FUTURA

THE JOURNAL OF THE BOEHRINGER INGELHEIM FONDS

VOL. 36 | 1.2021

How to map 37 trillion cells
A project to create an atlas of the human body

Projects, results, MD fellowships
New PhD projects, completed theses, and 2020 MD fellowships

A BIF fellow's guide to Dublin
The city of literature, pubs, and biological diversity

The cover illustration shows a simplified model of *Arabidopsis thaliana*, also dubbed the "botanical *Drosophila*". *A. thaliana* grows quickly, has a small genome, self-fertilizes, and is easily transformed by *Agrobacterium tumefaciens*. These valuable traits have made it a popular model organism for the study of topics such as growth and development.

FACTS

PROFILE OF *ARABIDOPSIS THALIANA*

HOW TO MAP 37 TRILLION CELLS

FELLOWS

NEW PHD PROJECTS, THIRD ROUND 2020

NEW PHD RESULTS

MD PROJECTS

FOUNDATION

"YOU CAN'T BLAME ANYONE ELSE"

PUBLISHING INFORMATION

Published by Boehringer Ingelheim Fonds (BIF)
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Production muehlhausmoers corporate communications gmbh, www.muehlhausmoers.com
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Printed by LUC GmbH, Hansaring 118, 48268 Greven, Germany

Images Boehringer Ingelheim Fonds, unless stated otherwise

Cover photos ESA/Hubble & NASA, A. Riess et al. (left); iStock/aldomurillo (middle); Sarah Power (right)

Publication date of current issue July 2021

WHAT ARE WE LOOKING FOR?

»What unites them is their thirst for knowledge, seemingly insatiable curiosity, and love for their topic.«

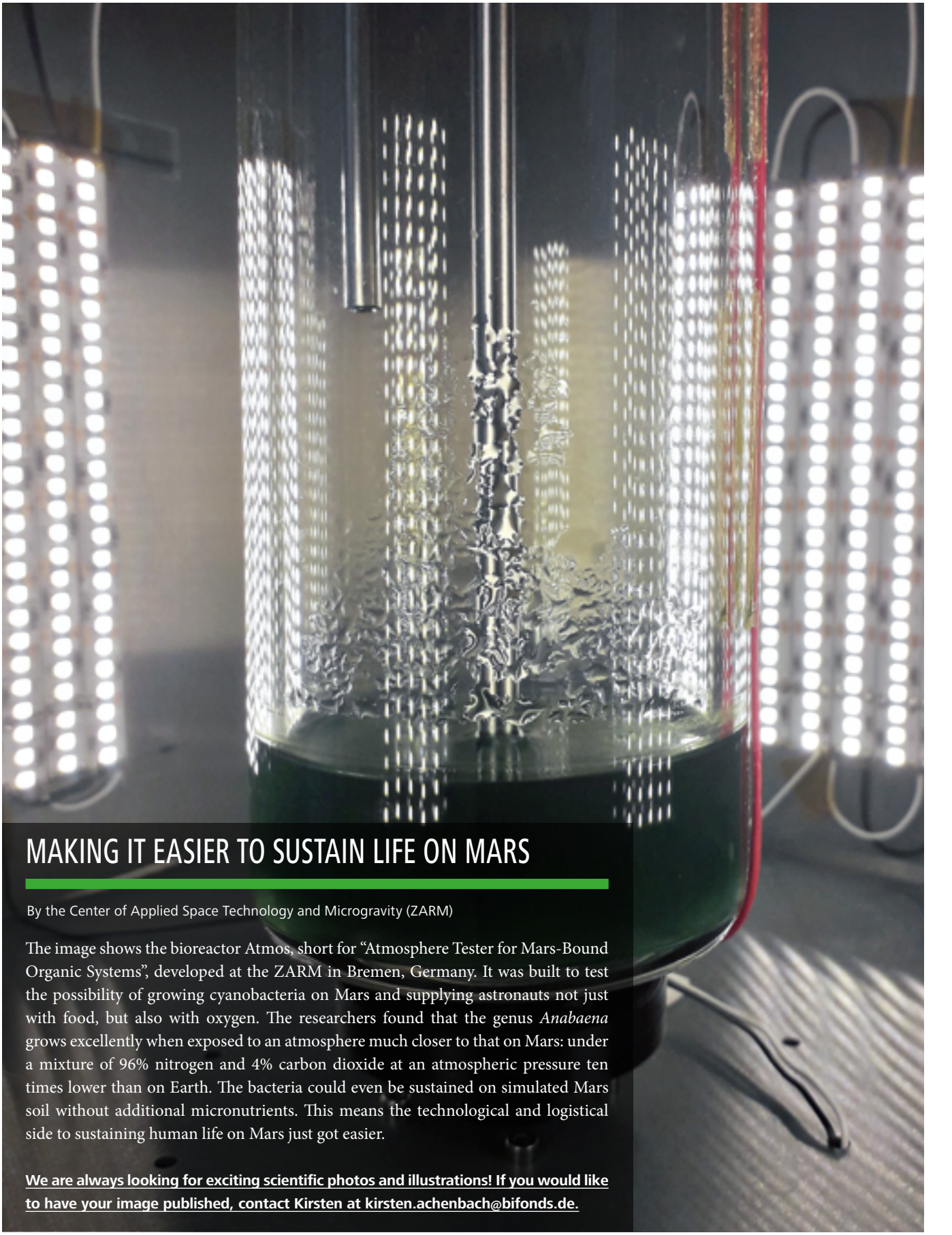
This question is often asked by applicants and fellows alike and, as is so often the case, the answer is not a simple one. We receive very impressive CVs in our PhD and MD fellowship programmes. And yes, the achievements they list are crucial for our decision. Together with the qualities that applicants show in their interviews, they are one of our three selection criteria. After all, we are looking for the most talented junior scientists. Nevertheless, applicants do not have to have outstanding marks across the board going back to first grade. What is important is that they have excelled when they follow their interests. Nor do we expect our fellows to have raced through their education. Depending on the education system the applicants come from, our formal requirements allow for one or two additional years to visit another laboratory or travel the world.

Are we impressed by references to Ivy League universities in a CV? It depends. Are they only meant to cast the candidate in a brilliant light? Or were the topic and laboratory well chosen? The same goes for mobility: again, the reason and amount are key. Internships and studies in many different countries might reflect wanderlust rather than the qualities we are looking for. On the other hand, in some cases there might be good reasons for staying put. Personal or national circumstances, for example, may not have allowed a candidate to study at the desired university or go abroad.

At BIF, we do not expect candidates to have already published paper(s). In our experience, relevant publications are rare exceptions and noted accordingly. For us, taking ownership of one's project – even a failed one – is more important than being co-author number nine, mentioned due to a minor contribution.

We are pleased to see the great diversity of our fellows, which we believe will foster their development, research, and the exchange between disciplines. Our programmes bring together fellows from some 60 countries. They include outspoken and cautious fellows, very junior researchers with great potential, and extraordinarily mature individuals who have already had some success in research before starting their PhD. Fellows who have wanted to become scientists since childhood meet colleagues whose passion for, say, immunology was sparked only at the end of their studies. Some fellows were highly focused during their education and became experts in a subject early on. Others regarded their studies as a chance to explore the breath of topics and techniques, freely following their curiosity and fascination for research.

What they all possess, despite their differences, is outstanding ability, motivation, creativity, drive, and perseverance – traits that are in high demand in any field of work. What unites them and contributes to the vibrant network of BIF fellows is their thirst for knowledge, seemingly insatiable curiosity, and love for their topic.



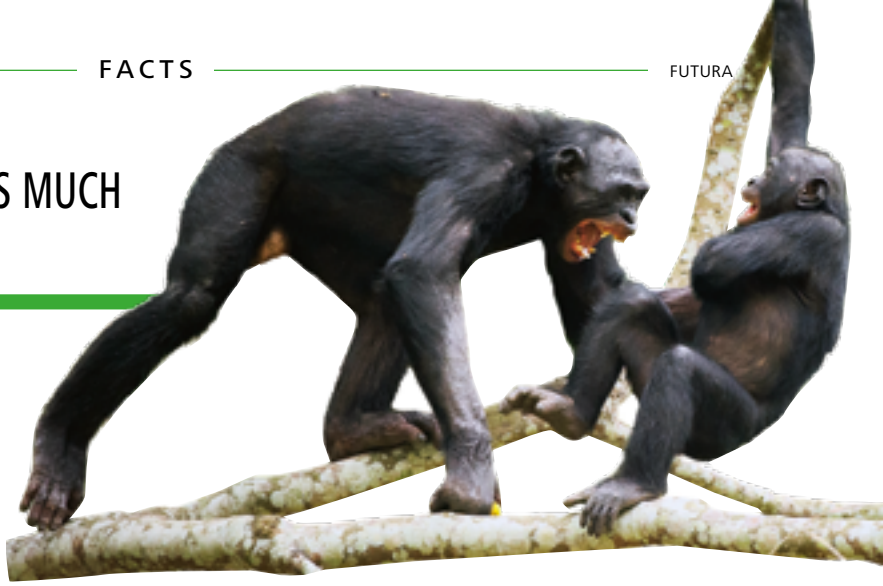
MAKING IT EASIER TO SUSTAIN LIFE ON MARS

By the Center of Applied Space Technology and Microgravity (ZARM)

The image shows the bioreactor Atmos, short for "Atmosphere Tester for Mars-Bound Organic Systems", developed at the ZARM in Bremen, Germany. It was built to test the possibility of growing cyanobacteria on Mars and supplying astronauts not just with food, but also with oxygen. The researchers found that the genus *Anabaena* grows excellently when exposed to an atmosphere much closer to that on Mars: under a mixture of 96% nitrogen and 4% carbon dioxide at an atmospheric pressure ten times lower than on Earth. The bacteria could even be sustained on simulated Mars soil without additional micronutrients. This means the technological and logistical side to sustaining human life on Mars just got easier.

We are always looking for exciting scientific photos and illustrations! If you would like to have your image published, contact Kirsten at kirsten.achenbach@bifonds.de.

CHIMPANZEES VALUE FRIENDS AS MUCH AS FAMILY IN FIGHTS

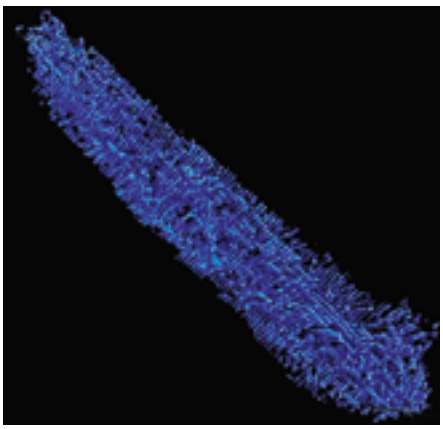


A 25-year study of three chimpanzee communities in Taï National Park in the Ivory Coast has revealed that when chimps fight neighbouring groups, it is not just family members who team up, but also their friends. The team analysed 500 hostile clashes between the chimp communities that resulted in serious injury or death of some animals. Given the high risk when going into battle, each chimp must make a decision about whether they will get involved in a fight. The researchers extracted factors that increased the likelihood of a chimp participating: one was a larger number of other chimps involved. Besides the size of the group, it also made a difference who was fighting. Chimps were more likely to jump into the fray when their maternal relatives were involved, but also when chimps fought who were unrelated, but part of their social group. It is thought that fights between social groups increase within-group bonding and thus reduce the chance of chimps being left alone in a fight. These strong social relationships observed in one of humans' closest relatives suggest that social bonds may have played a role in our own evolution as cooperative species and in our willingness to risk ourselves for others.

REFERENCE

Samuni L, Crockford C, Wittig, RM (2021) Group-level cooperation in chimpanzees is shaped by strong social ties. *Nat Commun* **12**, 539, https://doi.org/DOI:10.1038/s41467-020-20709-9

HEART MUSCLES USE MINI SUCTION PUMPS TO KEEP WORKING



A 3D reconstruction of the network of tubules criss-crossing a heart muscle cell.

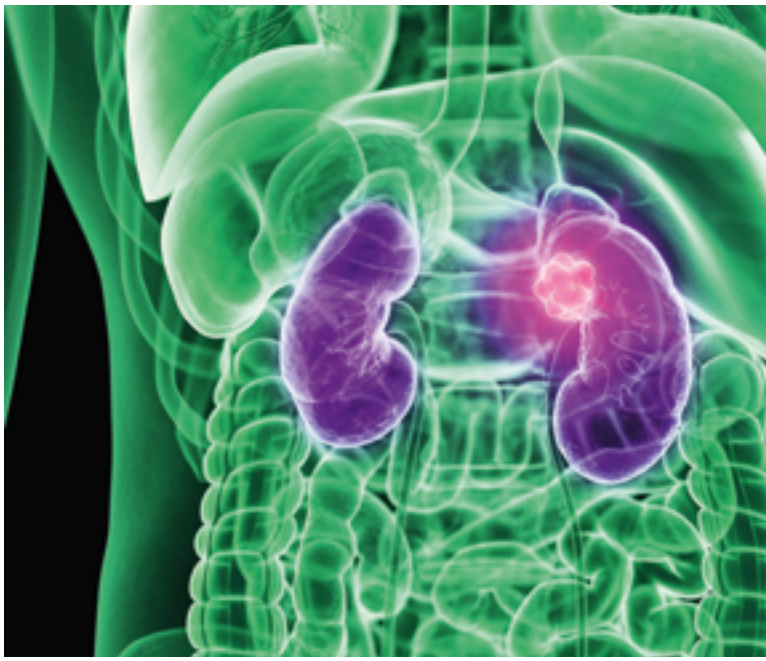
Every time the heart beats, ions travel into and out of muscle cells. This movement depends on the right ion concentration in the extracellular fluid. A crucial part of the extracellular fluid is located within a network of fine tubes that criss-cross heart muscle cells. In a healthy heart muscle cell, no point of the interior is more than a micrometre away from a tubule, close enough for fast ion exchange by diffusion alone. But until now it was not known how the extracellular fluid within the tubes themselves is refreshed. The tubes are too long for diffusion to work efficiently, but an active transport mechanism was unknown. Recently, researchers led by BIF alumnus Professor Peter Kohl at the University Heart Center Freiburg-Bad Krozingen, Germany, showed that the tubes are squeezed twice with every heartbeat. Every squeeze pushes the tubes' content out and every relaxation sucks fresh extracellular fluid into the tubes. Thus, even at a resting pulse of 60 beats per minute, the tube contents are partly exchanged twice per second, allowing for a fast recharge of the heart muscle cells for the next contraction. If heart rate goes up, so does the exchange in the tubes. However, heart disease often goes along with the remodelling of the heart's muscle tissue, leading to the network becoming less dense and less fine. Researchers suspect that this may impair the ion exchange and thus make heart muscles less effective.

REFERENCE

Rog-Zielinska EA, Scardigli M, Peyronnet R *et al* (2021) Beat-by-beat cardiomyocyte T-tubule deformation drives tubular content exchange. *Circulation Research* **128**: 203–215, https://doi.org/DOI:10.1161/CIRCRESAHA.120.317266

SILENT MUTATIONS CAN MAKE CANCER MORE AGGRESSIVE

A case of kidney cancer that killed a patient much sooner than expected has revealed the lethal effect of a so-called "silent mutation". Since silent mutations do not cause amino acid substitutions, many cancer genome studies looking for tumour-causing mutations tend to ignore them. Researchers from the German Consortium for Translational Cancer Research found a case of clear cell kidney cancer whose genetic profile predicted a survival of nearly ten years. Yet the patient died less than five years after diagnosis. When the researchers looked more closely, they found that the patient's kidney cells had a silent mutation in a tumour suppressor gene called BAP-1. This silent mutation causes exon 11 of BAP-1 to be left out. This error in the construction plan for BAP-1 creates a protein that is too short and quickly broken down. BAP-1's absence leads to a more aggressive tumour and a much shorter survival time for the patient. This finding suggests that silent mutations should be given more attention in cancer genome studies.



