

FUTURA

THE JOURNAL OF THE BOEHRINGER INGELHEIM FONDS

VOL. 32 | 1.2017



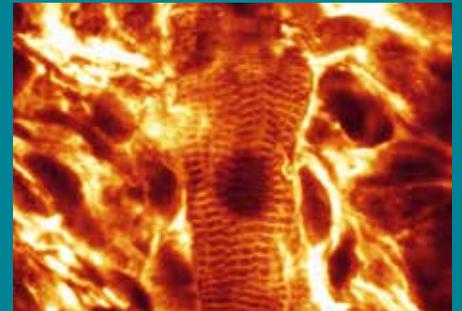
Autophagy, Again and Again

Nothing goes to waste: how cells have perfected the art of recycling



Projects, Results, MD Fellowships

New PhD projects, completed theses, and MD fellowships



Success in the Long Run

After 25 years, BIF alumnus Peter Kohl proved an exciting hypothesis



The cover illustration shows a simplified model of phage λ , a virus that invades bacterial cells, and its linear double-stranded DNA. The phage attaches to a bacterium's cell wall and then injects its DNA into the bacterium. It infects what is probably the best-known organism, *Escherichia coli*. Read more about phage λ in our new series on iconic model organisms on page 8.

FACTS

Science News 4

PROFILE OF PHAGE λ

A new series about some of the most iconic organisms and their role in research. In the first installment we introduce you to phage λ 8

AUTOPHAGY, AGAIN AND AGAIN

Recycling glass, paper, and plastic might seem to be a purely human endeavour, but cells have perfected their own form of recycling, called autophagy. 10

FELLOWS

NEW PHD PROJECTS, THIRD ROUND 2016

Fourteen applications for fellowships were approved and all were taken up. 15

NEW PHD PROJECTS, FIRST ROUND 2017

Thirteen applications for fellowships were approved and eleven were taken up. One is still pending. 29

PHD RESULTS

Eighteen fellowship holders give a brief account of their results. 41

MD PROJECTS

In 2016, BIF granted nine MD fellowships. 51

FOUNDATION

THE YEAR 2016 AT BIF

Facts and figures. 54

Papers in the spotlight: Susanne Falkner, Michael Hauer, and Christian Schmidt 56

Perspectives: from neurobiologist to medical writer 58

Profiles 59

A BIF fellow's guide to ... Szeged 60

Pay-off for tenacity and out-of-the-box-thinking 61

Upcoming events 61

PUBLISHING INFORMATION

Published by Boehringer Ingelheim Fonds
Stiftung für medizinische Grundlagen-
forschung

Schusterstr. 46–48

55116 Mainz

Germany

Tel. +49 6131 27508-0

Fax +49 6131 27508-11

E-mail: secretariat@bifonds.de

www.bifonds.de

Editor-in-Chief Dr Claudia Walther

Editors Kirsten Achenbach (BIF, executive
editor), Karsten Fiehe (muehlhausmoers
corporate communications gmbh)

Authors in this issue Kirsten Achenbach, Alison
Bell, Mitch Leslie, Ákos Nyerges, Dr Claudia
Walther, Sarah Williams

Translating, copy-editing, and proofreading Adam

Blauhut, Dr Caroline Hadley, Dr Alla

Katsnelson, Dr Susan Simpson

Production muehlhausmoers corporate
communications gmbh,

www.muehlhausmoers.com

Project management Karsten Fiehe

Art direction Britta Siebert

Printed by Druckhaus Cramer GmbH & Co. KG,
Hansaring 118, 48268 Greven, Germany

Images Boehringer Ingelheim Fonds, unless
stated otherwise

Cover photos: Jan von Holleben (left), BIF
(middle), Peter Kohl (right)

Publication date of current issue August 2017

BIF FUTURA is the journal of the Boehringer Ingelheim Fonds, a non-profit organization supporting basic research in biomedicine. Opinions expressed in BIF FUTURA cannot automatically be assumed to be the official standpoint of the Boehringer Ingelheim Fonds. This is particularly the case when the article is accompanied by the name of the author. Reproduction of articles or parts of the journal only with reference to and the permission of the foundation.

GIVE AND TAKE



“Peer review in all its forms is a fundamental feature of academia – and a system of give and take.”

One of the most frequently asked questions by applicants and BIF-fellows is: which scientist(s) evaluated my project? We won't tell. We believe – supported by others' experimental evidence – that the majority of reviews are more candid and critical when the authors are assured of confidentiality. Our fellowship programmes aim for excellency in terms of the candidate, proposed research project, and host laboratory; we therefore need critical and frank arguments from acknowledged experts in addition to the evaluations and panel discussions by our six internationally renowned scientists on the board making the final decision.

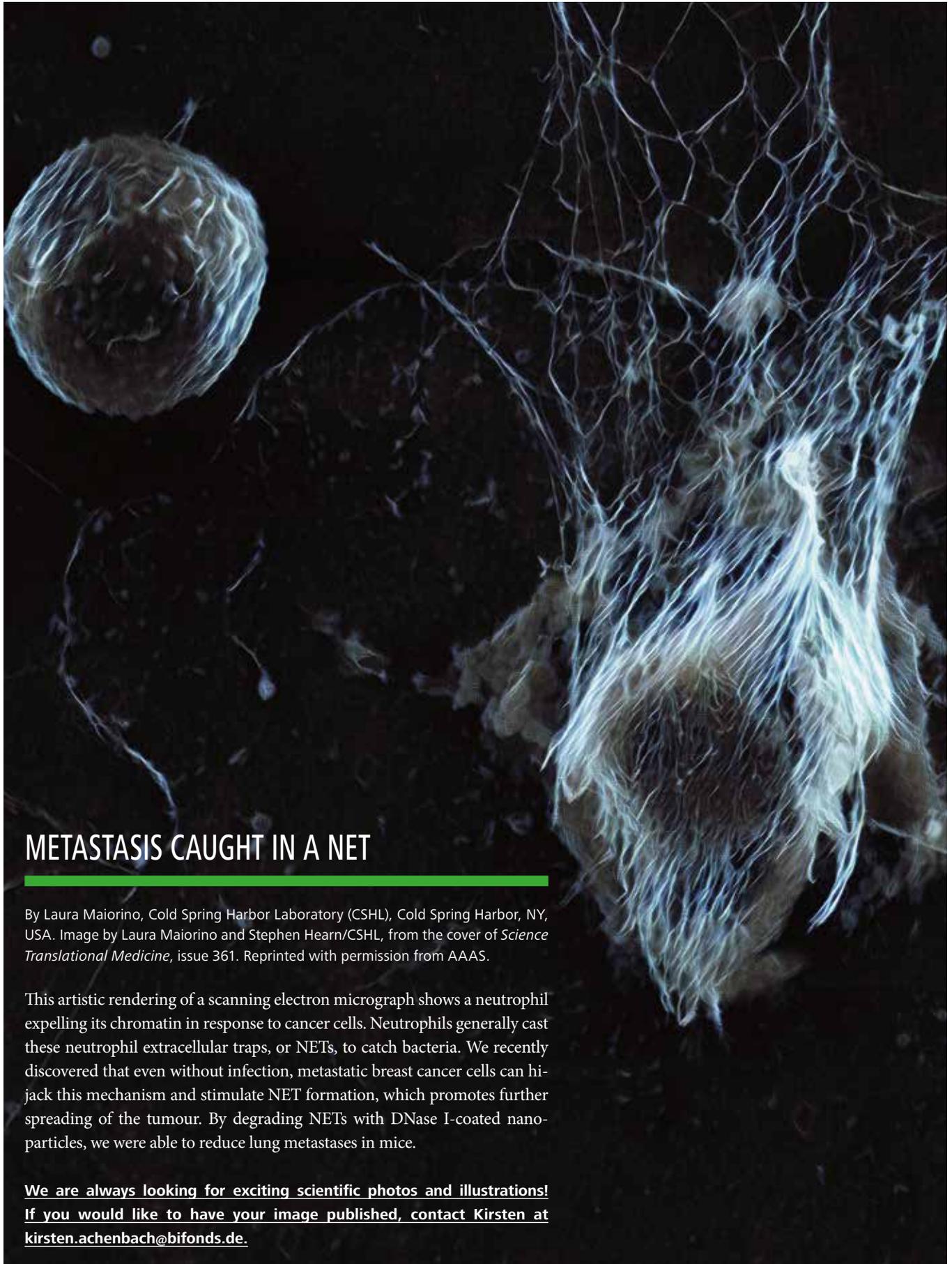
Confidentiality also protects the reviewers themselves: not all candidates and supervisors are good sports when receiving rejection letters – as funding organisations and editors well know. It is also important for senior postdocs or junior group leaders whose in-depth evaluation may have led to the rejection of a project in an established scientist's laboratory.

