LOUBIÈRE (STARK LAB)
HFSP Postdoc Fellowship

VICTORIA DENEKE (PAULI LAB)
HFSP Postdoc Fellowship

ANDREA PAULI
HFSP Young Investigator Grant

MELANIE DE ALMEIDA
(ZUBER LAB)
DOC Fellowship by the Austrian
Academy of Sciences (ÖAW)

DIEGO RODRIGUEZ TERRONES
(TANAKA LAB)
EMBO Fellowship

ELLY TANAKA
FEBS | EMBO Women in Science Award

LAURA LORENZO ORTS
(PAULI LAB)
Marie Skłodowska-Curie Fellowship

ANASTASIA CHUGUNOVA
(PAULI LAB)
Marie Skłodowska-Curie Fellowship

ULRICH HOHMANN (PLASCHKA LAB)
Marie Skłodowska-Curie Fellowship

LAURA LORENZO ORTS (PAULI LAB)
SNF Early Stage Fellowship

2021

TIM CLAUSEN
WWTF Grant

LOUISA HILL (BUSSLINGER LAB)
Ursula and Fritz Melchers Prize,
Austrian Society of Allergology and
Immunology

CLEMENS PLASCHKA
EMBO Young Investigator

ANNA OBENAUF
EMBO Young Investigator

MELANIE DE ALMEIDA (ZUBER LAB)
Mattias Lauwers Award

PHILIPP DEXHEIMER
(COCHELLA LAB)
Vienna BioCenter PhD Award

LISA HAAS (OBENAUF LAB)
Vienna BioCenter PhD Award

RUSHAD PAVRI
FWF Stand-alone Grant

WOUTER MASSELINK (TANAKA LAB)
FWF Stand-alone Grant

LOUISA HILL (BUSSLINGER LAB)
Kirsten Peter Rabitsch Award

KARL MECHTLER
APMA Lifetime Award and title
of Prof. h.c.

ANDREA PAULI
Elected EMBO Member

SABRINA HORN (PETERS LAB)
DOC Fellowship, Austrian Academy
of Sciences

FERNANDO BECERRIL PEREZ
(TANAKA LAB)
DOC Fellowship, Austrian Academy
of Sciences

FRANZISKA REITER (STARK LAB)
DOC Fellowship, Austrian Academy of Sciences

ELLY TANAKA
Corresponding Member of the Austrian Academy of Sciences (ÖAW)

FRIEDA LEESCH (PAULI LAB)
1st prize for PhD Student Talk at MicroSymposium on Small RNAs

JAN-MICHAEL PETERS
ERC Advanced Grant

JESSICA STOCK (PAULI LAB)
Christine Beattie Award of the International Zebrafish Society (IZFS)

JOHANNES SCHMÖLLERL (ZUBER LAB)
Young Investigator Award, Austrian Society for Hematology and Medical Oncology

SIMON NIMPF (KEAYS LAB)
Best Thesis Award 2020, Austrian Neuroscience Association

PAULINE JUNG (OBENAUF LAB)
President's Prize, Austrian Society for Dermatology and Venereology

LUKAS LEIENDECKER
(OBENAUF LAB)
President's Prize, Austrian Society for Dermatology and Venereology

MATTHIAS KOPANO VORLÄNDER
(PLASCHKA LAB)
Individual Fellowships | Marie Skłodowska-Curie Actions

DARIA RIABOV BASSAT
(PLASCHKA LAB)
Individual Fellowships | Marie Skłodowska-Curie Actions

ELAD BASSAT (TANAKA LAB)
Individual Fellowships | Marie Skłodowska-Curie Actions

DIEGO RODRIGUEZ-TERRONES
(TANAKA LAB)
Individual Fellowships | Marie Skłodowska-Curie Actions

KATHARINA LUST (TANAKA LAB)
Individual Fellowships | Marie Skłodowska-Curie Actions

JAKUB ZMAJKOVIC (ZUBER LAB)
Individual Fellowships | Marie Skłodowska-Curie Actions