The course of kidney cancer can be affected by a silent mutation.

REFERENCE

Niersch J, Vega-Rubin-de-Celis S, Bazarna A (2021) A BAP1 synonymous mutation results in exon skipping, loss of function and worse patient prognosis. *iScience* **24**, DOI: 10.1016/j.isci.2021.102173

THE ROLE OF INNATE IMMUNE CELLS IN NEURODEGENERATION

The older you get, the less you can usually rely on your brain to tell you such things as where your keys are or, more dramatically, who that nice young man is who calls you mother. During ageing, the axons conducting signals between nerve cells get damaged. It was known that the microglia cells of the innate immune system were involved. Researchers from the University of Würzburg, Germany, have now found that certain cells of the adaptive immune system called $CD8^+$ T cells can be part of the problem: they are more abundant in the brain of old mice and produce cytotoxic substances that damage nerve cells. Previously, the group had already discovered that in neurological disorders such as multiple sclerosis, these cells can aggravate disease. Now the group compared these cells in adult (one-year-old) and old (two-year-old) mice using gene expression analyses on single-cell level. In old mice, they found five groups of $CD8^+$ T cells that differed in abundance (up to a tenfold increase) and gene expression between adult and old mice. They showed that in old mice these cells express more cytotoxic effector molecules and possess more ageing-related markers. To test their results, the team studied mice that did not have these immune cells. These animals did not show the typical age-related damage and performed similar to much younger mice in motor and learning tasks. In addition, the team observed that in old mice, but not in adult ones, chronic inflammation led to more damage by the $CD8^+$ T cells. Why and how exactly this happens is not yet clear.

Finally, the team found a similar picture in regard to T cells when they compared autopsy samples from young and old people's brains. These results shed light on how the brain ages and open a possible window for therapeutic intervention to help us remember where we put the keys and, much more importantly, who our loved ones are.

REFERENCE

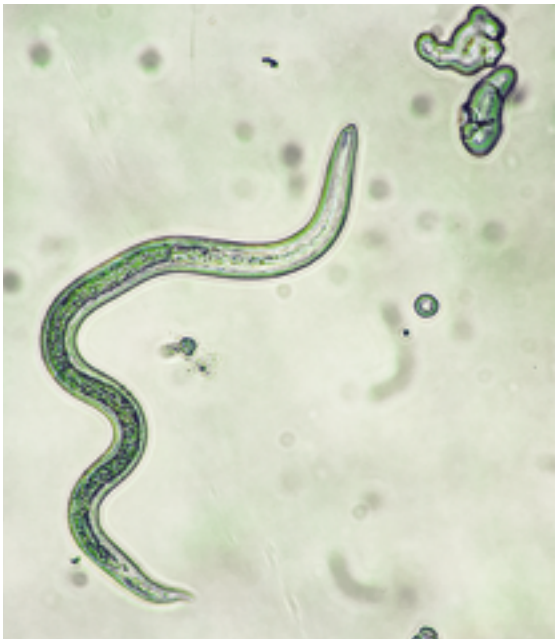
Groh J, Knöpper K, Arampatzi P, Yuan X, Lößlein L, Saliba A-E, Kastenmüller W, Martini R (2021) Accumulation of cytotoxic T cells in the aged CNS leads to axon degeneration and contributes to cognitive and motor decline. *Nat Aging* **1**: 357–367, https://doi.org/10.1038/s43587-021-00049-z

EXPELLING A WEB TO TRAP PARASITES

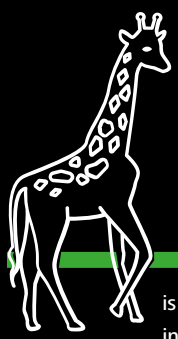
Filariae are nematodes that grow up to 70 cm in length and can live in human hosts for a long time. They cause serious diseases such as river blindness and elephantiasis – examples of the so-called neglected tropical diseases (NTDs), which affect around 80 million people living in the tropics. The nematodes are transmitted by mosquitoes, which pick up the less than a quarter millimetre small larvae (microfilariae) when they feed, and pass them on when they take their next meal. Two particular types of immune cells, called neutrophils and eosinophils, were known to play an important role in defending against filarial infections. Both types can sacrifice themselves in what is called extracellular DNA trap cell death (ETosis). They basically explode and fling their decondensed DNA at the parasites, trapping them in a web of DNA. For neutrophils, it was known that this web also contains antimicrobial peptides, thus trapping and killing the larvae in one throw. A team led by scientists from the University of Bonn, Germany, has now answered many questions regarding ETosis in eosinophils (EETosis): it is triggered when their Dectin-1 receptor comes in contact with the larvae. Their DNA web is composed of both mitochondrial and nuclear DNA, but mostly the first. However, they do not use antimicrobial peptides to fight the intruders. The researchers also showed *in vivo* that the DNA web helps mice to get rid of the parasites. As eosinophils of mice and man reacted to larvae of different species, the researchers postulate that EETosis is a conserved mechanisms helping to fight off these insidious parasites.

REFERENCE

Ehrens A, Lenz B, Neumann AL *et al* (2020) Microfilariae trigger eosinophil extracellular DNA traps in a Dectin-1-dependent manner. *Cell Reports* **24**: 108621, DOI: 10.1016/j.celrep.2020.108621



Immune cells sacrifice themselves to trap parasitic worms like this nematode in their expelled and uncoiled DNA.

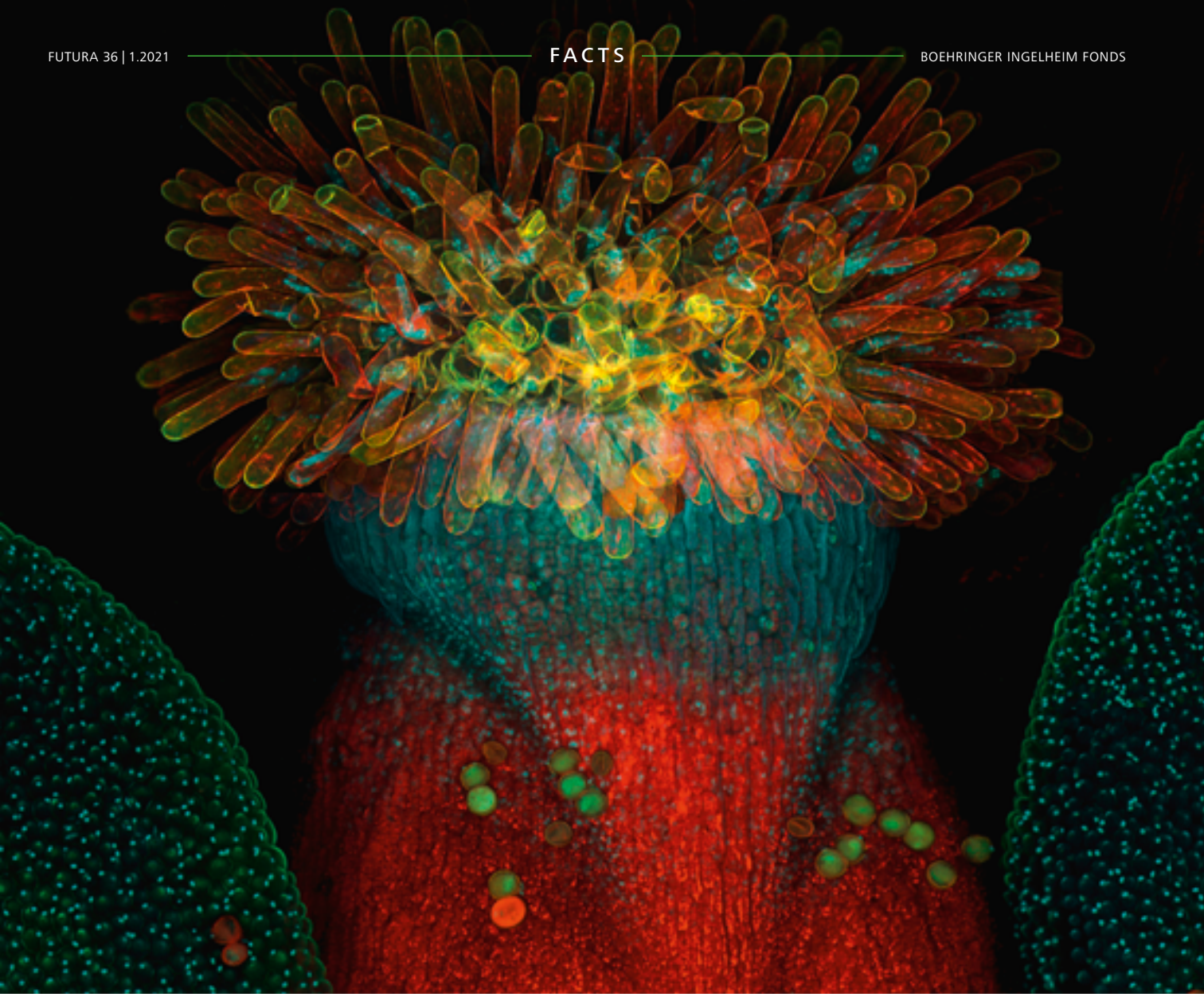


7+1

is the "functional" number of bones in a giraffe's neck. By using software originally designed for animated films such as *Shrek,* researchers have shown that the first thoracic vertebrae of a giraffe – despite bearing ribs – functions and moves as part of its unique elongated neck.

Source: Müller MA, Merten LJF, Böhmer C *et al.* (2021) Pushing the boundary? Testing the "functional elongation hypothesis" of the giraffe's neck. *Evolution* **75**: 641–655, DOI:10.1111/evo.14171

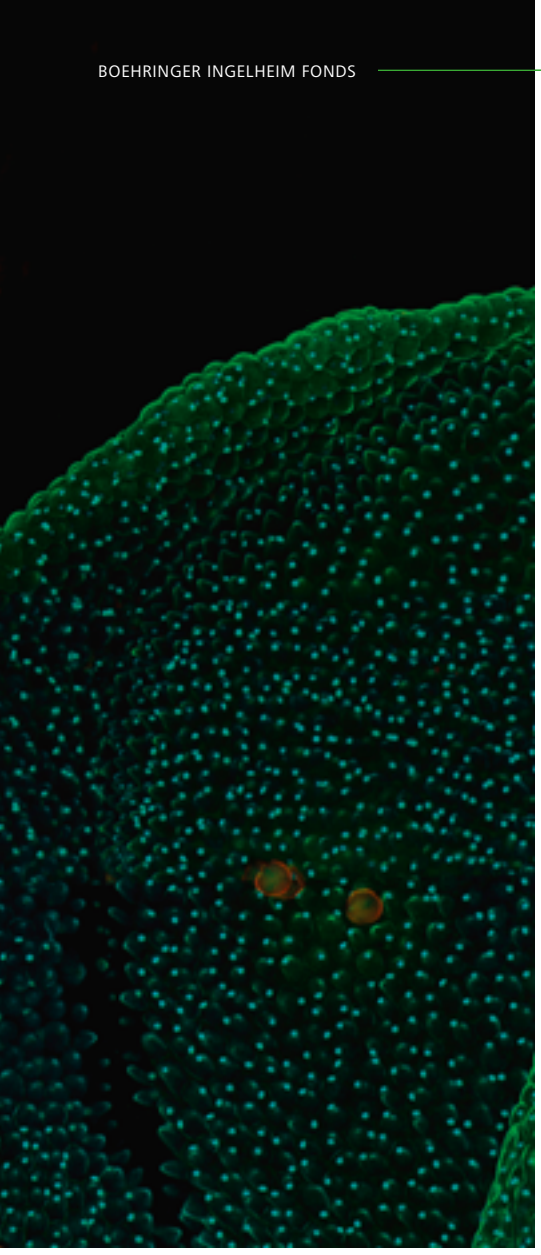
Photos: Adobe Stock/SciePro (top); iStock/dotana (right)



PROFILE OF ARABIDOPSIS THALIANA

By Mitch Leslie

While it seems unlikely that studying a plant will lead to insights into human biology, in the case of *Arabidopsis thaliana*, it turns out to be true: development and gene and protein regulation are just two of the many areas where important discoveries for human biology were first made in this unassuming plant.



A flower of *Arabidopsa thaliana* shows its beauty under the fluorescence microscope.

The first scientists to describe *Arabidopsis thaliana* were unimpressed by the small, white-flowered weed that is native to parts of Europe and Asia. "No particular virtues or uses", a British botanist wrote in the 1770s. But even today this "botanical *Drosophila*" is one of the most important model organisms still revealing fundamental information about growth, development, reproduction, metabolism, and defence in plants and animals.

Friedrich Laibach sparked a boom in *Arabidopsis* research in the 1940, when he realized how many virtues the little plant with only five small chromosomes had. It grows and reproduces quickly, producing its first seeds as little as six weeks after germination and yielding up to 10,000 of them in its lifetime. The plant requires little in terms of space, equipment, and care. Moreover, *Arabidopsis* usually self-fertilizes, a characteristic that can help researchers identify recessive genes and maintain mutant lines. These same traits now make it ideal for genome-wide association studies and research into the genotype-environment interactions relevant for all organisms. Scientists have developed methods for triggering mutations in the plant, enabling them to track down genes involved in processes such as flowering, metabolism, and innate immunity. Some names of orthologous genes in animals, such as CATERPILLAR, cannot deny their progeny. Three years after the *Arabidopsis* genome was sequenced in 2000, it became clear that most genes implicated in human disease have orthologues in this plant.

Despite these advances, *Arabidopsis* did not catch on as a model organism until scientists began to use new molecular biology tools in the 1980s. Researchers found that they could genetically modify *Arabidopsis* by infecting it with the bacterium *Agrobacterium tumefaciens*, which inserts its DNA – or genes that scientists are interested in – into the plant's cells. The plant's small genome, which is less than 1% the size of the wheat genome, simplified studies to map its genes and determine their functions in plants and animals.

Arabidopsis has enabled scientists to make several key discoveries about general biological processes, such as the innate immunity genes involved in inflammation, ubiquitination, the regulation of transcription via small double stranded RNAs, tumorigenesis, and lipid metabolism in metazoans. In the late 1990s and early 2000s, researchers identified the long-sought receptors that respond to auxins. These are the hormones that control all aspects of plant growth via ubiquitination and are important for animal and plant biologists alike. *Arabidopsis* remains the most important plant model and was the subject of more than 5,200 papers in 2020 alone.

WHO AM I? A FEW FACTS

- I first entered the lab in the early 1900s.
- I grow to about 20 to 25 cm tall.
- I live for one growing season.
- I prefer disturbed or poor soils.
- I work mostly in genetics, molecular biology, and developmental biology.
- I am still waiting for my first Nobel Prize.

Photos: AdobeStock/heitipaves (top); Adobe Stock/float (right)

The spiral galaxy NGC 5468 is about 100,000 light years across and contains roughly 200 billion stars; our body's cells outnumber the galaxy's stars by more than 100 to 1.

Photo: ESA/Hubble & NASA, A. Riess et al.

HOW TO MAP 37 TRILLION CELLS

By Liam Drew, PhD

Google Maps has become so quotidian that it is easy to forget what an audacious idea it once was. This software allows you to view maps of the Earth at whatever scale you want.

Wouldn't it be fabulous if a similar app existed for the human body – and instead of moving between buildings, you could explore the 37 trillion cells that form a human being?