External experts usually end their reviews with a recommendation about whether to fund or not to fund. However, their most important role is to thoroughly discuss and weigh the pros and cons of the proposed project. Our board members – each application is scrutinized by two of them – carefully consider and assess the external reviewers' arguments in light of their own evaluations. Are the arguments sound and fair? Is the review, for example, too optimistic or too critical? The board also sets the quality standards: what may seem worth funding to an external reviewer may not be exceptional enough according to BIF standards. However, the external reviewer's arguments are nevertheless helpful in the panel discussions. Importantly, the board is more likely to take risks than many external reviewers. If a high-risk project is original, has the potential for significant advances, and comes with a well-worked-out proposal discussing the risks properly, it may well be approved. Our board members are prepared to give applicants the benefit of the doubt.

Despite its limitations, peer review in all its forms is a fundamental feature of academia – and a system of give and take. Today, a scientist is asked for a review. A few months later, he or she may apply for a grant or have a junior scientist submit an application for a fellowship – maybe for a BIF programme – knowing that someone else will have to take on the role of reviewer. An oft-underestimated side effect for young PIs or senior postdocs: fair, in-depth reviews are a means of distinguishing oneself and help to build a good reputation.

We know that careful reviewing takes time and effort and therefore try to limit and spread the burden. We usually ask any one reviewer to provide an assessment at most once a year – and we inform them about the outcome. To the innumerable researchers who – in an honorary capacity – have helped us select the candidates with the best and most exciting projects, we extend our gratitude. Their in-depth, careful, fair, and critical evaluations show peer review at its best.

A handwritten signature in blue ink, appearing to read 'Cecilia Kuhl'.



METASTASIS CAUGHT IN A NET

By Laura Maiorino, Cold Spring Harbor Laboratory (CSHL), Cold Spring Harbor, NY, USA. Image by Laura Maiorino and Stephen Hearn/CSHL, from the cover of *Science Translational Medicine*, issue 361. Reprinted with permission from AAAS.

This artistic rendering of a scanning electron micrograph shows a neutrophil expelling its chromatin in response to cancer cells. Neutrophils generally cast these neutrophil extracellular traps, or NETs, to catch bacteria. We recently discovered that even without infection, metastatic breast cancer cells can hijack this mechanism and stimulate NET formation, which promotes further spreading of the tumour. By degrading NETs with DNase I-coated nanoparticles, we were able to reduce lung metastases in mice.

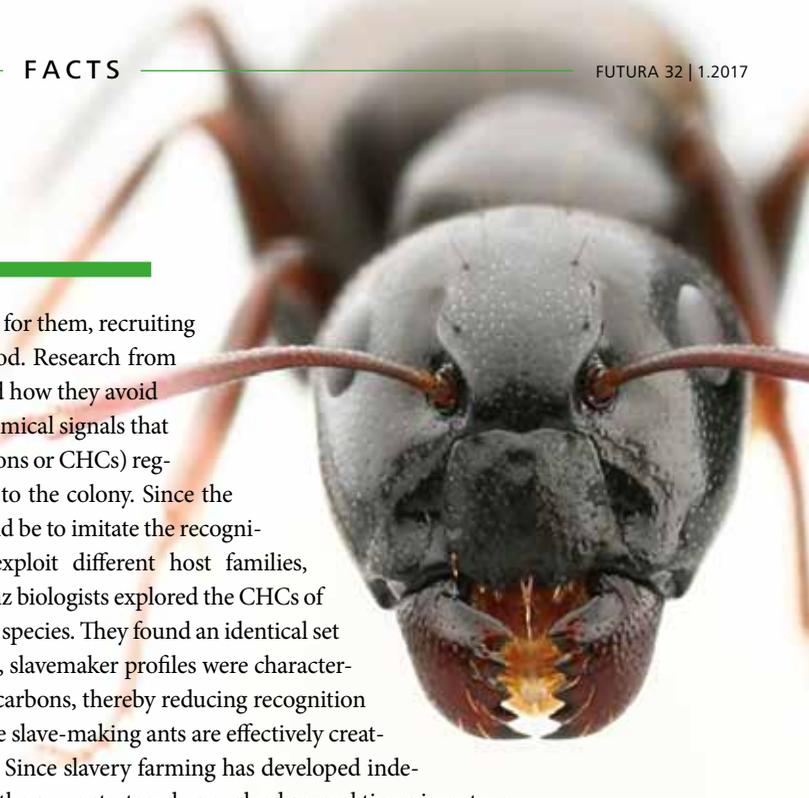
We are always looking for exciting scientific photos and illustrations!
If you would like to have your image published, contact Kirsten at kirsten.achenbach@bifonds.de.

STEALTHY SLAVE-MAKING ANTS CHEAT DETECTION

Slave-making ants subjugate other ant species to do their work for them, recruiting slaves by raiding surrounding colonies and enslaving the brood. Research from the Johannes Gutenberg University in Mainz has now revealed how they avoid detection. For ants, scent is an incredibly important sense. Chemical signals that grow from the body surface of the insects (cuticular hydrocarbons or CHCs) regulate, for example, that only nesting companions gain access to the colony. Since the slavemakers do not want to be recognized, one possibility would be to imitate the recognition profile of their victims. However, as they usually exploit different host families, a more universal strategy is needed. During the study, the Mainz biologists explored the CHCs of three species of slavemakers and their three closely related host species. They found an identical set of 35 hydrocarbons among the ants of all six species. However, slavemaker profiles were characterized by shorter-chained, less complex, and unbranched hydrocarbons, thereby reducing recognition cue quantity. By carrying less informative fragrance signals, the slave-making ants are effectively creating chemical stealth caps, avoiding detection by their victims. Since slavery farming has developed independently in this species group of ants, the analyses show that the same strategy has evolved several times in nature.

REFERENCE

Kleeberg I, Menzel F, Foitzik S (2017) The influence of slavemaking lifestyle, caste and sex on chemical profiles in *Temnothorax* ants: insights into the evolution of cuticular hydrocarbons. *Proc Biol Sci* **1850**: 20162249



The type III secretion system (T3SS) is a needle-like molecular machine that Gram-negative bacteria use to infect cells.