SEBASTIAN ISBANER
(BALZAROTTI LAB)
Individual Fellowships | Marie Skłodowska-Curie Actions

MATTHIAS MUHAR (ZUBER LAB)
Denise P. Barlow Award

2022

LUKAS LEIENDECKER (OBENAUF LAB)
Science Award of the Austrian Society for Dermatology and Venereology

TIM CLAUSEN
Allen Distinguished Investigator

MELANIE DE ALMEIDA (ZUBER LAB)
Angelika Amon Young Scientist Award

MORITZ GAIDT
Robert Koch Postdoc Award

FELIX HOLSTEIN (OBENAUF LAB)
Mattias Lauwers Award

JESSICA STOCK (PAULI LAB)
Vienna BioCenter PhD Award

JIRI WALD (MARLOVITS LAB)
Vienna BioCenter PhD Award

MELANIE DE ALMEIDA (ZUBER LAB)
Kirsten Peter Rabitsch Award

RUPERT MAYER
(PROTEIN CHEMISTRY FACILITY)
Best Presentation Award of the Single Cell Proteomics & Austrian Proteomics and Metabolomics Research Symposium

BERNARDO ALMEIDA (STARK LAB)
Life Science Research Awards Austria

JOHANNES ZUBER
Elected EMBO Member

ANNA OBENAUF
AAAS Martin and Rose Wachtel Cancer Research Award

ANNA OBENAUF
Alex's Lemonade Stand Foundation (ALSF) grant

ELLY TANAKA
Full Member of the Austrian Academy of Sciences (ÖAW)

FRANZISKA LORBEER (STARK LAB)
EMBO Fellowship

ELLY TANAKA
Marie Curie Innovative Training Network grant

ANDREA PAULI
ERC Consolidator Grant

ANNA OBENAUF
Elected among 10 Most Impactful Publicatons of 2021, European Association for Cancer Research

JAN MICHAEL PETERS, PETRA VAN DER LELIJ, RENPING QIAO COUDEVYLLE
(PETERS LAB)
ERC Proof of Concept Grant

VICTORIA DENEKE (PAULI LAB)
Trainee Science Communication Award, Society for Developmental Biology

SPONSORS AND PARTNERS

Research thrives in collaborative environments. Science at the IMP draws from many people and institutions – intellectually and financially.

The IMP thanks the following sponsors and funding organisations – first and foremost its main sponsor Boehringer Ingelheim – for their valuable investments and support:

Alex Lemonade Foundation
BMWFW – Federal Ministry of Science, Research and Economy
Boehringer Ingelheim
EMBO – European Molecular Biology Organization
ERC – European Research Council
European Commission
FFG – Austrian Research Promotion Agency
Fondation l'Oréal-UNESCO
FWF – Austrian Science Fund
HFSP – Human Frontier Science Program
Horizon 2020
LISAvienna – Life Science Austria
ÖAW – Austrian Academy of Sciences
Simons Foundation
SNF – Swiss National Science Foundation
Stadt Wien
Tempest
Universitätsklinikum Tübingen
Wirtschaftsagentur Wien
WWTF – Vienna Science and Technology Fund

FURTHER CHRONICLES

Keep exploring six years of research and progress. Extend your experience with the QR codes on this page for a celebration of the IMP's scientific contributions between 2017 and 2022.

Publications:

Seminars and talks:

Conferences:

Imprint

Published by
IMP – Research Institute of Molecular Pathology GmbH
Campus-Vienna-Biocenter 1
1030 Vienna, Austria
T +43(1) 79730-0
www.imp.ac.at

Responsible for the content
Jan-Michael Peters and Harald Isemann
Project Management
Mehdi Khadraoui, Benedikt Mandl
Scientific editor
Hannah Voak

Graphic Design
Gerhard BAUERund Barbara Lewall
Photography
Ludwig Schedl (lab photography),
Kurt Kuball (architectural photography)
Iris photography and processing
Johannes Tkadletz, Tibor Kulcsar

Printed by
Druckwerkstatt, Wien

ISBN 978-3-200-09679-0