Creating such a map is the ambition of Aviv Regev and Sarah Teichmann. Regev, now at Genentech, and BIF alumna Teichmann of the Wellcome Sanger Institute in Cambridge are the two computational biologists leading the Human Cell Atlas project (HCA). The Google Maps analogy was so irresistible that they could not help using it in October 2016, when they first outlined their goals at a meeting in London.

That big thinking had been sparked by the new ways in which cells could be defined using single-cell genomics and transcriptomics.

Methods for analysing the DNA or RNA of individual cells – the basis of the "resolution revolution" in genomics – had been developing in the late 2000s and early 2010s. The first genomics paper comprehensively cataloguing messenger RNA (whole transcriptomes) from single cells was published in 2009. That paper, however, characterized only a few cells. Both Teichmann and Regev started using these techniques soon after they were first developed, and saw that if they could be scaled, they could be used to create "a unified atlas of the cells of the human body".

A COLLABORATION OF CARTOGRAPHERS

Organization

- Around 2,000 contributing investigators from nearly 80 countries.
- Organized by a committee of 29 leading scientists, chaired by Sarah Teichmann and Aviv Regev.
- Eighteen biological networks for coordination of work on systems/organs/tissues, including development and organoids.
- Central data coordination platform.

Process

- Prospective contributors can join and contribute at any stage – from planning to post-publication (registration is simple at www.humancellatlas.org/join-HCA).
- Data collection through existing databases and the HCA data coordination platform with metadata and ethics standards (data.humancellatlas.org).
- Tissue samples include biopsies from healthy volunteers, resection tissue, *post mortem* samples from deceased organ donors, and human developmental samples.
- Over 65 papers published as part of the initiative since 2016.

Values

- Ethics and equity working groups to provide support for ethical tissue access and to ensure diverse ethnic and geographic groups are represented, participate, and benefit from the project.
- Support for outreach, data sharing, discussion, collaboration.
- Open science as cornerstone, data and methods freely accessible to everyone as early as possible.

Funding

- Several hundred million dollars.
- Significant supporters: Chan Zuckerberg Initiative, Wellcome, NIH, UKRI/MRC, British Heart Foundation, the European Commission, the Helmsley Charitable Trust, and others.

*as of April 2021

In 2014, Regev, then at the Broad Institute of MIT and Harvard, presented the atlas idea to the National Human Genome Research Institute (NHGRI), part of the American National Institutes of Health (NIH). At the same time, in the UK, Teichmann was co-leading the Sanger-EBI Single-Cell Genomics Centre, laying foundations to scale up the technologies and enable the possibility of mapping the human body.

In early 2016, the two of them joined forces to rally the international community around the vision of a human cell atlas.

Teichmann says the ultimate goal is to create reference atlases of healthy tissues, including developmental and male and female reproductive tissues. Using them, she explains, will be like zooming from a whole body or tissues into individual cells – or like moving from a Google map to a street view.

To achieve this, Regev and Teichmann envisaged a global community that would openly share expertise, resources, and data. At the London meeting in 2016, they proposed this and the scientific community immediately bought into it.

Five years on, the HCA is a well-funded collaboration expanding in multiple directions, involving around 2,000 researchers from almost 80 countries (See box above: "A Collaboration of Cartographers"). And with the transcriptomes of over 39 million cells from fifteen different organs sequenced, the consortium is well en route to version 1.0 of the atlas.

FROM CELLS TO ATLASES

In the current era of big science, there is something immediately appealing about a massive reference data set describing the human body's every cell type. Cells are the basic unit of life – and understanding how individual cells together form the functioning body of a multicellular organism requires knowledge of the diverse cells the body contains. Yet Teichmann says such an atlas has not traditionally occupied the imaginations of cell biologists.

She attributes this to the dominance of microscopy in cell biology. Ever since 1665 – when Robert Hooke first saw cells in a slither of cork using a new type of microscope – biologists have primarily described cell types according to their appearance. But microscopy-based biology has not lent itself to high-throughput science. "The HCA", Teichmann says, "is the marriage of the bioinformatics and genomics high-throughput community with cell biology studies, clinical research, and that imaging element."

This marriage is described in the consortium's white paper, which details two main complementary branches of investigation. The first entails using single-cell omics to catalogue all the body's cell types, tissue by tissue. To date, these efforts have been dominated by transcriptomics, but increasingly these characterizations will be supplemented by data from single-cell analyses of epi-

Photo: GettyImages/zak00

genomics, chromatin structure, and protein levels. The second branch involves spatial technologies to describe where the various cell types reside.

Highlighting the departure from traditional microscopy-based cell classification, mass single-cell transcriptomic profiling operates initially without reference to cellular morphology. Tissue samples are not looked at intact, but lysed to yield a cell suspension. The individual cells' mRNA content is then sequenced, and each cell is represented by its expression levels of the roughly 20,000 human coding genes.

A cell is then plotted as a position in 20,000-dimensional space. Such space exists only mathematically. To help imagine it, picture having three cells and mRNA levels for three genes. Cell A expresses the genes at 4, 5, and 12. Cell B has values of 1, 1, and 10. And Cell C: 4, 5, 1. You could then plot these cells as x, y, and z coordinates on a 3D graph. Note that cells A and C would have been indistinguishable with data only from genes one and two – but the third gene makes them clearly distinct points in 3D space.

Allowing for natural variability in expression levels, plotting data from many cells creates clusters in multidimensional space. Each cluster represents a cell type – with the size of each cluster corresponding to the abundance of that cell type. Plotting all the cells sampled from an organ or a body – i.e. millions to trillions of cells, represented on 20,000 axes – should reveal all the various cell types present.

When complete, this cellular catalogue – incorporating data from all the body's tissues – will be the endpoint of branch one. Then, branch two's spatial technologies will yield the final reference bodies, indicating the distributions of the characterized cell types across the body.

Emma Damm, a graduate student working with Teichmann, says these spatial methods, which are rapidly evolving, can currently be divided into two main categories. One uses fluorescent probes that bind to RNA within cells in slices of tissue. Essentially an advanced form of *in situ* hybridization, the approach has been honed so that multiple probes, labelled with different fluorophores, can distinguish hundreds of mRNAs: a tissue section glowing with different combinations of colours showing those RNAs' distribution.

To identify as many cell types as possible in a given tissue section, researchers analyse the expression profiles of the cell types present in the corresponding organ and design probes accordingly. "An area of development", Damm says, "is trying to identify the best set of marker genes to allow you to identify all the cell types in the tissue."

The second spatial technology uses sequencing and computation. Here, tissue slices are placed on grids of 50–100 µm diameter wells, and the cells situated above a well are then pulled down into the wells. Depending on cell size, each well in the grid will contain roughly 5–20 cells. Each well's RNA is barcoded to indicate where in the tissue it came from, then sequenced. Finally, machine learning algorithms analyse the wells' RNA content and compare each one to the profiles of the cell types defined in branch one. The algorithms then estimate the cell types present in every 50–100 µm spot of tissue, thereby creating a map of cell types.

These techniques, Regev says, "are essential to understand how cells work together in their environments, and to build comprehensive maps of the cells in the human body."

The HCA's first draft of the atlas will not be a whole-body map but rather cell type catalogues of twelve or more major organs containing some spatial characterization. It is expected within a year or two.

BIOLOGICAL INSIGHTS

So far, members of the HCA community have published more than 65 papers, which have included examinations of the lungs, colon, brain, liver, retina, pancreas, and heart. As was widely anticipated, single-cell transcriptomics has revealed a number of previously unknown cell types. Sometimes these were entirely novel; other times, well-known cell types were shown to actually comprise two or more subtypes.

The ultimate goal is to create reference atlases of healthy tissues, including developmental and male and female reproductive tissues.

The discovery of completely novel cell types is an exciting part of HCA research. As an example, Regev cites ionocytes in the lungs – these newly discovered, very rare cells may be important for cystic fibrosis biology, as they express the CTFR gene, mutations in which cause the condition.

New subclasses of neurons and dendritic cells were discovered in the retina and immune system, respectively. In both cases, these were unsuspected subdivisions of very well-studied cell types, which morphological studies had never previously indicated.

Among Teichmann's favourite examples of novel functional insights are those arising from her group's study of the first trimester of the maternal–foetal interface. "It wasn't really understood how maternal immune tolerance towards paternal antigens occurred," she says. "Our work really turned a light onto that topic." They showed, among other things, that the maternal side of the placenta, the decidua, contains layers of stromal cells which make unexpected contributions to immune system regulation.

"We also generated a general statistical framework to map the intercellular conversations that are happening in any tissue," she says of this placenta study. The group developed a computational tool – publicly available at www.cellphonedb.org – that predicts how ligands from one cell type activate receptors from other cell types and thus change their transcriptome. That is, cell-specific gene expression data can decipher important cell-to-cell communications.

"Computation is really at the heart of this project," Teichmann says. Innovative statistical analyses of big data impact every aspect of it. And this includes the challenge of distinguishing different cell types from distinct cell states.

Despite a colloquial understanding of the concept, precisely defining what "cell type" means is incredibly difficult. In general terms, it is considered a relatively stable and restricted phenotype that a cell assumes at a specified stage of its development. Conversely, cell states are considered different phenotypes that individual cells can dynamically move between.

Both states and types will, however, be characterized by different gene expression profiles, and thus correspond to different points on the multidimensional graph. Therefore, distinguishing between the two requires establishing how cells can move around that space.

Because cells of many types can assume multiple states, each state needs characterizing to avoid confusing different cell states for different cell types. Complicating matters, it is now known that at any stage in life, certain cells can differentiate into what has conventionally been considered a different type, further blurring the lines between what is defined as a state and a type. The more rigorously researchers interrogate these definitions, the more slippery they get.

Finally, numerous HCA studies have compared cell types in healthy and disease affected tissue, observing how novel cell types or states occur in pathological conditions. In addition, the consortium, notably, responded quickly to the Covid-19 pandemic, establishing which cell types are infected by the novel coronavirus and how this affected them.

FROM DESCRIPTION TO EXPERIMENTATION

To enable the construction of an entire human atlas, 18 HCA Biological Networks have been established for specific tissues. For example, the Developmental and Pediatric Networks are creating atlases that chart the cellular lineages that generate human cellular diversity. Also, there is the Organoid Cell Atlas, led by Professor Christoph Bock at the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences in Vienna.

Organoids are 3D cultures of cells that recapitulate – at micro- or mesoscopic levels – structural features of *in vivo* tissues. For some time, Bock used single-cell omics to investigate how human organoids respond when perturbed by genetic changes introduced by CRISPR technologies. As he explains, he felt that "the HCA, to achieve its goals, would have to go beyond descriptive profiling and take function into account."

Because organoids offer ways of doing functional assays on human tissues, Bock contacted Teichmann and Regev to discuss producing an Organoid Cell Atlas. By cataloguing the cell types present in organoids, researchers will see which are present and how they differ from those in intact systems. "The data we're generating will help make better organoids by seeing what is missing in the organoids, then adapting the conditions," Bock says.

More importantly for the overall HCA project, Bock envisages that hypotheses emerging from *in vivo* observations – including disease-related changes – will be testable using organoids. "The idea", he says, "is to build a computational platform: an organoid cell atlas portal that allows you to computationally go back and forth."

So far, the projects are merging well. "There's a certain feeling of community, that we're in this together and want to succeed at this as a global group," Bock says. He praises in particular how the HCA collaboration "has set a tone of how science is done in this field, which is something I very much appreciate, that it's about reproducibility, openness, and helping each other out."

This sentiment is echoed by Regev. When asked if she is surprised by how quickly the project has progressed, she says, "I am not, actually. I expected no less from these scientists – and I deeply believe in the power of scientific networks."

Please understand that in the interest of our fellows, we publish only results online, not descriptions of ongoing projects.

Therefore, this pdf continues with the section Results.

RESULTS The Boehringer Ingelheim Fonds funds excellent PhD students who are selected as much for their academic record as for their ambitious projects. Here, they present a synopsis of their findings, which aim to push the boundaries of our knowledge of the fundamental phenomena of human life.

Shared first authorship is indicated with an *.

DISSEMINATION OF ANTIBIOTIC RESISTANCE IN BACTERIAL GUT PATHOGENS

cf. BIF FUTURA, VOL. 33 | 1.2018

ERIK BAKKEREN
Discipline: Evolutionary Biologist, MSc
Institute: Institute of Microbiology, ETH Zurich, Switzerland
Supervisor: Prof. Wolf-Dietrich Hardt

Transmissible plasmids drive the dissemination of antibiotic resistance by moving genetic information between and within bacterial species. However, the factors that influence their spread in relevant ecological niches are poorly understood. In my PhD project, I aimed to quantify the spread of resistance plasmids and identify the factors that modulate plasmid spread in the gut. I used *Escherichia coli* clinical isolates harbouring extended-spectrum beta lactamase (ESBL) plasmids as donors and tested their transfer to other *E. coli* or *Salmonella enterica* serovar Typhimurium (STm) strains in mouse models for gut colonization. I found that ESBL plasmids transferred to the recipient strains in the absence of antibiotic selection, with up to 1% of the population obtaining a plasmid. Importantly, my collaborators showed that these plasmids spread similarly *in vitro*. This correlation between *in vitro* and *in vivo* spread suggests that predictive modelling could be used for high-throughput testing of plasmid-carrying clinical isolates against potential recipient strains, to understand how they might spread in the gut. To investigate the conditions that modulate plasmid transfer in the gut, I performed co-infection experiments with STm. STm is a gut pathogen that forms reservoirs of persister cells inside host tissues. These cells are phenotypically recalcitrant to antibiotic therapy. I found that plasmids inside STm persisters can be transferred to bacterial strains in gut lumen after antibiotic therapy. Vaccination can slow this process by blocking the establishment of persister reservoirs. Overall, I quantified the spread of clinically relevant antibiotic resistance plasmids, as well as demonstrating a role for persisters in promoting plasmid spread.

PUBLICATIONS

Benz F*, Huisman JS*, Bakkeren E*, Herter JA, Stadler T, Ackermann M *et al* (2020) Plasmid- and strain-specific factors drive variation in ESBL-plasmid spread *in vitro* and *in vivo*. *ISME J* **15**(3): 862–878: 10.1038/s41396-020-00819-4

Bakkeren E, Diard M, Hardt WD (2020) Evolutionary causes and consequences of bacterial antibiotic persistence. *Nat Rev Microbiol* **18**: 479–490

Bakkeren E, Huisman JS, Fattinger SA, Hausmann A, Furter M, Egli A *et al* (2019) *Salmonella* persisters promote the spread of antibiotic resistance plasmids in the gut. *Nature* **573**: 276–280

Further results of this project can be discussed on *BioRxiv*: doi: 10.1101/824821

OBESITY ALTERS MICROGLIA'S RESPONSE TO MYELIN DAMAGE AND PERTURBS MYELIN REGENERATION

cf. BIF FUTURA, VOL. 33 | 1.2017

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Discipline: Neuroscientist, MSc
Institute: German Centre for Neurodegenerative Diseases (DZNE), Munich, Germany
Supervisor: Prof. Mikael Simons

Damage to myelin, the lipid layer surrounding axons, triggers a regenerative process known as remyelination. This process is tightly regulated by microglia, which clear myelin and axonal debris and coordinate the recruitment and differentiation of new oligodendrocytes to regenerate myelin. In multiple sclerosis, remyelination decreases in efficiency or fails altogether, eventually leading to neurological decline and brain atrophy. Enhancing endogenous remyelination might prevent neuronal death. Recent studies demonstrate that diet could influence remyelination. The goal of my PhD project was therefore to study the effects of diet-induced obesity on remyelination. I fed wild-type mice a Western diet, which is high in calories, fat, and sugar. I used the mouse model of lysolecithin-induced demyelination, which creates a focal demyelination in the corpus callosum. I showed that mice fed a Western diet were characterized by poor remyelination and poor lipid processing by their microglia and macrophages. Moreover, I showed that transforming growth factor β (TGFβ) signalling is increased by the Western diet and prevents microglia and macrophages from activating their inflammatory repertoire upon demyelination. Such excessive TGFβ prevents the activation of the liver X receptor (LXR) pathway, which microglia and macrophages need after myelin intake. By hindering the LXR pathway, TGFβ prevents correct lipid handling by microglia and macrophages and jeopardizes remyelination. Indeed, deleting TGFβ signalling in microglia promoted lipid processing in microglia and subsequently enhanced remyelination in mice fed a Western diet. Based on these results, I propose that innate immune checkpoint mechanisms such as TGFβ signalling can perturb the microglial response to demyelination and limit remyelination potential. My results support earlier reports demonstrating that a temporally regulated switch in the microglia and macrophage phenotype is required to promote myelin repair. My work also demonstrates that obesity can perturb a regenerative response in the brain by disturbing the pro-regenerative functions of microglia.