RECREATING A MOLECULAR NEEDLE

Despite their complex structures, molecular machines are one of the most basic building blocks of life. The type III secretion system (T3SS) of so-called Gram-negative bacteria, including the pathogens of plague, cholera, typhoid, and *Salmonella*, is a good example of such a machine. Molecular structures resembling hollow needles torpedo the wall of a host cell and transport pathogenic proteins to spread infection. The over 20 proteins in this system are tightly regulated and vary depending on the surroundings of the bacteria. Can a much simpler version of this tool be recreated using synthetic biology? This was the question Thomas Marlovits from the Institute of Molecular Biotechnology (IMBA) in Vienna, Austria, asked himself. Using a bottom-up approach, he replaced coding and non-coding DNA or altered it with synthetic parts to create an ultra-simplified model of T3SS. “Over the course of this three-year study, many rounds of debugging were needed to generate a fully functional system,” explains Marlovits. The development of the simplified T3SS reveals that none of the intrinsic regulatory features of the system are required to generate a functional needle complex. The refactored T3SS could serve as new tool in biotechnology.

REFERENCE

Song M, Sukovich DJ, Ciccarelli L, Mayr J, Fernandez-Rodriguez J, Mirsky EA *et al* (2017) Control of type III protein secretion using a minimal genetic system. *Nat Comm* **8**: 14737

TROJAN FISH INVADE THE MEDITERRANEAN

The mystery of how some invasive species spread in the world's oceans may have been partly solved, thanks to research from an international team with the participation of the GEOMAR Helmholtz Centre for Ocean Research in Kiel. Red sea rabbitfish invaded the Mediterranean through the Suez Canal in the 20th century, followed soon after by unicellular organisms from the Indo-Pacific ocean known as foraminifera. Why did the invasion happen in parallel? The research revealed the so-called "Trojan fish" effect, finding evidence of a possible invasion path in the gut of fish. In the stomach and faeces of freshly caught rabbitfish from the Mediterranean, the team found the small organisms – some alive. Although they are plant-eaters, rabbitfish accidentally devour these organisms from the sea floor while feeding. Some of them survive the trip through the fish's digestive system and thus become transplanted to new habitats. While the dispersal of plants and animals by fish – so-called ichthyochory – has been known in lakes and rivers, the present study is the first to look into fish transporting new species in the ocean.

REFERENCE

Guy-Haim T, Hyams-Kaphzan O, Yeruham E, Almogi-Labin A, Carlton JT (2017) A novel marine bioinvasion vector: ichthyochory, live passage through fish. *Limnology and Oceanography Letters*, DOI: 10.1002/lol2.10039



NICHE HOMES AT HYDROTHERMAL VENTS

Conditions at deep-sea hydrothermal vents are hardly conducive to easy living – here, water heated in the Earth's interior spews forth at temperatures of several hundred degrees Celsius. On top, with no light, photosynthesis is impossible. Despite these challenges, bacteria have found their own ecological niche. Two main types of bacteria populate the vents – the so-called SUP05 gammaproteobacteria as well as the epsilonproteobacteria of the genera *Sulfurovum* and *Sulfurimonas*. These microorganisms use chemical compounds, such as methane or sulphide, to grow. In this extreme habitat, conditions change drastically on very small scales – a few centimetres make all the difference. The biologist Dimitri Meier and his colleagues from the Max Planck Institute for Marine Microbiology in Bremen, Germany, set out to understand how resident microorganisms adapt. Samples of gas, water, and different surfaces in the immediate vicinity of vents were collected in the Manus Basin (Papua New Guinea). Tests found that life at the vents is clearly defined. The SUP05 bacteria are adapted to low sulphide concentrations farther away from the vent, while epsilonproteobacteria can live closer to the vent due to their enhanced surface attachment and stress resistance mechanisms. This also had an impact on their diversity: SUP05 bacteria were represented by just a few, very closely related species, while there were many different species of epsilonproteobacteria, albeit with comparable genetic make-up.

REFERENCE

Meier DV, Pjevac P, Bach W, Hourdez S, Girguis PR, Vidoudez C *et al* (2017) Niche partitioning of diverse sulfur-oxidizing bacteria at hydrothermal vents. *ISME J* 11: 1545–1558

3.48

BILLION YEARS

That's the age of hot spring deposits in the Pilbara region of Australia. They contain fossils that are the earliest known trace of microbial life on land.

This means that there has been life on earth some 580 million years earlier than previously thought.

Source: Djokic T, Van Kranendonk MJ, Campbell KA, Walter MR, Ward CR (2017) Earliest signs of life on land preserved in ca. 3.5 Ga hot spring deposits. *Nat Commun* 8: 15263



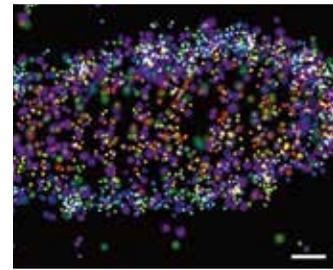
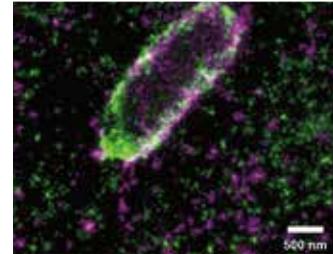
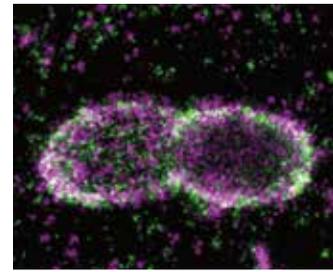
Collecting samples at hydrothermal vents in the Manus Basin.

CELLS VS BACTERIA, CLOSE-UP

How do cells fight bacteria? An interdisciplinary team from Goethe University Frankfurt, Germany, led by Mike Heilemann and BIF alumnus Ivan Dikic, have studied cellular defence mechanisms against *Salmonella* through super-resolution microscopy, visualizing protein patterns at the near-molecular level. Like many bacteria, *Salmonella* has developed clever mechanisms to survive and grow within host cells. Cells themselves have in turn developed effective defence strategies, flagging bacteria within the cell with a protein called ubiquitin (Ub). The attached flags contain chains of differently linked Ub molecules, relaying specific signals from the surface of the bacteria into the cell. By using single-molecule localization microscopy, the team visualized the balance and nanoscale distribution pattern of linear Ub chains formation at the surface of *Salmonella* bacteria. The research revealed that linear Ub chains trigger the degradation of bacteria and kick off an inflammatory signalling cascade which results in restricting bacterial proliferation. In addition, the researchers identified the enzyme Otulin as an important regulator capable of limiting the inflammation induced by linear ubiquitin chains – a very important notion considering the fact that excessive inflammation is one of the major causes of tissue damage.

REFERENCE

van Wijk SJ, Fricke F, Herhaus L, Gupta J, Hötte K, Pampaloni F, Grumati P, Kaulich M, Sou Y, Komatsu M, Greten F, Fulda S, Heilemann M, Dikic I (2017) Linear ubiquitination of cytosolic *Salmonella* Typhimurium activates NF- κ B and restricts bacterial proliferation. *Nat Microbiol*, DOI: 10.1038/nmicrobiol.2017.66



Salmonella bacterium in a human cell, surrounded by different ubiquitin chains (purple and green).