PUBLICATION

Bosch-Queralt M, Cantuti-Castelvetri L, Damkou A, Schifferer M, Schlepckow K, Alexopoulos I *et al* (2021) Diet-dependent regulation of TGFβ impairs reparative innate immune responses after demyelination. *Nat Metab* **3**(2): 211–227

INVESTIGATIONS INTO THE ROLE OF TPL-2 AND ABIN-2 IN INFECTION

cf. BIF FUTURA, VOL. 32 | 1.2017

FELIX BREYER
Discipline: Biochemist, BSc
Institute: The Francis Crick Institute, London, UK
Supervisor: Prof. Steve Ley

Treatment of bacterial diseases represents a major unmet medical need. Antimicrobials are widely used to treat bacterial infections. However, antimicrobial resistance has become widespread, giving rise to "superbugs" that are resistant to traditional antibiotics. In the innate immune response, pathogens such as bacteria are killed by immune cells including macrophages. During phagocytosis, bacteria are internalized into membrane-bound vacuoles called phagosomes. In a complex maturation process, they undergo sequential membrane fusion events with the endosomal compartment and ultimately with lysosomes to form a phagolysosome. The mature phagolysosome efficiently degrades internalized bacteria. Tumour progression locus 2 (TPL-2), a protein kinase, plays an important role in mediating inflammatory immune responses to bacteria, viruses, and fungi by regulating the expression of immune genes. TPL-2 forms a complex with A20-binding inhibitor of NF-κB 2 (ABIN-2), an adaptor protein with an unknown physiological function. During my PhD, I used a combination of analytical biochemistry, cell biology, and proteomics to investigate whether TPL-2 or ABIN-2 regulate phagocytosis in macrophages. I found that both of these proteins promote phagosome maturation in macrophages. Genetic inactivation of TPL-2 catalytic activity or ABIN-2 ubiquitin binding substantially altered the composition of the phagosome proteome in mouse macrophages. Consistent with these findings, I showed that TPL-2 catalytic activity and ABIN-2 ubiquitin binding are required for efficient killing of internalized *Staphylococcus aureus* by macrophages. Genetic inactivation of the two proteins' functions impaired maturation of *S. aureus* phagosomes and led to increased bacterial burden. By identifying novel signalling pathways that promote phagosome maturation and pathogenic bacterial killing by macrophages, my research may lead to the development of novel therapeutic approaches for acute bacterial infections.

PUBLICATIONS

Breyer F, Härtlova A, Thurston T, Flynn HR, Chakravarty P, Janzen J *et al* (2021) TPL-2 kinase induces phagosome acidification to promote macrophage killing of bacteria. *EMBO J*, doi: 10.15252/embj.2020106188

Ventura S*, Cano F*, Kannan Y*, Breyer F, Pattison MJ, Wilson MS *et al* (2018) A20-binding inhibitor of NF-κB (ABIN) 2 negatively regulates allergic airway inflammation. *J Exp Med* **215**: 2737–2747

UNDERSTANDING POST-TRANSCRIPTIONAL MECHANISMS OF RNA DEGRADATION DURING EMBRYOGENESIS

cf. BIF FUTURA, VOL. 32 | 1.2017

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Supervisor: Dr Andrea Pauli

In embryogenesis, a single cell gives rise to a complete organism. As this process is transcriptionally silent, it relies on the post-transcriptional regulation of maternally provided RNAs. As embryogenesis proceeds, RNAs are degraded and new transcripts are generated during the maternal-to-zygotic transition (MZT). Although mechanisms of RNA degradation have been described, they do not fully account for the extent of RNAs removed during the MZT. In my PhD project, I investigated alternative pathways of RNA decay during the zebrafish MZT. First, I analysed RNA sequencing and ribosome profiling data to quantify RNA and translation level, respectively, from 2–4-cell to 5-day-old zebrafish embryos. I found a correlation between the presence of upstream open reading frames (uORFs) – which are repressive elements – in maternal transcripts and the degradation of these RNAs during the MZT. This led me to hypothesize that uORFs might play a role in promoting maternal RNA decay. I combined an RNA reporter library with high-throughput sequencing to assess whether the uORFs are necessary (in the case of the mutated uORFs) or sufficient (in the case of inserted uORFs) in promoting RNA decay. Analysis of reporter RNA levels over time revealed that uORFs are neither necessary nor sufficient to promote maternal RNA decay. However, inducing uORF translation during embryogenesis promoted efficient RNA degradation. These results suggest that the presence of uORFs in maternal transcripts does not indicate functionality, and that efficient uORF translation is necessary to promote RNA decay during the MZT. In the second part of my project, I characterized a component of the global RNA degradation machinery in eukaryotes, the superkiller 7 protein, Ski7. I found that Ski7 is highly expressed during early embryogenesis. Combined phenotypic and time-course RNA sequencing analysis of *ski7* mutants revealed that Ski7 is not essential during embryogenesis. However, it is involved in shaping the maternal transcriptome during egg formation and potentially in regulating the stress response during embryogenesis. Overall, my results provide insight into mechanisms of post-transcriptional regulation of gene expression during the MZT.

PUBLICATION

Cabrera Quio LE, Schleiffer A, Mechtler K, Pauli A (2021) Zebrafish Ski7 tunes RNA levels during the oocyte-to-embryo transition. *PLoS Genet* **17**(2): e1009390

DEVELOPING A LIVE-CELL IMAGING TECHNIQUE TO VISUALIZE MAMMALIAN FOETAL OOCYTE MEIOSIS

cf. BIF FUTURA, VOL. 32 | 1.2017

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Institute: Max Planck Institute for Biophysical Chemistry, Goettingen, Germany
Supervisor: Dr Melina Schuh

Oocytes are female germ cells that mature into fertilizable eggs through meiosis. In mammals, oocytes form in the ovaries during foetal development. Foetal oocytes progress through the first stage of meiosis, termed prophase, before entering a prolonged state of cellular arrest; meiosis only resumes following puberty. During prophase, maternal and paternal chromosomes pair and align, and then chromosome recombination is induced. These prophase processes are crucial for increasing genetic diversity in offspring and for decreasing the risk of chromosome segregation errors during later meiosis. Studies of mammalian oocyte meiosis focus mainly on the stages following meiotic resumption. This is largely due to the establishment of techniques that enable live-cell microscopy of adult oocytes. By contrast, our understanding of foetal oocyte meiosis is derived from fixed-cell studies, which produce less dynamic and temporal information about how molecular processes proceed in foetal oocytes. During my PhD, I developed a technique to study mouse foetal oocytes through live-cell microscopy. After dissecting ovaries from mouse embryos, I isolated a single-cell suspension of foetal oocytes through chemical-based disaggregation of ovarian tissue. Following this, I used electroporation to transiently introduce plasmids encoding fluorescently tagged proteins into these foetal oocytes. I then cultured the transfected foetal oocytes *ex vivo* on a layer of pre-seeded somatic cells. I found that this cell-culture method supports oocyte survival *ex vivo* while also allowing subcellular events during meiotic prophase to be visualized with ease using confocal microscopy. This live-cell imaging technique is expected to allow for more comprehensive investigations into dynamic meiotic prophase events, such as homologous chromosome pairing. Such studies could provide further insight into how chromosomal mis-segregations arise in oocytes, a leading cause of pregnancy loss and several congenital diseases.

PUBLICATIONS

The results of this project have not yet been published.

MAKING SENSE OF VARIATIONS IN THE TRANSCRIPTIONAL IDENTITY OF CORTICAL NEURONS

cf. BIF FUTURA, VOL. 31 | 1.2016

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Supervisor: Dr Solange Brown

Proper function of the neocortex depends on a wide variety of neuronal cell types. A central goal of neuroscience has been to describe the diversity of these cell types, but the correlations between morphological, physiological, and molecular neuronal identities, as well as the distinction between neuron types and states, remain poorly understood. In my PhD project, I compared expression profiles of layer 6 corticothalamic neurons (L6CThNs). This heterogeneous population of neocortical neurons has been defined by anatomical, functional, and gene expression studies, which makes them well suited for investigating relationships between transcriptional subtypes and other cellular properties. By combining single-cell RNA sequencing with an enrichment strategy that preserved axonal target information, I identified two distinct L6CThN subtypes whose transcriptional profiles reflected their long-range projection targets and laminar position within layer 6. These two L6CThN subtypes also exhibited divergent signatures of neuronal activity, both at baseline and following manipulation of sensory input. Subtype biases in the choice of response following sensory manipulation increased transcriptional heterogeneity within each type and reinforced the transcriptional differences between the two L6CThN subtypes. My results demonstrate that scRNA-seq can be used to identify the independent contributions of multiple biological signals that together determine transcriptional heterogeneity within and across neuronal populations.

PUBLICATIONS

Whilden C*, Chevée M*, Seong Yeol An C, Brown SP (2021) The synaptic inputs and thalamic projections of two classes of layer 6 corticothalamic neurons in primary somatosensory cortex of the mouse. *J Comp Neurol*, in press

Frandolig JE, Matney CJ, Lee K, Kim JK, Chevée M, Kim SJ *et al* (2019) The synaptic organization of layer 6 circuits reveals inhibition as a major output of a neocortical sublamina. *Cell Rep* **28**: 3131–3143

Minamisawa G, Kwon SE, Chevée M, Brown SP, O'Connor DH (2018) A non-canonical feedback circuit for rapid interactions between somatosensory cortices. *Cell Rep* **23**: 2718–2731

Chevée M, Robertson JDJ, Cannon GH, Brown SP, Goff LA (2018) Variation in activity state, axonal projection, and position define the transcriptional identity of individual neocortical projection neurons. *Cell Rep* **22**: 441–455

MUTANT mRNA DECAY TRIGGERS TRANSCRIPTIONAL ADAPTATION TO MUTATIONS

cf. BIF FUTURA, VOL. 32 | 1.2017

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Supervisor: Prof. Didier Stainier

Robustness to mutations promotes organisms' well-being and fitness. The increasing number of mutations in various model organisms and in humans that show no obvious phenotype has renewed interest into how organisms adapt to gene loss. Genetic compensation of deleterious mutations by transcriptional upregulation of related genes (also known as transcriptional adaptation) has been reported in many species. However, the molecular mechanisms underlying transcriptional adaptation were unclear. The goal of my PhD project was therefore to investigate this phenomenon. I developed and analysed multiple models of transcriptional adaptation in zebrafish and mouse cell lines. First, I showed that transcriptional adaptation is not caused by loss of protein function, which indicates that the trigger lies upstream. As mutations often lead to transcripts that are degraded by the mRNA surveillance machinery, I investigated this process. Using genetic and pharmacological approaches, I found that degradation (also described as decay) of mutant mRNA is required to trigger transcriptional upregulation of the related gene. Next, I generated alleles that fail to transcribe the mutated gene. I found that these alleles do not display a transcriptional adaptation response, and they exhibit more severe phenotypes than those observed in alleles displaying mutant mRNA decay. Transcriptome analysis of the alleles displaying mutant mRNA decay revealed upregulation of a significant proportion of genes that had sequence similarity to the mutated gene's mRNA. This suggests that mRNA decay intermediates could induce transcriptional adaptation via sequence similarity. Further mechanistic analyses suggested that RNA decay factor-dependent chromatin remodelling and repression of antisense RNAs might also be implicated in the transcriptional adaptation response. In addition to identifying a novel role for mutant mRNA decay in protecting against mutations, my results have implications for understanding disease-causing mutations. They could also help in the design of mutations that lead to minimal genetic compensation induced by transcriptional adaptation, which would facilitate the study of gene function in model organisms.

PUBLICATION

El-Brolosy MA, Kontarakis Z, Rossi A, Kuenne C, Günther S, Fukuda N *et al* (2019) Genetic compensation triggered by mutant mRNA degradation. *Nature* **568**: 193–197

HUMAN GUANYLATE BINDING PROTEINS ARE KEY PLAYERS IN CELL-INTRINSIC IMMUNITY

cf. BIF FUTURA, VOL. 32 | 2.2017

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Discipline: Molecular Biologist, MSc
Institute: The Francis Crick Institute, London, UK
Supervisor: Dr Eva-Maria Frickel

Multicellular organisms use cell-intrinsic immunity to combat intracellular pathogens. Most cell-intrinsic defence mechanisms are induced by cytokine exposure. IFNγ signalling induces the expression of guanylate binding proteins (GBPs), which modulate processes such as autophagy and regulated cell death. GBPs can also disrupt pathogen-containing vacuoles. Little was known about the role of human GBPs in cell-intrinsic immunity. My PhD project focused on identifying new roles of human GBPs upon infection of macrophages with the apicomplexan parasite *Toxoplasma gondii* (Tg) or the Gram-negative bacterium *Salmonella* Typhimurium (STm). I developed an artificial intelligence-based image analysis pipeline (Host Response to Microbe Analysis, or HRMAn) to study host-pathogen interactions. Using HRMAn, I showed that GBP1 can accumulate at the intracellular site of infection, which triggers several downstream processes. In Tg infection, GBP1 disrupted pathogen vacuoles and the parasites themselves, releasing Tg DNA into the host cell cytosol. This in turn induced apoptosis via the DNA-sensing protein AIM2 assembling an atypical canonical inflammasome that contained ASC (apoptosis-associated Speck-like protein containing a caspase activation and recruitment domain) and caspase-8. In STm infection, GBP1 recruitment to cytosolic bacteria created a novel immune signalling platform containing active caspase-4, which led to activation of the non-canonical inflammasome and increased pyroptosis. I also identified a feedback loop between caspase-1 and GBP1 that controls inflammation and tissue homeostasis. My work reveals new roles for human GBPs and IFNγ signalling in the regulation of cell death and, for the first time, links a GBP to the induction of apoptosis to counteract pathogen survival.