NEW PH SENSOR CAN BE USED TO TRACK TUMOUR METABOLISM

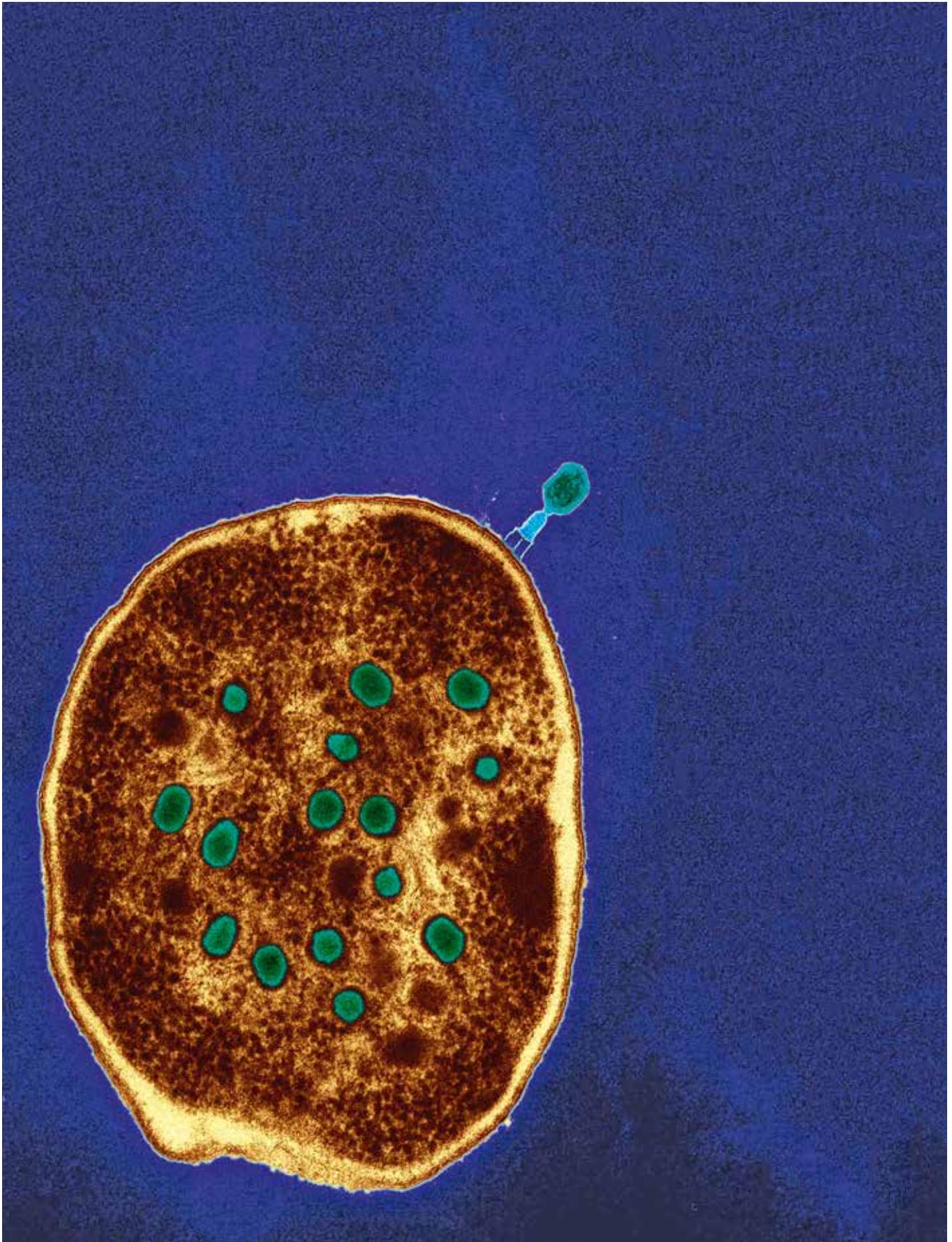


Much more complicated than using a pH test strip: measuring medically relevant pH values inside the body of living organisms.

Some diseases, such as tumours, disturb the body's acid-base balance. Tissues surrounding them are usually slightly more acidic than healthy tissue. Therefore, a pH-imaging method would make it possible, for instance, to show the metabolic processes of tumours. It could also help to quickly find out how effective a treatment is. A successful therapy might change the metabolism of the tumour and thereby the pH value of surrounding tissue, before the tumour itself starts to shrink. The first pH-imaging method for living tissues has now been developed by a team from the Technical University of Munich (TUM). It is based on magnetic resonance imaging (MRI), a non-invasive, radiation-free method, in combination with an injection of zymonic acid. This non-toxic substance is highly sensitive to pH changes and can be made to emit signals detectable with an MRI scan. Although the method still needs to be refined, the team showed that it can detect medically relevant pH changes in the body, promising to be a valuable tool for medical imaging.

REFERENCE

Düwel S, Hundshammer C, Gersch M, Feurecker B, Steiger K, Buck A *et al* (2017) Imaging of pH in vivo using hyperpolarized ^{13}C -labeled zymonic acid. *Nat Comm*, DOI: 10.1038/NCOMMS15126



Phages (green) on the surface of an *Escherichia coli* cell inject genetic material into the bacterium.

PROFILE OF PHAGE λ

By Mitch Leslie

The article “The Supermodels of Biological Research” in the second issue of *Futura* in 2016 was the starting point for a series of portraits of some of the most iconic organisms and their role in research. In this issue we introduce phage lambda.

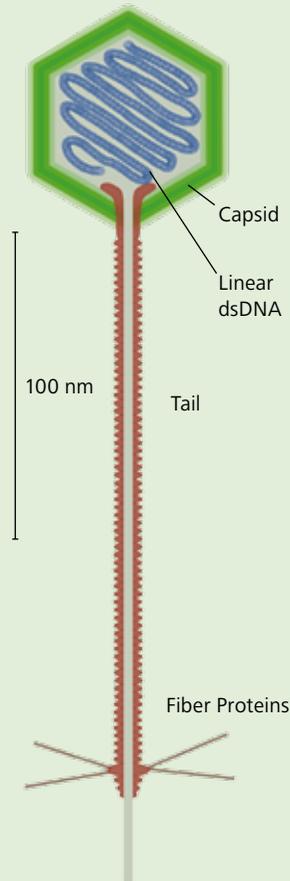
One day in the late 1940s, microbiologist Esther Lederberg accidentally discovered one of the most important model organisms in a lab refrigerator at the University of Wisconsin. Lederberg noticed that colonies of the bacterium *Escherichia coli* she had been studying looked “nibbled,” as if something was killing the cells. The culprit was an unknown type of bacteria-attacking virus, which she named phage λ . At the time, the structure of DNA was still a mystery, and phage λ became indispensable for molecular biologists trying to answer questions such as how genes work, how chromosomes are organized, and how to manipulate organisms’ DNA. The Cold Spring Harbor Laboratory in New York became a hub for research on phage λ and similar viruses by scientists such as the Nobel prize-winner Alfred Hershey.

Phage λ has several qualities that make it a good model. It is simple, easy to raise, and prolific, producing a new generation in 45 minutes. In addition, it infects what is probably the best-known organism, *E. coli*. One of the biggest discoveries involving phage λ stemmed from another of its attributes: its ability to hide out in, rather than kill, its host. Studying how the virus shuts down most of its genes helped Jacques Monod and Francois Jacob of the Pasteur Institute in France uncover the lac operon, the first example of a switch for turning genes off and on. The scientists shared the 1965 Nobel Prize in Physiology or Medicine for the discovery.

The question of why some bacteria are “immune” to phage λ sparked the award-winning research of Werner Arber, then at the University of Geneva in Switzerland, who shared the 1978 Nobel prize in Physiology or Medicine. The reason, his lab showed, was that the bacteria produce restriction enzymes that attack the viral DNA. These DNA-cutting enzymes became essential tools for genetic engineering. As researchers began to tinker with organisms’ DNA, phage λ proved useful as a cloning vector to ferry DNA sequences into bacterial cells. Phage λ has remained a go-to model organism. By exploiting its *exo* and *bet* genes, researchers developed a more efficient and versatile genetic engineering technique known as recombinering. These genes allow researchers to insert an engineered DNA sequence seamlessly into bacterial DNA using a re-

pair mechanism called homologous recombination. Investigations into why some mutant *E. coli* are resistant to phage λ sparked the discovery of molecular chaperones, proteins that help other proteins fold into the right shape. And scientists still rely on the virus to study everything from HIV vaccines to the evolution of new species.

WHO AM I? SOME FACTS



- I weigh about 64 million daltons – less than 1/100,000,000,000,000th of a gram – and have 48,502 base pairs.
- In 1977, my DNA was the first double-stranded DNA to be sequenced.
- I may or may not be alive – researchers still disagree.
- I need *Escherichia coli* cells to reproduce.
- I work mostly in molecular biology.
- I helped researchers to win two Nobel Prizes.
- My name is lambda (11th letter in the Greek alphabet), because I was thought to be similar to the kappa (10th letter) particle in *Paramecium*.



Recycling precious materials is nothing new – cells have been doing it for ages.

AUTOPHAGY, AGAIN AND AGAIN

By Sarah Williams

Recycling glass, paper, and plastic may be a purely human endeavour, but cells have perfected their own form of recycling, called autophagy. Once dubbed a “garbage pathway”, autophagy is now turning out to be far more complex – and have further-reaching impacts on health and disease – than scientists ever guessed.

You are made of stardust. The atoms in your big toe, your brain, and the overwhelming majority of everything in between predate you by billions of years, hailing from the Big Bang and from distant, ancient stars. These atoms have been recycled again and again – making their way through time until they ended up in your body.

Inside all living cells, these cosmically born atoms make up the building blocks of proteins, fats, sugars, and DNA. When they have served their purpose, these macromolecules are recycled. Again and again, molecules are broken down into their components to build entirely new structures.

Christian de Duve coined the term autophagy – literally “self-eating” – more than 50 years ago to describe the process in which eukaryotic cells gather up molecules and whole organelles from inside the cell to be reduced to their building blocks. Today, it is clear that autophagy is vital to the normal functioning of cells. The recycling process, among other things, helps cells balance sources of energy when they are under stress. For example, mitochondria – which rely on oxygen to generate energy – undergo autophagy when there is a shortage of oxygen.

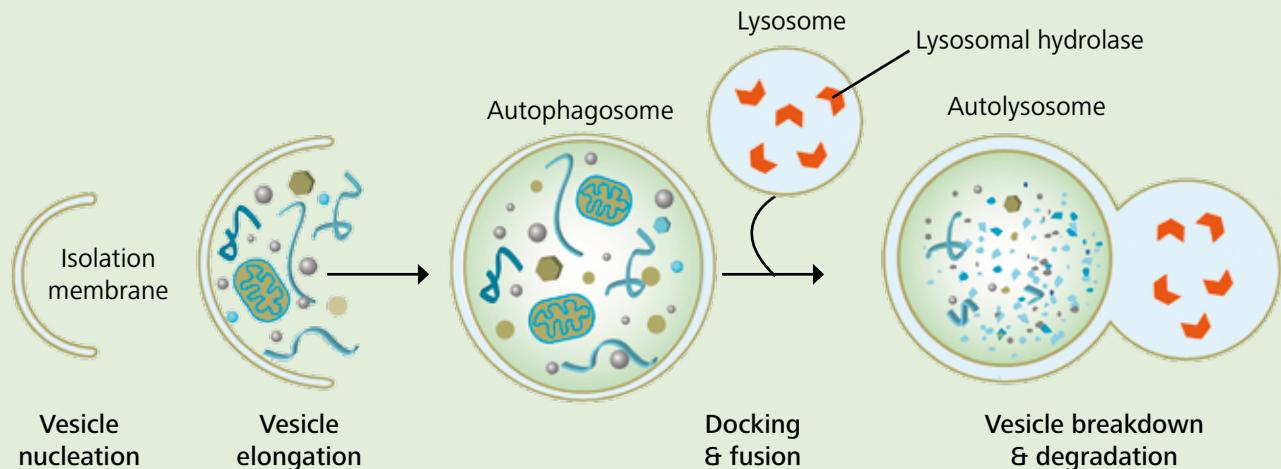
For nearly three decades, though, autophagy was shrugged off as a “garbage pathway,” and studied only tangentially. Most assumed that autophagy had little effect on how cells functioned,

and technically, it was difficult to study autophagy – there were no methods to track the process other than watching it under a microscope, and no known autophagy genes. Instead, researchers focused their attention on ubiquitin-mediated protein degradation, which had clear implications for controlling levels of regulatory proteins in cells.

In the 1990s, though, a series of discoveries helped pique scientists’ interest in autophagy. In the span of just a few years, Yoshinori Ohsumi, then at the University of Tokyo in Japan, developed an assay for measuring autophagy in yeast cells, and used the approach to discover 15 autophagy-related genes, the ATG genes. His group went on to uncover mammalian versions of the yeast genes, paving the way for researchers to start making links between autophagy and human health. When, in 1999, Beth Levine of the University of Texas Southwestern Medical Center in the USA reported that Beclin-1, a mammalian autophagy gene, could inhibit tumour formation – the first link between autophagy and disease – the field really took off. In 2016, Ohsumi won the Nobel Prize for his pioneering work.

While basic questions remain about autophagy and its regulation, scientists have identified a number of pathways that intersect with it and countless links between autophagy and human disease have been uncovered. →

HOW MACROPHAGY WORKS



Macroautophagy was the first type of autophagy to be discovered as it can be observed under the microscope.

De Duve first described autophagy in liver cells. They use it as a way to get energy from unneeded molecules when levels of nutrients in the liver decrease – between meals, for instance. During the first eight hours of nutrient deprivation, the liver breaks down proteins, including many found in energy-generating mitochondria, which are themselves engulfed through autophagy. After eight hours of starvation, the liver’s autophagy begins to focus on lipids, engulfing lipid droplets – stores of triglycerides and cholesterol. Immediately after a meal – when blood sugar levels rise again – autophagy is curbed.

Research since de Duve’s time has revealed reasons other than starvation that cells throughout the body turn on autophagy. Proteins, organelles, or DNA molecules that are damaged can all spur the process. Misfolded proteins, in particular, might disrupt a cell’s normal functioning if allowed to accumulate. Infection, oxidative stress, or a lack of oxygen can also trigger autophagy, using the programme as a way both to destroy invading bacteria or viruses and conserve energy to avoid cell death.

“Autophagy is often a survival mechanism,” says Ioannis Nezis of the University of Warwick in the UK. “A cell is eating [parts of] itself as a last effort to survive under stress.” In other cases, a cell may need to destroy proteins or other molecules to massively shift its shape or function, as happens often during

development, stem cell differentiation, and immune responses. And even in the absence of these triggers, it seems the cell is constantly engaged in low levels of “quality control” autophagy to help encourage the turn-over of long-lived proteins and organelles which may have accumulated damage over time.

Not all autophagy, though, looks the same; three different forms have been described. All three forms of autophagy involve moving cellular components to the lysosome, a compartment in the cell that is optimized for digesting molecules. With a low pH – like the stomach – this membrane-bound section of the cell contains enzymes that can break apart proteins, carbohydrates, lipids, and nucleic acids. But there are large differences between types of autophagy when it comes to how materials get to and into the lysosome and how much is moved in one go.

When most scientists talk about autophagy, they really mean macroautophagy – it is the best understood of the three varieties, and the longest studied. It is also the kind that is easiest to watch under a microscope: a double membrane begins to assemble, first forming a shape like a catcher’s mitt that can grab nearby material floating in the cell’s soupy cytoplasm. As the membrane – called the phagophore or isolation membrane – continues to grow, it closes around the material to be broken down, forming a sphere known as an autophagosome. The autophagosome – a floating recycling bin of sorts – moves to the

lysosome with its cargo, which can include multiple different types of molecules in one load. The autophagosome then fuses with the lysosome. The resulting vesicle is called an autolysosome; everything in it is broken down by the lysosomal hydrolase.