PUBLICATIONS

Fisch D, Clough B, Domart M-C, Encheva V, Bando H, Snijders AP *et al* (2020) Human GBP1 differentially targets *Salmonella* and *Toxoplasma* to license recognition of microbial ligands and caspase-mediated death. *Cell Rep* **32**: 108008

Fisch D, Bando H, Clough B, Hornung V, Yamamoto M, Shenoy AR, Frickel E-M (2019) Human GBP1 is a microbe-specific gatekeeper of macrophage apoptosis and pyroptosis. *EMBO J* **38**: e100926

Fisch D*, Yakimovich A*, Clough B, Wright J, Bunyan M, Howell M *et al* (2019) Defining host–pathogen interactions employing an artificial intelligence workflow. *Elife* **8**: e40560

CONNECTIVITY AND COMPUTATIONAL DIVERSITY OF L5 PYRAMIDAL NEURONS IN THE VISUAL CORTEX

cf. BIF FUTURA, VOL. 31 | 2.2016

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Discipline: Neuroscientist, MSci
Institute: The Francis Crick Institute, London, UK
Supervisor: Dr Ede Rancz

Sensory areas in the cerebral cortex receive connections that convey either sensory signals (feedforward) or a range of contextual information that is critical for perception (feedback). Thick-tufted layer 5 (ttL5) pyramidal neurons play an important role in integrating these multimodal signals. These cells have large morphologies that support thousands of synaptic inputs from across the brain. They express multiple ion channels that enable nonlinear dendritic operations, such as sustained potentials underlying spike bursts. Their axons also form the largest output pathway from the cerebral cortex, making ttL5 neurons ideal for combining brain-wide signals to drive behaviour. In my PhD project, I used patch-clamp recordings to characterize a genetically labelled population of ttL5 neurons in the mouse medial secondary visual cortex (V2). I found that these neurons lack some of the nonlinear functions typical of ttL5 neurons in other brain regions, which suggests that they may perform fundamentally different computations. I used morphological reconstructions and computational modelling to reveal a general principle through which variations in dendrite length can account for this diversity. The location of synapses along the dendritic tree of ttL5 neurons is also known to strongly influence how inputs are combined. A lab member had used rabies tracing to identify the brain-wide pre-synaptic inputs to ttL5 neurons in V2, which originated in several regions associated with both sensory and cognitive functions. Based on this data, I combined optogenetics and spatially patterned optical stimulation to map the subcellular distribution of synapses from these regions along the ttL5 dendrites. I found that different neural pathways are highly specific in their targeting of dendrites and have synaptic distributions that often deviate from canonical long-range connectivity rules. My work provides evidence that contradicts classical theories of hierarchical connectivity and computational uniformity in the cerebral cortex. It also lays the groundwork for future experiments investigating the function of ttL5 neurons in higher-order cortical circuits.

PUBLICATIONS

Galloni AR, Laffere A, Rancz EA (2020) Apical length modulates dendritic excitability in L5 pyramidal neurons. *Elife* **9**: e55761

Further results of this project can be discussed on *BioRxiv*: doi: 10.1101/2021.01.31.429033

IMMORTALIZATION BY MUTATIONS IN THE TELOMERASE GENE PROMOTER

cf. BIF FUTURA, VOL. 31 | 2.2016

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Institute: Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA
Supervisor: Prof. Dirk Hockemeyer

One of the hallmarks of cancer cells is immortality: the capacity to divide indefinitely. Normally, cells are limited in their ability to proliferate by the progressive shortening of their telomeres. To become immortal, most cancer cells aberrantly express the enzyme telomerase, which counteracts telomere shortening. Point mutations in the promoter of the telomerase reverse transcriptase gene (TERT) are the most common non-coding mutations in cancer and have been associated with increased telomerase levels. However, it remained unclear what effect TERT promoter mutations (TPMs) have on cells' capacity to proliferate and when during tumorigenesis they are acquired. The goal of my PhD project was to determine at what stage of cancer formation TPMs are selected for and how they contribute to the immortalization of pre-cancerous cells. By analysing samples of precursor and advanced melanomas from the same malignant lesions, my collaborators and I found that TPMs are some of the first mutations that occur during melanoma formation, but they do not prohibit telomere shortening. Using long-term isogenic genome-edited cells with or without the TPM, I found that the presence of a TPM did not prohibit telomere shortening but nevertheless led to the immortalization of a bulk cell population. The immortalization by TPMs was characterized by two phases. In the first phase, telomerase was gradually upregulated, and telomeres continued to shorten. Telomerase expression levels were sufficient only to extend the cellular lifespan so that pre-cancerous cells could keep dividing longer. In the second phase, due to excessive telomere shortening, the resulting deprotected telomeres caused genomic instability by telomere fusions. In this phase, the presence of TPMs supported the further upregulation of telomerase to ensure sustained proliferation. In summary, my results offer explanations for how TPMs can support immortalization and how tumour cells can end up with extremely short telomeres despite elevated telomerase levels.

PUBLICATIONS

Lorbeer FK, Hockemeyer D (2020) TERT promoter mutations and telomeres during tumorigenesis. *Curr Opin Genet Dev* **60**: 56–62

Chiba K*, Lorbeer FK*, Shain AH, McSwiggen DT, Schruf E, Oh A *et al* (2017) Mutations in the promoter of the telomerase gene TERT contribute to tumorigenesis by a two-step mechanism. *Science* **357**: 1416–1420

MECHANISTIC INSIGHTS INTO A NOVEL ANTIBIOTIC AGAINST MRSA

cf. BIF FUTURA, VOL. 32 | 2.2017

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Discipline: Biochemist, MSc
Institute: Technical University of Munich, Germany
Supervisor: Prof. Stephan A. Sieber

Multidrug-resistant bacteria are a growing threat to human health. The Sieber group created a new molecule, PK150, that has antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and other Gram-positive pathogens. PK150 can also kill persister cells – dormant cells that normally resist drug treatment. The group observed no development of resistance to PK150 under laboratory conditions. Mode-of-action analysis using chemical proteomics suggested that the primary target of PK150 in *S. aureus* is signal peptidase Ib (SpsB), an essential enzyme in the bacterial secretory pathway. Unexpectedly, PK150 stimulates, rather than inhibits, enzyme activity. The goal of my PhD project was to study PK150's effect on SpsB and its antibacterial mechanism in general. Using a chemical probe that was more refined than the one used previously, I identified demethylmenaquinone methyltransferase (MenG) as an additional target of PK150. MenG catalyses the last step in the biosynthesis of the essential electron carrier menaquinone, so its blockage disrupts bacterial energy metabolism. I confirmed the inhibitory effect of PK150 on MenG using biochemical assays. In addition, I discovered that MenG might also be targeted by the structurally related compound triclocarban. This antibacterial chemical of unknown mechanism of action was formerly used as a disinfectant. Unlike PK150, however, it does not modulate SpsB. Lastly, I found that PK150 can be rendered active against Gram-negative bacteria if their outer membrane is permeabilized. However, the underlying targets are likely distinct from those of *S. aureus*. In particular, I found that PK150 does not activate the homologous signal peptidase in *Escherichia coli*. My work has provided new insights into the multifaceted mode of action of a novel antibacterial compound and thereby laid the groundwork for its development into an antibiotic drug.

PUBLICATIONS

Le P*, Kunold E*, Macsics R*, Rox K, Jennings MC, Ugur I *et al* (2020) Repurposing human kinase inhibitors to create an antibiotic active against drug-resistant *Staphylococcus aureus*, persisters and biofilms. *Nat Chem* **12**: 145–158

Macsics R, Hackl MW, Fetzer C, Mostert D, Bender J, Layer F *et al* (2020) Comparative target analysis of chlorinated biphenyl antimicrobials highlights MenG as a molecular target of triclocarban. *Appl Environ Microbiol* **86**: e00933-20

CONFORMATION OF SISTER CHROMATIDS IN THE REPLICATED HUMAN GENOME

cf. BIF FUTURA, VOL. 32 | 1.2017

MICHAEL MITTER
Discipline: Cell Biologist, MSc
Institute: Institute of Molecular Biotechnology (IMBA), Vienna, Austria
Supervisor: Dr Daniel Gerlich

A human cell packs two metres of DNA into a nucleus measuring only a few hundred micrometres in diameter. DNA compaction cannot happen randomly, because many cellular processes need quick access to different parts of the genome. When the genome is replicated during the cell cycle, the amount of DNA in the already packed nucleus doubles. Moreover, the genome performs other functions, most importantly the repair of DNA breaks using the second sister chromatid as a template. During this process, how sister chromatids fold relative to each other – that is, the precise orchestration of relative structure – is critical to ensure successful DNA repair and to avoid disastrous outcomes upon failure, such as cell death or cancer. However, there were no methods to look at the relative structure of sister chromatids, which precluded experiments that measure its influence on genomic processes. In my PhD project, I set out to develop such a method. I used synthetic nucleotides to make the two sister chromatids distinguishable. Specifically, I found a nucleotide that is similar to natural thymidine but can be chemically modified in HeLa cells. In a sequencing experiment, this modification marks the position of the synthetic nucleotide in the genome. Using this approach, I applied sequencing techniques to generate the first map of relative sister chromatid structure in HeLa cells. This map shows that sister chromatids are globally aligned during the G2 cell-cycle stage – a genomic location on one sister chromatid is in the physical vicinity of the same region on the other sister chromatid. At a local level, however, the sister chromatids are not perfectly aligned – some regions are close together, while others are far apart. Perturbation experiments showed that cohesin, a protein complex involved in DNA folding, is responsible for this organization. A stable population of cohesin is responsible for global alignment, whereas a dynamic population causes local separation. This new technology for distinguishing sister chromatids, as well as my genome-wide map of sister chromatid interactions, will contribute to our understanding of many processes that rely on shaping genome structure throughout the cell cycle.

PUBLICATION

Mitter M, Gasser C, Takacs Z, Langer HC, Tang W, Jessberger G *et al* (2020) Conformation of sister chromatids in the replicated human genome. *Nature* **586**: 139–144

UNRAVELLING HOW ANCILLARY NON-SPECIFIC NUCLEASES BOLSTER TYPE III CRISPR-CAS IMMUNITY

cf. BIF FUTURA, VOL. 32 | 1.2017

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Institute: The Rockefeller University, New York, NY, USA
Supervisor: Prof. Luciano Marraffini

To protect against genetic parasites, bacteria use diverse defence mechanisms, such as CRISPR-Cas. Of the six types of CRISPR-Cas system, type III systems are uniquely able to degrade both the DNA and RNA of an invader using the main Cas complex. In addition, this complex synthesizes cyclic oligoadenylate (cOA) second messengers during targeting. In my PhD project, I characterized the downstream accessory effector nucleases that are activated by cOA signalling, as well as their effect on immunity. Csm6 (*cas* subtype *Mycobacterium tuberculosis* 6) is the most common ancillary gene found in type III systems. I showed *in vivo* that Csm6 is a non-specific RNase that degrades both host and parasite transcripts. This creates a transient growth defect, which is relieved when the Cas complex clears the invader. I found that Csm6 was sometimes essential – against targets that were difficult for the Cas complex to eliminate – but at other times was dispensable for immunity. Many type III loci contain different genes that are also predicted to be activated by cOA. I pursued a gene, which I named cOA-activated RNase and DNase 1 (Card1), that contains a restriction endonuclease-like domain (predicted to cut DNA). I showed that *in vitro*, the addition of cOA activated both single-stranded DNase activity and single-stranded RNase activity in Card1. *In vivo*, Card1 enhanced anti-bacteriophage protection and induced a growth arrest that can eventually lead to cell death. My work shows how type III CRISPR-based immunity is achieved by a "two-hit punch", whereby the sequence-specific targeting of the main Cas complex is combined with the non-specific nuclease activity of an accessory nuclease (Csm6 or Card1).

PUBLICATIONS

Rostøl JT*, Xie W*, Kuryavyi V, Maguin P, Kao K, Froom R *et al* (2021) The Card1 nuclease provides defence during type-III CRISPR immunity. *Nature* **590**: 624–629

Rostøl JT, Marraffini LA (2019) Non-specific degradation of transcripts promotes plasmid clearance during type III-A CRISPR-Cas immunity. *Nat Microbiol* **4**: 656–662

Rostøl JT, Marraffini LA (2019) (Ph)ighting phages: how bacteria resist their parasites. *Cell Host Microbe* **25**: 184–194

Niewoehner O*, Garcia-Doval C*, Rostøl JT, Berk C, Schwede F, Bigler L *et al* (2017) Type III CRISPR–Cas systems produce cyclic oligoadenylate second messengers. *Nature* **548**: 543–548

SEQUENCE SPACE AND PROPERTIES OF XPO1-DEPENDENT NUCLEAR EXPORT SIGNALS

cf. BIF FUTURA, VOL. 32 | 1.2017

OLEH RYMARENKO
Discipline: Molecular Biologist, MSc
Institute: Max Planck Institute for Biophysical Chemistry, Goettingen, Germany
Supervisor: Prof. Dirk Görlich

Exportin 1 (XPO1) mediates one of the most versatile protein transport pathways in the eukaryotic cell. It exports hundreds of different proteins from the nucleus to the cytoplasm. XPO1 recognizes its cargos by binding to the nuclear export signals (NESes) – short continuous sequences of specific amino acid composition. Despite decades of research, the features that define the ability of a sequence to recruit XPO1 remain poorly characterized. To address this question, I combined filamentous phage display and high-throughput DNA sequencing to interrogate the sequence space of the most common type of NES. This allowed me to quantitatively assess the preference of XPO1 for different residues at different positions of an NES. I discovered that XPO1 has strong and specific preferences for residues at so-called spacer positions, which were previously neglected in defining the NES motif. Furthermore, I showed that the spacer residues contribute significantly to NES activity *in vivo*. Altering the spacer composition was sufficient to inactivate a potent NES. Using the preferences I discovered, I engineered novel NES-like sequences. First, I created sequences that share no identity with the prototype NES yet act as XPO1-dependent NESes *in vivo*. Next, I designed peptides that bind XPO1 with much higher affinity than previously described NESes. These peptides exhibited unique behaviour *in vivo*, including inhibition of XPO1-mediated nuclear export in the cell. I structurally characterized the interaction of one of these NES-like peptides with XPO1. The interaction mimicked the binding of physiological NESes. I showed that the high binding affinity is due to the optimal fit of the peptide to the NES binding site. Finally, I used the preference data to construct an algorithm for sequence-based NES prediction. Such an approach has the important advantage of being essentially unbiased in comparison to existing alternatives. The results of this project should prove useful for identifying NESes in proteins and for the rational manipulation of protein localization. In addition, the high-affinity NES-like peptides that I designed could be used as an alternative to small molecule export inhibitors.

PUBLICATIONS

The results of this project have not yet been published.

RNA-BINDING PROTEINS GOVERN PROTEOSTASIS TO BALANCE NEURONAL ACTIVITY

cf. BIF FUTURA, VOL. 30 | 2.2015

RICO SCHIEWECK
Discipline: Biochemist, MSc
Institute: Department for Cell Biology and Anatomy, University of Munich (LMU), Germany
Supervisor: Prof. Michael Kiebler

All neuronal processes involving higher cognition rely on the balance between excitation and inhibition (E/I) of neuronal networks. An imbalance is thought to cause neurological and neuropsychiatric diseases such as epilepsy and autism. To allow for the complex interplay between ion channels, receptors, and signalling molecules that is necessary for E/I balance, neurons need to maintain protein homeostasis (proteostasis). Identifying global regulators of proteostasis is key to understanding the molecular basis of E/I balance. RNA-binding proteins (RBPs) are promising candidates, as they control numerous transcripts and exploit different modes of action to monitor their localization and expression. The goal of my PhD project was to investigate the physiological role of RBPs in neuronal proteostasis. I focused on Pumilio2 (Pum2) and Staufen2 (Stau2), which interact with each other in neurons and share a sizeable fraction of their mRNA targets. Proteomics and transcriptomics revealed that their impact on protein expression is highly selective: Pum2 enhances translation and protein expression without significantly affecting the overall transcriptome, but Stau2 inhibits protein expression mostly through altering the corresponding mRNAs. Pum2 and Stau2 affect distinct pathways: Pum2 mainly regulates neuronal inhibition, while Stau2 balances synaptic plasticity. My work suggests that RBPs could have distinct regulatory roles in maintaining E/I balance. Understanding the dynamics of E/I regulation will help us to understand multigenic diseases such as epilepsy and autism.