For a number of years, scientists assumed that macroautophagy was always non-selective; the phagophore seemed to just grab whatever was nearby in its attempts to salvage energy. But we now know that the phagophore can also enclose materials that are specifically chosen to be recycled. For instance, when a cell's RNA damage repair response senses a double strand break, a selective form of macroautophagy is activated to destroy the damaged DNA. In other cases, the cell uses this type of specific macroautophagy to recycle entire organelles, and each subtype has its own name: mitophagy to digest mitochondria and ribophagy to digest ribosomes, for instance.

In the mid-1990s, just as Ohsumi was identifying the ATG genes required for macroautophagy in yeast, scientists including Ana Maria Cuervo from the Albert Einstein College of Medicine in New York City, USA, also revealed an entirely new type of autophagy, called chaperone-mediated autophagy (CMA) specific to mammals. CMA transports proteins that have a sequence of five specific amino acids that are kept hidden within the proteins' structures at most times. The sequence can be exposed, however, when a protein is misfolded due to errors in the folding process or unfolded due to damage – which in either case is potentially dangerous to the body. When this happens, the cytosolic heat shock protein Hsc70 binds the exposed sequence. Together, Hsc70 and the target protein move to the lysosome, where Hsc70 helps the complex to dock at the lysosomal membrane. There, a separate molecule, LAMP-2A, which extends through the lysosomal membrane, pulls the tagged protein directly into the interior of the lysosome without the help of a vesicle.

Like macroautophagy, CMA was first discovered in liver cells, the champions of recycling in our bodies. But CMA has more recently been shown to be critical to clearing damaged proteins from cells in other parts of the body, too. In the brain, where new cells are rarely formed, removing damaged proteins from existing cells before they can trigger cell death is particularly vital. As opposed to macroautophagy, in which functioning proteins may be non-specifically swept into an autophagosome, CMA helps ensure that only damaged proteins, with the five-amino-acid sequence exposed, are moved to the lysosome.

There is a third type of autophagy, called microautophagy, which researchers are still working to understand. Materials can be moved to the lysosome using Hsc70, as in CMA, or through other means, and the process can be either specific or non-specific, like macroautophagy. But once the molecules are near the lysosome, they are not moved across the lysosome membrane by

LAMP-21 as happens in CMA. Instead, they are internalized when the lysosomal membrane blebs inward, forming a vesicle. In general, these vesicles – which form at the surface of the lysosome once materials are already in the vicinity – are smaller than the vesicles which form in the cytosol to move materials to the lysosome in macroautophagy. So far, most of what is known about microautophagy comes from studies in yeast. The three types of autophagy operate using their own machinery, but are far from independent, researchers are discovering.

In some cases, when one form is blocked, the others might increase their activity. Other times, the same mechanisms – cellular damage or starvation, for examples – boost levels of all the types of autophagy at once.

To study autophagy, researchers have developed systems that let them track where and when autophagy is occurring by watching the programmes in action in isolated cells and tissue cultures. The methods are letting them move past basic observations on how and when autophagy occurs to ask more complex questions about how it is regulated. “Both too much and too little autophagy is a problem,” says Daniel Klionsky of the University of Michigan in Ann Arbor, USA, who studies →

When most scientists talk about autophagy, they really mean macroautophagy – it is the best understood of the three varieties, and the longest studied. It is also the kind that is easiest to watch under a microscope.

macroautophagy in yeast. “And there’s a huge range of mechanisms that cells are using to regulate all the genes and proteins involved in the process.”

Klionsky has been on the hunt for regulatory genes that impact the expression of the known ATG genes. To study the potential effects of genes identified in screens, he often turns to a fusion protein that is used widely in the field: a combination of green fluorescent protein (GFP) and Atg8, a protein that remains associated with the autophagosome from its formation until its fusion with the lysosome. Scientists can see fluorescent dots when Atg8 is produced and integrated into an autophagosome.

In one recent *Current Biology* paper, Klionsky’s team uncovered the role of a gene known as Pho23 in autophagy using this approach in yeast. They found that Pho23 controls the expression of the autophagy gene ATG9, which, in turn, regulates the frequency of autophagosome formation.

Compared to macro- and microautophagy, studying CMA is complicated by the fact that yeast and *Drosophila* do not have a CMA pathway; it is specific to mammals. But in mammalian cells, Cuervo’s team has developed their own approach. They couple a fluorescent protein and the five-amino-acid sequence that targets proteins for CMA. When the proteins are added to rat or mouse cells, they move toward the lysosome and, depending on levels of LAMP-2A, are eventually pulled inside the organelle and degraded. In one experiment, Cuervo used this fluorescence approach on a variety of cell types, including neuroblastoma, hepatoma, and breast adenocarcinoma, to compare their rates of CMA when the cells were removed from protein-rich serum, a stressful change that often triggers autophagy. The hepatoma and breast adenocarcinoma cells, the researchers found, had higher basal rates of autophagy but upregulated the process less than other cells when starved of serum. The implications of this on cancer treatment are not yet clear.

Cuervo and others have also discovered that as humans age, levels of autophagy in their cells go down. In flies and worms, mutations that dampen or knock out autophagy decrease lifespan. Proteins involved in various steps of all three types of autophagy seem to work less well – or have lower levels – in older animals. Cuervo has shown that for CMA this can be attributed to a particular receptor on the surface of lysosomes that becomes less stable with age. Reduced autophagy could thus be one reason why the risk of some diseases increases with age.

New research about autophagy, therefore, has the potential to offer valuable insights into human health – and maybe even lead to new drugs. If scientists can learn how to turn up autophagy in specific cells and tissues, they may be able to stop the accumulation of damaged proteins and help cells metabolize correctly. The first could be a boon for treating Alzheimer’s, Parkinson’s, and Huntington’s diseases, the latter could be useful for

treating metabolic disease including diabetes. The accumulation of damage in the mitochondria of cells has been implicated in insulin resistance, and some researchers have suggested that ramping up autophagy to remove defective mitochondria could help treat or prevent diabetes. In addition, since Levine’s first paper on Beclin1, scientists have uncovered a complex relationship between autophagy and cancer, with the early formation of tumours often linked to a suppression of autophagy, and more advanced tumours relying on high rates of autophagy to survive stress. To date, no drugs on the market have been developed specifically to target autophagy, but some previously approved ones – including metformin and lithium – interact with proteins involved in autophagy and have been shown to change levels of autophagy in cells.

But with hypotheses on the roles of autophagy in human health and disease, scientists keep hitting the same brick wall. There is currently no way to track autophagy in living animals, let alone people. “This is one of the biggest challenges right now,” says Klionsky. “If you want to modulate autophagy for therapeutic purposes in humans, you want to do know if you’re actually changing the process. Did your treatment actually do what you wanted?”

As researchers continue to study the basics of autophagy, they are continually reminded that the process is more than just destruction. After materials are broken down in the lysosome, molecules like amino acids and nucleic acids are transported back into the cytoplasm, ready to build new structures. “The beauty of it is that you both destroy unwanted things and use the pieces of what you’re breaking down to synthesize new things,” says Cuervo. ←

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.