PUBLICATIONS

Schieweck R, Ninkovic J, Kiebler MA (2020) RNA-binding proteins balance brain function in health and disease. *Physiol Rev*, doi: 10.1152/physrev.00047.2019

Pernice HF, Schieweck R, Jafari M, Straub T, Bilban M, Kiebler MA, Popper B (2019) Altered glutamate receptor ionotropic delta subunit 2 expression in Stau2-deficient cerebellar Purkinje cells in the adult brain. *Int J Mol Sci* **11**: e1797

Berger SM, Fernández-Lamo I, Schönig K, Fernandez Moya SM, Ehses J, Schieweck R *et al* (2017) Forebrain-specific, conditional silencing of Staufen2 alters synaptic plasticity, learning, and memory in rats. *Genome Biol* **18**: 222

Follwaczny P*, Schieweck R*, Riedemann T, Demleitner A, Straub T, Klemm AH *et al* (2017) Pumilio2-deficient mice show a predisposition for epilepsy in mice. *Dis Model Mech* **10**: 1333–1342

USING NUCLEOPORIN-BINDING NANOBODIES TO TRACK OR TRAP NUCLEAR PORE COMPLEX ASSEMBLY

cf. BIF FUTURA, VOL. 32 | 2.2017

MIREIA SOLA
Discipline: Molecular Biologist, MSc
Institute: Max Planck Institute for Biophysical Chemistry, Goettingen, Germany
Supervisor: Prof. Dirk Görlich

The nuclear pore complexes (NPCs) are multiprotein complexes that control the transport of cargoes from the nucleus to the cytoplasm. Newly formed NPCs are inserted into an intact nuclear envelope during interphase. In higher eukaryotes, NPCs also assemble concomitantly with the re-formation of the nuclear envelope upon mitotic exit. Both pathways are still poorly understood, mainly due to technical difficulties in capturing the intermediate stages of NPC assembly. The goal of my PhD project was to use nanobodies to follow NPC assembly and also to block this process at discrete intermediate stages. Nanobodies, which are single-domain antibodies with unique biochemical features, have become a promising research tool. I used two types: tracking and trapping. Tracking nanobodies bind soluble nucleoporins (Nups) and intact NPCs, thereby allowing NPC assembly to be followed using fluorescence microscopy. By contrast, trapping nanobodies recognize Nup epitopes that are masked during NPC assembly, but they do not stain intact NPCs. Trapping nanobodies bind Nups, thus preventing essential Nup-Nup interactions. This arrests the NPC assembly process at specific intermediates, causing them to accumulate. To target conserved and thus functionally relevant Nup epitopes, I applied a cross-specific phage display strategy that alternates between different Nup species in consecutive selection rounds. Next, I assembled NPCs from *Xenopus* egg extracts in the presence of all selected nanobodies. In this system, nanobodies recognizing Nup93, Nup98, and Nup155 trapped NPC assembly at different discrete stages. To investigate interphase NPC assembly, I established a novel assay in which frog NPCs are inserted into the nuclear envelope of permeabilized human cells. By exploiting the species-specificity of Nup-binding nanobodies, I could easily identify the newly inserted frog NPCs. Finally, I showed that the anti-Nup98 nanobody blocks two well-known essential Nup-Nup interactions. This proves that trapping nanobodies can be used to map Nup regions essential for the formation of functional NPCs. My work opens new avenues for a biochemical dissection of NPC self-assembly and could be extended to investigate the assembly of other intricate protein complexes.

PUBLICATIONS

The results of this project have not yet been published.

MODELLING WILMS TUMOUR IN HUMAN KIDNEY ORGANOIDS

cf. BIF FUTURA, VOL. 31 | 2.2016

VERENA WAEHLE
Discipline: Molecular Biologist, MSc
Institute: Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland
Supervisor: Dr Jörg Betschinger

Wilms tumour is the most widespread kidney cancer in children. It is thought to arise from impaired nephrogenesis leading to overgrowth of immature nephron progenitor cells (NPCs) at the expense of functional kidney tissue. Numerous genetic alterations associated with Wilms tumour have been identified. Various model systems have been developed, but most of them fail to recapitulate Wilms tumorigenesis. During my PhD, I aimed to develop an *in vitro* model for Wilms tumour by introducing known mutations into kidney organoids derived from human pluripotent stem cells. Using inducible overexpression constructs, I found that the microRNA-processing genes *LIN28A/B* and a Wilms tumour-specific variant of the NPC transcription factor SIX2, $SIX2^{Q177R}$, failed to induce Wilms tumour phenotypes. By contrast, ectopic activation of canonical WNT signalling – which I induced by chemically stabilizing β-catenin, thus mimicking the effect of β-catenin mutations found in Wilms tumour – and CRISPR-Cas9-mediated knockout of the tumour suppressor *WT1* during organoid development led to overgrowth of NPCs at the expense of differentiated nephron structures, a phenotype reminiscent of Wilms tumorigenesis. Focussing on *WT1* knockout organoids, I discovered that the progression of mutant NPCs was stalled at the onset of nephrogenesis during their mesenchymal-to-epithelial transition. Using RNA sequencing and comparison with published gene sets of foetal kidney cell types, I confirmed that *WT1* knockout resulted in impaired differentiation, persistence, and enhanced proliferation of NPCs. Importantly, gene expression changes in *WT1* knockout organoids recapitulated alterations in human Wilms tumour, especially the upregulation of genes related to myogenesis, which is specific to a subclass of Wilms tumours. Using serial passaging and *in vitro* transplantation experiments, I found that cells derived from *WT1* knockout organoids can be propagated in the presence of immature and differentiated wild-type cells without additional growth factors. Thus, I developed the first human kidney organoid model for Wilms tumour. My work complements the current toolbox of preclinical Wilms tumour models and sets the stage for extending the use of kidney organoids in kidney cancer research.

PUBLICATIONS

Further results of this project can be discussed on *BioRxiv*: doi: 10.1101/2021.02.02.429313

IMMUNOREGULATORY MECHANISMS IN ALLERGIC AIRWAY INFLAMMATION

cf. BIF FUTURA, VOL. 31 | 1.2016

ANTONIA WALLRAPP
Discipline: Immunologist, MSc
Institute: Evergrande Center for Immunologic Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA
Supervisor: Prof. Vijay K. Kuchroo

Asthma is characterized by chronic inflammation of the airways, which can lead to severe bronchoconstriction and shortness of breath. The immune response is initiated by alarmins, such as the cytokine interleukin 33 (IL-33), that are released by epithelial cells in response to stress or damage and that activate immune cells, including type 2 innate lymphoid cells (ILC2s). However, how the release of alarmins and the function of ILC2s are regulated has not been well investigated. My preliminary data showed that mice lacking the surface molecule C-type lectin domain family 1 member b (Clec1b) spontaneously develop airway inflammation. In my PhD project, I found that the interaction of Clec1b on alveolar macrophages and its ligand podoplanin on alveolar epithelial type 1 cells controls the development of airway inflammation by regulating the function of alveolar macrophages and the expression of IL-33 by alveolar epithelial type 2 cells. In addition, I performed single-cell RNA sequencing of ILCs isolated from the lungs of mice challenged with PBS or the alarmins IL-25 or IL-33. It revealed that ILC2s express receptors for the neuropeptides neuromedin U (Nmu) and calcitonin gene-related peptide (CGRP), which suggests that neurons regulate the function of ILC2s. Whereas Nmu synergizes with IL-25 to induce proliferation, thus expanding the subset of highly proliferative and inflammatory ILC2s that drive airway inflammation, CGRP limits the development of airway inflammation by negatively regulating proliferation and cytokine production of ILC2s. My work identified novel pathways that regulate the production of epithelial cell-derived IL-33 and the effector function of ILC2s. Since these pathways play an important role in the development of lung inflammation, they might serve as potential targets for therapies.

PUBLICATIONS

Wallrapp A*, Burkett PR*, Riesenfeld SJ*, Kim SJ, Christian E, Abdulnour RE *et al* (2019) Calcitonin gene-related peptide negatively regulates alarmin-driven type 2 innate lymphoid cell responses. *Immunity* **51**: 709–723.e6

Wallrapp A, Riesenfeld SJ, Burkett PR, Kuchroo VK (2018) Type 2 innate lymphoid cells in the induction and resolution of tissue inflammation. *Immunol Rev* **286**: 53–73

Wallrapp A*, Riesenfeld SJ*, Burkett PR*, Abdulnour RE, Nyman J, Dionne D *et al* (2017) The neuropeptide NMU amplifies ILC2-driven allergic lung inflammation. *Nature* **549**: 351–356

MD FELLOWS 2020

With its MD fellowships, the Boehringer Ingelheim Fonds helps outstanding medical students to pursue an ambitious experimental project in basic biomedical research. Candidates study in Germany and change institution and city for at least ten months to work in an internationally renowned laboratory. In 2020, eight fellows were granted an MD fellowship. Here, we present the six fellows who were able to take up their fellowships despite the restrictions due to the pandemic.

AURELIA BLEINROTH

Using gray hair to determine cancer radio-resistance mechanisms

SEBASTIAN KUNZ

The role of the transcription factor ICER (inducible cAMP early repressor) in immune evasion of glioblastoma

FELIX RADTKE

Heterogeneity through differentiation in murine neutrophils

VIKTORIA STROHMENGER

Cellular distribution and gene expression in human hearts with hypertrophic cardiomyopathy compared to healthy ones

FELIX SÜMPELMANN

Proteomic analysis of human microglia phagosomes in health and disease

JONAS ZIMMER

Macrophage heterogeneity complicates atherosclerotic plaque reversal

USING GRAY HAIR TO DETERMINE CANCER RADIO-RESISTANCE MECHANISMS

AURELIA BLEINROTH

Duration: 10/20–08/21

Project at: Harvard Medical School, Massachusetts General Hospital, Charlestown, MA, USA

Supervisor: Professor Jonathan Hoggatt, PhD

Home University: Witten/Herdecke University

THE ROLE OF THE TRANSCRIPTION FACTOR ICER (INDUCIBLE CAMP EARLY REPRESSOR) IN IMMUNE EVASION OF GLIOBLASTOMA

SEBASTIAN KUNZ

Duration: 08/20–08/21

Project at: University Medical Center Mainz, Institute for Immuniology, Germany

Supervisor: Professor Dr Tobias Bopp

Home University: University Medical Centre Mannheim

HETEROGENEITY THROUGH DIFFERENTIATION IN MURINE NEUTROPHILS

FELIX RADTKE

Duration: 02/20–01/21

Project at: Harvard Medical School, Brigham and Women's Hospital, Boston, MA, USA

Supervisor: Professor Peter Nigrovic, MD

Home University: Heidelberg University Hospital

CELLULAR DISTRIBUTION AND GENE EXPRESSION IN HUMAN HEARTS WITH HYPERTROPHIC CARDIOMYOPATHY COMPARED TO HEALTHY ONES

VIKTORIA STROHMENGER

Duration: 02/21–12/21

Project at: Harvard Medical School, Department of Genetics, Boston, MA, USA

Supervisor: Johannes G. Seidman, PhD

Home University: University Hospital of Munich (LMU)

PROTEOMIC ANALYSIS OF HUMAN MICROGLIA PHAGOSOMES IN HEALTH AND DISEASE

FELIX SÜMPELMANN

Duration: 10/20–10/21

Project at: Massachusetts Institute of Technology (MIT), Whitehead Institute for Biomedical Research, Cambridge, USA

Supervisor: Professor Dr Rudolf Jaenisch

Home University: Münster University Hospital

MACROPHAGE HETEROGENEITY COMPLICATES ATHEROSCLEROTIC PLAQUE REVERSAL

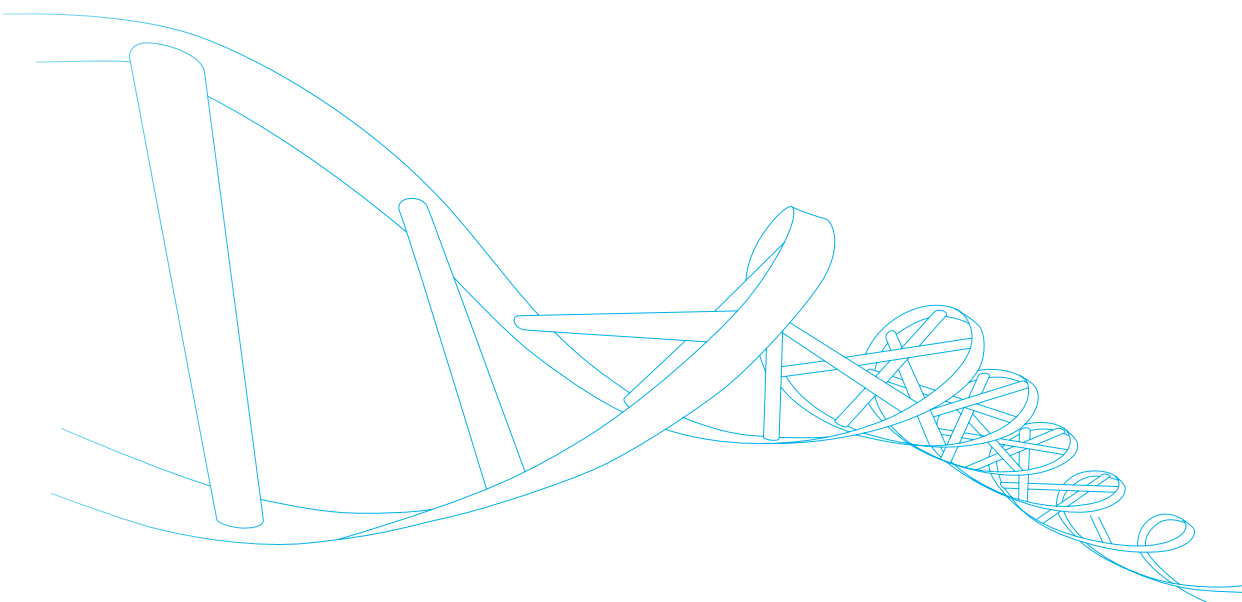
JONAS ZIMMER

Duration: 6/21–5/22

Project at: Harvard Medical School, Brigham and Women's Hospital, Boston, MA, USA

Supervisor: Professor Elena Aikawa, MD, PhD

Home University: Medical Faculty of Heidelberg



THE FOUNDATION The Boehringer Ingelheim Fonds (BIF) is a public foundation – an independent, non-profit organization for the exclusive and direct promotion of basic research in biomedicine. The Foundation pays particular attention to fostering junior scientists. From the start, it has provided its fellowship holders with more than just monthly bank transfers: seminars, events, and personal support have nurtured the development of a worldwide network of current and former fellows.

"YOU CAN'T BLAME ANYONE ELSE"

Thinking about starting your own company? We interviewed two BIF alumni who have chosen this path: Carsten Linnemann, who recently founded his third biotech start-up, Neogene Therapeutics, and Sebastian Virreira-Winter, who is still in the early stages of entrepreneurship with his company OmicEra Diagnostics.

By Kirsten Achenbach

NEOGENE THERAPEUTICS

Carsten Linnemann studied in Bielefeld, Germany, and received his PhD in immunology from the University of Leiden, where he worked in the laboratory of Ton Schumacher at the Netherlands Cancer Institute. Carsten and Ton's fruitful collaboration continued through Carsten's postdoc, and they still work together today. They have co-founded two biotech companies. Their first, T-Cell Factory B.V., was acquired by Kite Pharma Inc. in 2015, and their latest, Neogene Therapeutics, founded in 2018, attracted 110 million dollars in venture capital last year. With Neogene, Carsten wants to take T cell therapy for cancers to the next level by using patient tissue from routine biopsies to create wholly personalized T cells to attack solid tumours. Carsten is Neogene's president and chief executive officer.

CARSTEN LINNEMANN

Did you always dream of founding a company?