STEFANIE BUSCHOR

Characterization of the early dynamics of *Citrobacter rodentium* infection 42

MICHAEL-JOHN DOLAN

Cracking the circuits for innate behaviour in *Drosophila* 42

TILMAN FLOCK

The inner workings of activation and selectivity in G-protein-coupled signalling 43

CLÉMENCE FOLTZ

Immune-mediated control of *Toxoplasma* infection by the ubiquitin ligase TRIM21 43

JAN HERUDEK

Characterizing the function of promoter upstream transcripts 44

LUKAS HUTTER

Mitotic exit: basic principles at work 44

AGNIESZKA JANSKA

The role of DNA Pol epsilon in DNA replication 45

ROOS KARSEMEIJER

Preventing pathogenic DNA repair at dysfunctional telomeres 45

PHILIPP KOLDEWEY

Mechanistic and structural characterization of the molecular chaperone Spy 46

MEHRPOUYA BALAGHY MOBIN

Vigilin – a lock keeper for fat 46

SIMON NEYER

Structural mechanisms of RNA polymerase I transcription initiation and elongation 47

MICHAŁ PASTERNAK

RNAi screen for novel regulators of mammalian meiosis 47

PRIYANKA SAHASRABUDHE

Analysis of the Hsp90 co-chaperone network dynamics 48

JULIANE SCHMIDT

Local regulation of DNA methylation by the transcription factor REST 48

HEENA SHARMA

Rapid rotation of the ribosomal subunits 49

CARINE STAPEL

Zygotic genome activation at single-molecule resolution 49

SEBASTIAN VIRREIRA WINTER

Spatiotemporal analysis of glutathione oxidation in murine macrophages 50

FRIEDERIKE ZUNKE

Analysis of the lysosomal transport receptor LIMP-2 and its role in Parkinson's disease 50

CHARACTERIZATION OF THE EARLY DYNAMICS OF *CITROBACTER RODENTIUM* INFECTION

cf. BIF FUTURA, VOL. 28 | 2.2013

STEFANIE BUSCHOR

Discipline: Biochemist, MSc

Institute: Institute of Infectious Diseases,

University of Bern, Bern, Switzerland

Supervisor: Prof. Siegfried Hapfelmeier



Intestinal infection by attaching and effacing (A/E) pathogens such as enteropathogenic *Escherichia coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) is a worldwide health issue and is frequently fatal. *Citrobacter rodentium* is a mouse pathogen that shares its key mucosal virulence mechanism with EHEC and EPEC and is therefore commonly used as a surrogate model. Many factors of the innate and adaptive immune system play a crucial role in the host defence mechanisms against *C. rodentium*, but very little is known about key factors during the very early phase of infection. In this study, we found that events during the first few hours determine the outcome and the severity of a *C. rodentium* infection. In mice lacking a competing gut microbiota (germ-free mice), we found an inverse dose-severity correlation such that larger infectious doses of bacteria led to a milder disease. We speculated that this is partly due to a self-limitation of the bacteria themselves, as they are less virulent when administered in high densities. We were able to prove this by measuring expression levels of the master regulator of the virulence island *ler* by quantitative PCR *in vitro* and *in vivo*. In addition, innate immunity triggered within the first few hours of infection effectively prevents luminal bacteria from re-infecting the colonic epithelium at later stages. This protective immune response includes upregulation of the chemokine CXCL1 and the subsequent recruitment of neutrophils and monocytes to the colon. Only virulent *C. rodentium* but not the avirulent Δler mutant is able to induce this rapid response. Furthermore, the innate immune signalling molecules MyD88 and Trif are indispensable for the observed host response, as mice lacking these two molecules cannot mount an early immune response and therefore become heavily infected. These findings highlight the importance of the first few hours of this type of infection for the disease outcome and may be relevant for new approaches to prevent EPEC infection in young children.

PUBLICATIONS

Cuenca M, Pfister SP, Buschor S, Bayramova F, Hernandez SB, Cava F *et al* (2016) D-alanine-controlled transient intestinal mono-colonization with non-laboratory-adapted commensal *E. coli* strain HS. *PLoS One* **11**: e0151872

CRACKING THE CIRCUITS FOR INNATE BEHAVIOUR IN *DROSOPHILA*

cf. BIF FUTURA, VOL. 27 | 3.2012

MICHAEL-JOHN DOLAN

Discipline: Biologist

Institute: MRC Laboratory of Molecular Biology,

University of Cambridge, Cambridge, UK

Supervisor: Dr Gregory Jefferis



The olfactory system provides an excellent model for how sensory information is transformed into behaviour because its neural circuits are conserved across the animal kingdom. In my PhD, I aimed to dissect the function of two higher brain regions in *Drosophila* – the mushroom body (MB) and the lateral horn (LH). In *Drosophila*, olfactory stimuli are first processed in the brain's antennal lobe, then travel to the MB and the LH, which are thought to mediate learned and innate behaviour respectively. In the corresponding mammalian brain pathway, neurons from the olfactory bulb project to the piriform cortex and the olfactory amygdala. The function of the MB is well understood: By silencing the neurons of the MB with genetic tools that switch certain cell types on or off, previous studies showed that without MB neuronal signalling flies could respond to odours but could not learn. However, no such tools existed for the LH, so its role in olfactory behaviour remains unknown. I first developed a new genetic tool that can control gene expression in specific sets of neurons and other tissues in the fly. Next, I developed a collection of transgenic lines, each allowing control of a specific type of neuron in the LH. Using these lines, I generated an atlas of higher olfactory processing regions in the fly brain. I integrated these data with a published anatomical analysis of the MB and discovered higher order olfactory processing regions that may take information from both the MB and the LH. Finally, my laboratory used these lines to investigate the LH's role in learning and memory. We identified an LH cell-type that was essential for olfactory memory recall, implying that the LH functions beyond innate behaviour. The tools and data I produced will help elucidate the function of the LH, and the principles we discover may apply to analogous regions in the vertebrate brain.

PUBLICATIONS

Dolan M-J*, Luan H*, Shropshire W, Sutcliffe B, Cocanougher B, Scott R *et al* (2017) Ciliating neuron-specific genetic manipulations in *Drosophila* using a split GAL4 repressor. *Genetics* **26**: 755–784

Dolan M-J, Huovalia P, Jefferis G. (2015) Insect Olfaction: Telling food from foe. *Curr Biol* **25**: R995–R998

Kohl J, Ng J, Cachero S, Ciabatti E, Dolan M-J, Sutcliffe B, Tozer A *et al* (2014) Ultrafast tissue staining with chemical tags. *Proc Natl Acad Sci USA* **111**: E3805–E3814

THE INNER WORKINGS OF ACTIVATION AND SELECTIVITY IN G-PROTEIN-COUPLED SIGNALLING

cf. BIF FUTURA, VOL. 28 | 2.2013

TILMAN FLOCK

Discipline: Molecular Biotechnologist, MSc
 Institute: MRC Laboratory of Molecular Biology,
 University of Cambridge, Cambridge, UK
 Supervisor: Dr M. Madan Babu



G-protein-coupled receptors (GPCRs) comprise the most medically relevant human receptor family, with over 800 different members. The selective coupling of GPCRs to specific G proteins is critical for triggering the appropriate cellular response to extracellular stimuli. During my PhD, I developed a multi-scale computational approach to (1) investigate the atomic architecture of G proteins and GPCRs by comparing ~100 G-protein structures, and (2) study the evolutionary history of more than 1,000 G-protein sequences from ~66 species. I showed that, despite their diversity, G proteins share the same molecular architecture of activation. By mapping clinical and genetic variations to this activation model, I explained the molecular origins of disease-related mutations in G proteins. I also elicited the molecular details of how, despite their conserved mechanism of activation, different GPCRs selectively activate different G proteins to trigger particular signalling pathways. I revealed a “selectivity barcode” that is present on each of the 16 human G proteins but absent from the receptors. Through ancestral reconstruction of the evolutionary history of GPCRs, I found that different receptors have evolved unique solutions to read the G-protein barcodes. Hence, while the G proteins have been conserved throughout evolution, new organism-specific GPCRs have dynamically evolved to couple to an existing G protein and trigger a specific signalling pathway. In addition to explaining how GPCRs became the most diverse human receptor family, this discovery could be used to minimize the cross-reactivity of drugs targeting the GPCR by selectively blocking specific G-protein interactions.