No, quite the opposite. I was planning on doing another postdoc, despite the feeling that I was where I wanted to be personally and scientifically. My supervisor Ton and I were tremendously productive together and I felt at the top of my game. However, remaining in the same laboratory would have greatly limited my chances in academia. We both felt that it was bizarre that the system encouraged me to leave when everything else told me to stay. When Ton asked me one day to co-found a company, I

I think one way to handle a fear of failure is to define failure.

didn't hesitate for a second before saying yes, even if I had no exact understanding what I was saying yes to.

What was the biggest challenge?

Communication. Acknowledging this was key to getting ahead. I finally understood how important the message from the BIF communication seminars is on the need to make the science exciting and understandable to your target audience. Getting the facts across is just the first step. If you want someone to invest what might possibly be millions of dollars, you need to clearly say where you're going, what you need to do to get there, and evoke enthusiasm for your idea and yourself as the person willing and able to overcome all the obstacles. There's a strong component of personal conviction. For me, it was helpful to enter business plan competitions and talk to people with business backgrounds.

What was the most unexpected thing you learned?

How important a network is. As a scientist, you have a grasp of the science, but you most likely don't know the right people. Investors are very much in demand and to get through to them, you need to develop a network. That was the biggest advantage we had when founding Neogene. Normally, you have a seminal finding in academia, maybe even a patent that you build your business around. For Neogene, all we had was a concept on paper. We approached the industry veterans Arie Belldegrun and David Chang, who had been my mentors at Kite Pharma. Our goal was to have an initial discussion about the concept of Neogene. Instead, after 45 minutes, we had their backing. This only happened because they knew us and believed we could do this. And, of course, because the idea behind it is so exciting. Normally, this business is a lot harder, and you need a lot of stamina and the belief in yourself and your idea. So if any BIF fellow wants to take that path, they can always contact me.

Why is the concept of Neogene so exciting?

We take material from the biopsies of different solid cancers and look for cancer-associated mutations manifesting as targets on the surface of the cells, so-called neo-antigens. There are hundreds to thousands of these potential targets per sample, with an overlap of less than 1% between patients. With our technology we aim to engineer the patient's T cells to recognize these targets arising from cancer mutations and thus to enable them to attack the tumour cells.

How far along are you?

It took about 18 months to fully validate the concept. We're now aiming to file a clinical trial application by the end of the year and be in the clinic in 2022. Because fully personalized approaches are still a relatively new concept, there's a lot of work on all levels – for example, when it comes to regulatory and manufacturing aspects.

For Neogene, you collected 110 million dollars from investors in 2020. When you think of this kind of money, can you still sleep at night?

If such sums keep you awake at night, you can't be the CEO of a cell therapy company. Of course, I take the responsibility seriously and get advice from experts and learn as much as possible. I think one way to handle a fear of failure is to define failure. Failure to deliver on an operational level because people don't do their jobs is dramatic. But if the biology is stacked against you, there's nothing you can do. Everyone knows it's a risky business and many projects will fail. What builds your reputation in this business is that you deliver on what you say you will do. This is probably the biggest difference to academia. Negative results from a grant project often harm you the next time around. Academia still has no good mechanism to give credit for rigorously executing a study that does not yield exciting results.

How many hours do you work?

As many as needed! I enjoy being exposed to all parts of the business – zooming in on detailed aspects as needed. I have an impact on our strategy, our plan, and on executing it. Together with the team, I take responsibility for securing the financial basis for the company and building the business relationships. At the same time, I still try to join every research meeting. But being in the lab has become so rare that people start taking pictures and making fun of me when I show up. This business is about getting things done. The moment action needs to be taken – whether it's repairing the coffee machine or getting the meeting room ready – and you say, "I'm the CEO, this is below me," you know you're in the wrong job.

Photo: Neogene Therapeutics

OMICERA DIAGNOSTICS

SEBASTIAN VIRREIRA-WINTER

Sebastian Virreira Winter studied molecular biomedicine at the University of Bonn and the Whitehead Institute of Biomedical Research in Boston, USA. He received his PhD in immunology from the Humboldt University in Berlin and then completed a postdoc at the Max Planck Institute of Biochemistry, where he worked in the laboratory of Professor Matthias Mann, one of the leading experts in proteomics. He is currently the chief technological officer of the proteomics start-up OmicEra Diagnostics, which he founded in 2019 with two other postdocs from the Mann laboratory. OmicEra aims to revolutionize medical diagnostics by implementing high-end mass spectrometry-based proteome analysis of human samples, ranging from fluids to frozen tissues.

When did you first dream of founding your own company?

During my graduate studies at the Whitehead Institute. The MIT campus in Boston has the highest density of start-ups worldwide. It seems everyone has a company or even several. My PI had one, all the other PIs had one. It's just everywhere. That's very different from Bonn, where I studied. Yes, the chances of making it are small, but if you do, you can work on very cool things.

You decided very early that you wanted to found your own company. Why?

In academia, everybody is focused on their own career. There's a lot of pressure. It's very important that you publish and have a significant position in the list of authors. In the company environment, the team spirit is better: people work on different aspects of the same mission. In addition, I really enjoy the agile world of a small company, the strong team spirit. In a big company, you're a small cog in the wheel; in a small company, your own impact is much more direct and noticeable.

What steps did you take to found your own company?

During the second part of my PhD programme, we started a collaboration with the laboratory of Matthias Mann. The possibilities of mass spectrometry and proteomics fascinated me, and I joined his lab for a postdoc, even though I was quite sure that I'd leave academia eventually. Most people would say that in that case a postdoc is a waste of time, but it offered me the chance to learn from one of the best groups in the world. My project was to develop the so-called EASI-tag, a novel tool to improve peptide and protein quantification by mass spectrometry. It meant dropping my biological interests for a time, really digging into the technology, and developing it further. This took me from being just another user to knowing the technology inside out. After around one and a half years, we started a clinical project to discover biomarkers for Parkinson's patients: developing new methods for sample analysis for urine and cerebrospinal fluids. Without these two sides, the technological and the clinical, I wouldn't be OmicEra's chief technological officer today.

What does OmicEra Diagnostics do?

We offer complete proteome analyses of body fluids and tissues, from designing studies and obtaining and preparing samples to analysing and interpreting data. Our clients are not only large pharmaceutical companies that are seeking expertise in the proteomics of clinical samples, but also smaller biotechs and even individual clinician scientists. Proteomics is a relatively young field and a highly technical process that requires very specific knowledge. All four of our founders are proteomics experts who together cover all aspects of the technology. We work closely with mass spectrometry manufacturers to further improve the capabilities of the machines, which gives us a technological edge. We've automated much of the process and developed efficient workflows that result in high reproducibility, improved sensitivity, and higher throughput. We can now obtain complete proteomes with about 10,000 proteins with very high precision. We've developed novel informatics approaches to find new biomarkers

Photo: OmicEra

I really enjoy the agile world of a small company, the strong team spirit.

for disease and health. In short, we want to be the best-performing mass spectrometry platform for the analysis of clinical samples. Our goal is to revolutionize medical diagnostics.

What was your biggest challenge when founding OmicEra?

Finding lab space, which we never thought would be a problem since Martinsried is such a hub for biotech. At first, we could only get two rooms, with about 20 square metres each in the Innovation and Start-up Center for Biotechnology (IZB), a state-funded start-up centre. This was obviously not enough. We called about 100 companies and real estate agents. One agent told us that he had demand for up to 40,000 square metres of lab space a year, but could only offer about 2,000. In the end, we got lucky and found an old office building that we were able to renovate and convert into a modern lab and office space.

What was the most challenging thing you had to learn?

To get more organized, more structured personally, but also as a team. Everyone has their own system of lab notebooks, of organizing their experiments. But in a company, you can't have five ways of doing things, you have to have guidelines on how it is done. We also had to learn to be more professional with regard to project management – for example, by organizing our internal and external meetings so that we didn't end up with many short time slots that fracture the day. As a team, we've instigated daily scrum meetings to get each other up to speed. Juggling many diverse tasks such as accounting, administration, the management of people, and the science was also challenging at first. I now reserve certain days for specific tasks. It would be good to emphasize such skills in the academic curriculum as well. After all, data lose value over time.

What is the one piece of advice you would like to give other BIF fellows who are thinking about founding their own company?

It's imperative to find the right team to do it with, people you work well with and who have the same vision. Having the idea isn't that hard. We got lucky, as three of us shared an office in Matthias's group, which at that point had about 50 people. We had already been working together for two years when we decided to take this step.

Are you working on the coronavirus?

We're doing two studies on the proteome from blood samples of corona patients. One will be the largest blood proteome study ever published, analysing 2,700 samples from patients. We also have the clinical data, which often isn't documented or is difficult to access. We can therefore correlate the proteomics data with the standard clinical data. Among other things, we hope to validate biomarkers flagged by a much smaller study that predict which people will get really sick. This would help clinicians to chart therapies for COVID-19 patients.

What's been your biggest joy so far?

The team – everyday every one of us enjoys going to work. We all believe that there is great potential for this technology to revolutionize diagnostics.

What do you like least about having your own company?

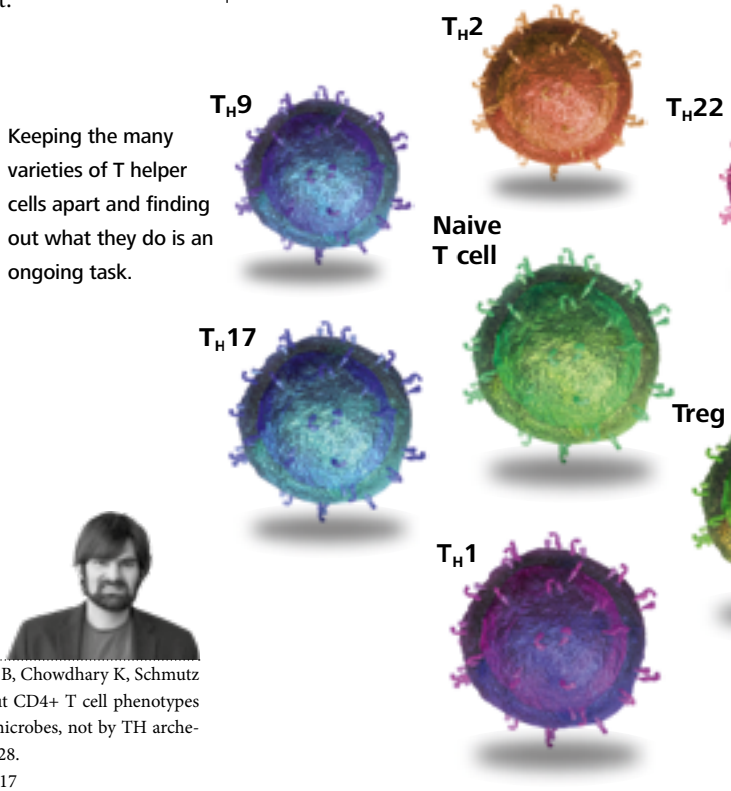
If something doesn't go well, it's our fault – you can't blame anyone else.

PAPERS IN THE SPOTLIGHT

In "Papers in the spotlight", we present papers from current fellows and recent BIF alumni. The selection criteria are based not only on scientific merit but also on the general interest of the topic. If you would like to see your paper discussed here, send an email to kirsten.achenbach@bifonds.de.

SINGLE CELL TRANSCRIPTOMICS REFUTES T HELPER CELL PARADIGM

Almost 40 years ago, the T helper cells T_H1 and T_H2 were first stipulated to be different cell types with specific functions, distinguished by their expression of different cytokines *in vitro*. Since then, a growing number of additional T helper cell types, such as T_H9 and T_H17, have been defined in this way. However, from the very beginning, it has been questioned whether all these cells are really different cell types. Because these T helper cells, which in their activated state are called T effector cells, have very few surface markers to distinguish them, it has been difficult to be sure. Evgeny Kiner from the group of Diane Mathis and Christophe Benoist at Harvard Medical School, Boston, USA, and others have now shown that the classical distinction between T effector cells may not be so black and white *in vivo*. The authors used single-cell RNA transcriptomics to analyse the transcribed genes and chromatin states of the gut T cells of mice. The method easily distinguished the four well-established subgroups of gut T cells – two types of regulatory T cells, naive T conventional cells, and T effectors cells. However, with this method, the authors were unable to resolve T effector cells any further. They infected groups of mice with four different pathogens known to cause different T cell responses. They found, for example, that T_H1 cells from a mouse infected with *Salmonella enterica* were much closer to T_H17 cells from a mouse infected with the same pathogen than to T_H1 cells from mice infected by a different pathogen. Evgeny notes: "Cytokine expression is certainly important functionally, but it is not the defining feature of these cells *in vivo*." Even when the authors looked at clones derived from one progenitor cell, these did not express just one cytokine or the other. "Our findings once again show that *in vitro* studies are essential models that can teach us a lot, but they do not necessarily translate into real biology *in vivo* and especially not in the clinic," comments Evgeny. "These findings are not unexpected, but many people told us they are glad that someone finally said it."



Keeping the many varieties of T helper cells apart and finding out what they do is an ongoing task.

REFERENCE

Kiner E, Willie E, Vijaykumar B, Chowdhary K, Schmutz H, Chandler J *et al* (2021) Gut CD4+ T cell phenotypes are a continuum molded by microbes, not by TH archetypes. *Nat Immunol* **22**: 216–228.

Evgeny Kiner, fellow 2015–2017

HOW GUTSY BACTERIA HELP CANCER THERAPIES

It is known that certain bacteria, especially from the gut, are essential for the success of cancer therapies, including so-called immune checkpoint blockade (ICB) therapies. The why of it, however, has been unclear. Alumnus Lukas Mager, now a postdoc in the lab of Kathy McCoy at the University of Calgary, Canada, has shown that certain bacteria increase T_H1 cell differentiation during ICB therapy by producing high levels of inosine plus a further co-stimulus. Increasing T_H1 cell response is one of the goals in cancer therapies such as ICB, as these cells kill tumour cells.

Photos: Alamy Stock Foto/Stocktrek Images, Inc. (middle); Marzia Munafo (right)

Inosine is a metabolite produced not only by eukaryotes, but also by bacteria, many of which live in the guts of mice and humans. However, only a few bacteria produce enough inosine to help to fight a tumour. Additionally, inosine needs to pass the gut barrier to have an effect. A leaky gut barrier is generally considered a negative side effect of ICB therapy, but it helps inosine to pass into the body. The authors found that, once there, inosine is recognized by T cells and induces transcription of the genes important for T_H1 cell differentiation by leading to phosphorylation of the transcription factor CREB. However, inosine alone is not enough. Quite the opposite: by itself, inosine even hinders T_H1 cell differentiation. A further stimulus is crucial to unleashing its anti-tumour effect. The ICB-boosting bacteria studied by the team provide this further stimulus as well. The team has not yet identified it, but they have found a substitute: "When we used inosine and the oligodeoxynucleotide CpG to treat mice with bladder, bowel, or skin cancer, the ICB therapy showed much better results." This work can make cancer therapies more effective for a larger number of people.

REFERENCE

Mager LF, Burkhard R, Pett N, Cooke NCA, Brown K, Ramay H *et al* (2020) Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Science* **369**: 1481–1489

Lukas Mager, fellow 2012–2014

PROTEIN ASSEMBLY: JOINED AT BIRTH

Imagine you need to find a specific person at a rally with millions of people. This is similar to the task faced by most protein molecules within the cytoplasm: they need to find one or more specific partners to form functional complexes. As Matilde Bertolini from Bernd Bukau's group at Heidelberg University, Germany, has now shown, some protein molecules solve this problem by binding to their partners before they are (figuratively speaking) fully born. Until recently, this "co-co" assembly was only a hypothesis. But Matilde and her fellow authors found that it not only exists but occurs in more than 10% of human proteins, especially in homomeric protein complexes, which contain two or more moieties of the same protein.

Matilde developed disome selective profiling (DiSP), a technique enabling her to find ribosomes connected to each other via partially translated parts of protein molecules. First she lysed the cells and destroyed all the mRNA that was not sheltered within ribosomes. After separating the single ribosomes (monosomes) from the connected ribosomes (disomes), she then extracted the mRNA snippets sheltered within and sequenced them separately. By comparing the sequences, she was able to work out where and when the nascent protein molecules had started to connect. Most often this happened at coiled-coil motifs, followed by globular domain motifs. In addition, Matilde showed in a separate experiment that the two protein molecules of the protein complex lamin A are translated by a disome sitting on the same mRNA. This is an elegant design, as lamin complexes made up of different isoforms, such as lamin A and C, are non-functional. Co-co assembly from the same mRNA molecule can also mitigate the effects of negative dominant mutations. It prevents wild-type protein molecules from being wasted by binding to mutated variants, and thus leads to more functional complexes.

Coiled coil motifs of nascent proteins zip together during co-co assembly.

Altogether, the authors conclude co-co assembly is common, faster, more efficient, and more precise because it eliminates (i) diffusion, (ii) the need for assembly factors, and – when done from the same mRNA – even (iii) the chance of binding to wrong isoforms. The authors therefore propose that most co-co assembly happens on the same mRNA.

REFERENCE

Bertolini M, Fenzl K, Kats I, Wruck F, Tippmann F, Schmitt J *et al* (2021) Interactions between nascent proteins translated by adjacent ribosomes drive homomer assembly. *Science* **371**: 57–64

Matilde Bertolini, fellow 2018–2019

PERSPECTIVES

FROM PHD TO CARTOGRAPHER OF HUMAN CELLS

In this section, we introduce BIF alumni from various scientific backgrounds and professional contexts. They describe their career paths, highlighting important steps and decisions that helped them to reach their current position.

INTERVIEW WITH SARAH TEICHMANN, PHD, CO-FOUNDER OF THE HUMAN CELL ATLAS, FELLOWSHIP: 1996–1999

Sarah Teichmann, a native of Germany, received her PhD in biochemistry in 2000 for her work on bioinformatics in the lab of Cyrus Chothia at Cambridge University, UK. As excellent opportunities arose locally, she stayed in the Cambridge area, working as programme leader at the MRC Laboratory for Molecular Biology (2001–2012), research group leader at the EMBL-EBI, and senior group leader and currently head of cellular genetics at the Wellcome Sanger Institute (like the EMBL-EBI, located just outside Cambridge). She is also director of research at the Cavendish Laboratory (the Physics Department of Cambridge University) and senior research fellow at Churchill College. In 2016, she co-founded the Human Cell Atlas (HCA) initiative, which aims to map every cell type in the human body using single-cell transcriptomic technologies and spatial methods (see page 10). Sarah has received many accolades for her contributions to understanding protein complex assembly and gene regulatory networks. They include an EMBO Gold Medal and election as a fellow to the Academy of Medical Sciences and the Royal Society. Her lab motto is "Be bold, be brilliant, be kind".

What is the greatest challenge you face as coordinator of the HCA?

Mapping every cell type in the human body is an enormous project that requires global collaboration. There was no template for this, so we've had to create organizational strategies, especially to ensure the data is in a format that can be easily used and accessed. Being open to everyone and well organized at the same time is wonderful and very challenging. It results in a very dynamic process.

Why do you think the HCA is necessary?

A Human Cell Atlas of every cell type in the human body will allow unprecedented analyses of cells and tissues. Such an ambitious goal – there are around 37 trillion cells in the human body – requires a global approach and collaboration between people from many different disciplines, ranging from biological research and computational methods to technological improvements. With the HCA, we're transforming our understanding of biology, in both health and disease.

What does the UK do exceptionally well in regard to science?

The UK is very good at bringing expertise together in one place. Take the Wellcome Genome Campus with over 2,000 people, all focusing on bioinformatics and genomics and discussing, collaborating, and developing ideas. This interplay and the sheer size of the community is why I decided to stay here at two crucial junctures in my career.

Is there an aspect of science that you think needs to be recognized more?

Mentoring and supporting researchers is a huge part of running a scientific group, but doing this well is generally not recognized or rewarded by institutions, perhaps because it's hard to quantify. The question of how to recognize individuals' contributions to teamwork is also challenging and needs to be addressed.

What advice would you give young scientists just starting out in their careers?

Collaborate not just within your field but outside of it – the earlier, the better. Science is becoming less about locking yourself in your lab. You need to go out and find meaningful partners: collaboration and teamwork accelerate science.

What personal skills have you developed during your career?

I started off as a PhD student and postdoc in bioinformatics and was able to thrive at these career stages with quite an introverted personality. Since then, I've developed networking skills and now really enjoy talking to people and bringing people together. There's real strength and inspiration in interactions and interdisciplinary collaboration. These skills can be the key to success.

You've been called unstoppable – do you see yourself this way?

No, not at all! It's funny and encouraging to be called that. There have been a lot of things in my life that have slowed me down, such as changing institutes and having kids. However, I've always kept going – perseverance is my middle name.

Photo: Wellcome Sanger Institute

PROFILES

ALEXANDER BATES
Institute: Harvard Medical School, Boston, MA, USA
Fellowship: 2016–2018

LISA TRAUNMÜLLER
Institute: Harvard Medical School, Boston, MA, USA
Fellowship: 2014–2017

The Human Frontier Science Program has awarded long-term fellowships to Alexander Bates and Lisa Traunmüller. Their projects are titled, respectively, "A Cartesian Coordinate System for Generating Flexible Internal Goals" and "Contribution of Activity-Regulated Neuropeptide Function to Synaptic Plasticity and Memory". Because the grants are meant to allow researchers to train in a new field in an outstanding laboratory of their choice in another country, both Alexander and Lisa have joined Harvard Medical School. In addition, Alexander received the 2020 postgraduate award of the British Neuroscience Association, and Lisa won the 2020 FENS-Kavli Network of Excellence PhD Thesis Prize.

PROFESSOR MARÍA TERESA PISABARRO
Institute: TU Dresden, Germany
Fellowship: 1994–1997

María Teresa Pisabarro has been appointed professor at Technische Universität Dresden, where she heads the Structural Bioinformatics and Computational Biology Group. This unit's current research focuses on the discovery and functional characterization of novel proteins, the rational engineering of proteins with improved functional properties, and the *de novo* design of molecular scaffolds for a variety of biotechnological and biomedical applications.

ASSISTANT PROFESSOR HAUKE HILLEN
Institute: University of Göttingen, Germany
Fellowship: 2014–2016

Hauke Hillen has been appointed junior professor of protein biochemistry at the University of Göttingen. In parallel to his position as group leader at the MPI for Biophysical Chemistry, he will now study the molecular biology of mitochondria to understand how their genome is expressed. He will also focus on the regulation and coordination of this process and examine how it is embedded in the cellular context.

ASSISTANT PROFESSOR IVANA GASIC
Institute: University of Geneva, Switzerland
Fellowship: 2010–2012

Ivana Gasic has joined the Cell Biology Department of Geneva University as assistant professor, a position financed by an Eccellenza Professorial Fellowship from the Swiss National Science Foundation. Having recently discovered how tubulin protein quantity feeds back into its encoding mRNA stability via a specificity factor, she plans to study tubulin quantity control and microtubule homeostasis.

As we have just learned, early last year Ivana received a 100,000-dollar Breakthrough Scientist Award for this work from the Damon Runyon Cancer Research Foundation. The prize goes to holders of the prestigious Damon Runyon Fellowship who are deemed "most likely to make paradigm-shifting breakthroughs that transform the way we prevent, diagnose, and treat cancer." The foundation is dedicated to funding brilliant scientists who do high-risk, high-reward research related to fighting cancer.

INES DRINNENBERG
Institute: Institut Curie, Paris, France
Fellowship: 2007–2010

Ines Drinnenberg is one of just 30 scientists to be named EMBO Young Investigators. The award comes with four years of financial and practical support as well as membership in the programme's growing network. "These 30 life scientists have demonstrated scientific excellence and are among the next generation of leading life scientists," says EMBO director Maria Leptin. Ines's research focuses not only on the different types of centromeres organisms have evolved to ensure the faithful segregation of sister chromatids during cell division, but also on the additional key players involved.

LEIF LUDWIG
Institute: Berlin Institute of Health (BIH) and Berlin Institute of Medical Systems Biology (BIMSB), Berlin, Germany
Fellowship: 2011–2012

Our MD programme alumnus Leif S. Ludwig has been awarded an Emmy Noether Group Leader Fellowship by the Deutsche Forschungsgemeinschaft (German Research Foundation) to study stem cell dynamics and mitochondrial genetics in hematopoiesis. His group is one of four junior research groups in the focus area "Single Cell Approaches to Personalized Medicine", which is supported by both the BIMSB/Max Delbrueck Center and the BIH/Charité. The aim of this area is to translate state-of-the-art single-cell multi-omic technologies into clinical applications.

Photo: Bates: private; Traunmüller: private; Hillen: private; Gasic: University of Geneva; Drinnenberg: Aida de la Cruz; Ludwig: Felix Petermann, MDC

A BIF FELLOW'S GUIDE TO ...

DUBLIN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Travelling is fun – especially if you get insider tips from locals! In each edition of FUTURA, one fellow shows you around his or her city. In this edition your guide is Sarah Power. She reports from Dublin, Ireland, a UNESCO City of Literature with a stunning coastline.

FACTS & FIGURES

Country: Republic of Ireland
Population: about 4.9 million
Area: about 70 km²
Students: about 225,628
Famous for its coast and the Book of Kells
Website: www.visitdublin.com

BEST SIGHTS

Trinity Long Room Library: one of the world's most beautiful libraries, with many rare ancient texts such as the Book of Kells.
Dublin Castle: one of the most important buildings in Irish History.
Phoenix Park: one of Europe's largest enclosed urban parks with hundreds of deer.
EPIC The Irish Emigration Museum: discover how a small island had a big impact on the world.

NIGHTLIFE

The Blind Pig: underground speakeasy and cocktail bar from prohibition times.
Sophie's: a glassed-in rooftop bar with a 360° view of the city.
Temple Bar 4**:** the most famous bar in Ireland with live Irish music every night.
Johnny Foxes: Ireland's "highest pub".

ACTIVITIES

Winter: take the Guinness brewery tour and learn how to pour your own pint of Guinness. Check out the 360° view from the rooftop bar.
Spring: get a boat/kayak lift to Dalkey Island 3 and try some "bel gelato" in Dalkey Village.
Summer: hike up the Howth Cliff Walk 2 for panoramic views of the coastline or brave a dip at Balscadden Bay Beach. Take a boat to Ireland's Eye from Howth Pier and finish off your day with some food at Howth Market.
Fall: watch an Irish Gaelic football or hurling match (free at lower levels).

WHERE TO STAY

Luxury: the Marker Hotel, beautiful rooftop view overlooking the waterfront 1.
Mid-range: Drury Court Hotel, located in the middle of the cultural quarter.
Budget: Dublin City Hotel, close to all the nightlife.

RESTAURANTS

Traditional: the Winding Stairs, named after the Yates poem. Look out at H'Penny Bridge and check out the bookstore below.
Italian: Gigi Ranelagh, delicious fresh homemade pasta and the best Aperol Spritz.
Vegetarian/vegan: Glas Restaurant, stylish food with amazing flavours.
Brunch: Brother Hubbard North and Two Boys Brew, both cool and cosy cafes.
Pastries: try Bread 41's signature cruffin.
Coffee: Clement & Pekoe, Kaph, Bear Market, Café Di Napoli, Nicks, and 3fe.

Contributors wanted! If you would like to introduce your city, send an email to kirsten.achenbach@bifonds.de

Sarah Power is 27 years old and studies at Trinity Biomedical Sciences Institute in Dublin. Her supervisor is Professor Tomas Ryan, PhD.

Photos: private

PROFILES

PROFESSOR FRAUKE GRÄTER
Institute: Heidelberg Institute for Theoretical Studies (HITS) and Heidelberg University, Germany
Fellowship: 2002–2005

PROFESSOR CHRISTINE SELHUBER-UNKEL
Institute: Heidelberg University, Germany
Fellowship: 2004–2006

Frauke Gräter and Christine Selhuber-Unkel have each been awarded an ERC Consolidator Grant. Frauke aims to find out how proteins are designed to withstand mechanical forces. Such stress is known to rupture chemical bonds, producing so-called mechanoradicals. She will study whether these radicals influence the ageing of collagen in our body. In 2021, she was appointed to the rotating two-year position of scientific director of the HITS and also joined the editorial board of *Biophysical Journal*.

Christine's project "Photomechanical Writing of Cell Functions" (PHOTOMECH) aims at regulating cell functions through external physical forces. To this end, she will combine photoswitchable materials with a complex optical system that uses intensive laser light pulses. This laser is intended to be used as a "pen" to write cell functions in three dimensions and enable the growth of cell tissues. This is her fifth ERC Grant.

JOHANNES LE COUTRE
Institute: University of New South Wales, Australia
Fellowship: 1992–1995

Johannes has been named fellow of the Royal Society of New South Wales, Australia, in recognition of his significant and valued contributions to food science and the translation of research from academia to industry.

INTRODUCING TEAM MEMBER ANDREA EPPERLEIN

Andrea Epperlein joined the BIF in January 2020 and is now responsible for accounting and various administrative tasks. She ensures that fellows and travel grant holders all over the world receive their stipends and grants securely and on time. She also supports Iris Bodenbender in organizing the BIF's office. Andrea was born in Leipzig in 1971. She completed a training programme in office administration and added a graduate degree in business administration to her résumé in 2008. Before joining the BIF, she worked as assistant to the general manager of a non-profit organization and as head of the medical writing service of a hospital. Of her new job, she says: "I especially like the international work, the flat hierarchy, the small, collegial team, and getting a glimpse of scientific discoveries." In her spare time, Andrea likes to bike, hike, do gardening, and – in the quieter hours – read a good book or solve Sudokus.

UPCOMING EVENTS

9–10 JULY, 12–13 NOVEMBER 2021
Meetings of BIF's Board of Trustees
The trustees decide on the allocation of fellowships, review the proposals for the International Titisee Conferences, and handle all the foundation's matters of fundamental importance.

14–20 AUGUST 2021
Progress seminar, Hirschegg, Austria
During the progress seminar you will have ample opportunity for scientific exchange and networking with around 50 other current BIF fellows in Europe.

10–12 SEPTEMBER 2021
European alumni seminar, Glashütten, Germany
Annual meeting of former BIF PhD and MD fellows based in Europe. This year's seminar topic is "Imagine".

24–28 SEPTEMBER 2021
North America meeting, USA
We bring together alumni and current fellows working in North America to discuss science and get to know each other.

6–10 OCTOBER 2021
122nd International Titisee Conference, Titisee, Germany
The 122nd ITC – "Space, Time, and Life" – will be chaired by Frank Jülicher (Dresden, Germany) and Marcos Gonzales-Gaitan (Geneva, Switzerland).

27–31 OCTOBER 2021
123rd International Titisee Conference, Titisee Germany
The 123rd ITC – "Life 2.0: From Designing the Molecules of Life to Designing Life" – will be chaired by Tobias Erb (Marburg, Germany) and Marileen Dogterom (Delft, The Netherlands).
ITC Participation is by invitation only.

Need more details or an update on upcoming events? Check our website at www.bifonds.de

Photos: Gräter: HITS/A. Mück; Selhuber-Unkel: T. Schwerdt; Le Coutre: Froniers Media SA

ISSN 0179-6372

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