PUBLICATIONS

Flock T, Hauser SH, Lund N, Gloriam DE, Santhanam B, Babu MM (2017) Selectivity determinants of GPCR-G protein binding. *Nature* **545**: 317–322

Sun D, Flock T, Deupi X, Maeda S, Matkovic M, Mendieta S *et al* (2015) Probing Gai1 protein activation at single-amino acid resolution. *Nat Struct Mol Biol* **22**: 686–694

Flock T, Ravarani CN, Sun D, Venkatakrishnan AJ, Kayikci M, Tate CG *et al* (2015) Universal allosteric mechanism for Ga activation by GPCRs. *Nature* **524**: 173–179

IMMUNE-MEDIATED CONTROL OF *TOXOPLASMA* INFECTION BY THE UBIQUITIN LIGASE TRIM21

cf. BIF FUTURA, VOL. 27 | 2.2012

CLÉMENCE FOLTZ

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



In parasitic infections, both the host and the pathogen must evolve and adapt in order to survive. The vacuolar parasite *Toxoplasma* invades nucleated host cells, where it resides and replicates within a parasitophorous vacuole (PV). The major mediator of the host defence against *Toxoplasma* is the cytokine interferon gamma (IFN γ). Following infection, IFN γ upregulates the p47 immunity-related GTPases and the p65 guanylate-binding proteins (GBPs), which accumulate at the PV of the avirulent type II and III, but not the virulent type I, strains of *Toxoplasma*, mediating its disruption and orchestrating the clearance of the parasite. However, the molecular mechanisms underlying the host-pathogen interaction and responses remain unknown. My PhD aimed to identify what host factors play a role at the PV and are involved in mediating resistance against *Toxoplasma*. Coupling immunoprecipitation assays with confocal imaging, I identified the E3 ubiquitin ligase TRIM21 as a novel interaction partner of GBP1. TRIM21 and GBP1 were co-recruited to the PV of avirulent but not virulent *Toxoplasma*. In addition, avirulent – but not virulent – *Toxoplasma* was ubiquitinated in an IFN γ - and TRIM21-dependent manner, with TRIM21 partly mediating Lys63 ubiquitin linkages. Using replication assays and electron microscopy, I found that TRIM21 induces the restriction of parasite early replication without interfering with vacuolar disruption. I also demonstrated by enzyme-linked immunosorbent assay that TRIM21 affects the secretion of pro-inflammatory cytokines. TRIM21^{-/-} mice were highly susceptible to *Toxoplasma* infection and had lower serum levels of pro-inflammatory cytokines, which were associated with higher parasite burden in the periphery and in the brain. My work identifies TRIM21 as a modulator of *Toxoplasma* resistance *in vivo*, thereby extending host innate immune recognition of eukaryotic pathogens to include E3 ubiquitin ligases.

PUBLICATIONS

Haldar AK, Foltz C, Finethy R, Piro AS, Feeley EM, Pilla-Moffett DM *et al* (2015) Ubiquitin systems mark pathogen-containing vacuoles as targets for host defense by guanylate binding proteins. *Proc Natl Acad Sci USA* **112**: E5628–E5637

CHARACTERIZING THE FUNCTION OF PROMOTER UPSTREAM TRANSCRIPTS

cf. BIF FUTURA, VOL. 29 | 1.2014

JAN HERUDEK

Discipline: Biochemist, MSc

Institute: Centre for mRNA Biogenesis and Metabolism,

Department of Molecular Biology,

Aarhus University, Aarhus, Denmark

Supervisor: Prof. Torben Heick Jensen



Genome transcription in humans produces an enormous amount of non-coding RNA (ncRNA). Promoter upstream transcripts (PROMPTs) represent a group of ncRNAs that are produced immediately upstream of protein-coding gene promoters. Because PROMPTs are normally rapidly degraded after synthesis by the ribonucleolytic RNA exosome complex, their biological function is still unclear. Although the close proximity of PROMPTs to their associated protein-coding genes may indicate a role in *cis* gene regulation, recent efforts failed to confirm such a hypothesis. Protein-coding gene pairs are often arranged in a divergent (head-to-head) orientation and are co-localized with promoters that are very close to each other. PROMPT transcription could therefore influence expression of the neighbouring protein-coding gene in *trans*, regardless of whether the PROMPTs directly regulate their own associated protein-coding genes. The main goal of my PhD project was to determine whether PROMPT transcription itself can modulate the expression of neighbouring protein-coding genes in a divergent orientation. By applying genome-wide sequencing methods, I found that the distance between divergent promoters strongly correlates with the expression, stability, and length of their associated PROMPTs. If the promoters are sufficiently separated (>1 kb apart), the high number of polyadenylation signals causes early transcription termination, and canonical PROMPTs are subsequently degraded by the RNA exosome. However, if the promoters are positioned more closely (300 bp–1 kb apart), the polyadenylation signals are depleted in these genomic regions and the PROMPTs are stabilized. These non-canonical PROMPTs grow longer and overlap the neighbouring gene, effectively creating new transcription start sites and generating longer messenger RNA isoforms for the neighbouring protein-coding genes. In addition to revealing that the human genome is more complex than previously appreciated, my results show that the transcription of ncRNA may shape the transcriptome.

PUBLICATIONS

Chen Y, Pai AA, Herudek J, Lubas M, Meola M, Järvelin AI *et al* (2016) Principles for RNA metabolism and alternative transcription initiation within closely spaced promoters. *Nat Genet* **48**: 984–994

MITOTIC EXIT: BASIC PRINCIPLES AT WORK

cf. BIF FUTURA, VOL. 29 | 2.2014

LUKAS HUTTER

Discipline: Systems Biologist, DPhil

Institute: Department of Biochemistry,

University of Oxford, Oxford, UK

Supervisor: Prof. Bela Novak



Nothing in mitosis makes sense except in the light of post-translational modification. To successfully divide and temporally coordinate the underlying processes, cells harness a complex machinery of kinases and phosphatases that activate and inactivate the right enzymes at the right time. At the beginning of this epic tug-of-war known as mitosis, the kinases have the upper hand and promote dramatic changes in the cellular architecture, such as the condensation of the chromatin, the assembly of the mitotic spindle, and the breakdown of the nuclear envelope. At the end of mitosis, following the separation of sister chromatids, phosphatases gain the upper hand and restore the cellular architecture. The phosphatase PP2A:B55 plays a crucial role at this stage, but little is known about what precisely its substrates are, how they are recognized, and how temporal ordering is established. To address these questions, I worked with the group of Francis Barr at the University of Oxford to analyse the dephosphorylation kinetics of HeLa cells *in vitro* by tracking the relative phosphorylation state of proteins as cells exit mitosis. To identify novel substrates of PP2A:B55, we performed the study under unperturbed conditions, as well as under conditions that either hypo- or hyper-activate PP2A:B55. I expressed the expected phosphorylation signature of PP2A:B55 substrates as a mathematical model and fitted the model to the entire dataset. This enabled us to identify 300 novel substrates of PP2A:B55 with high confidence. Moreover, we identified a bipartite polybasic recognition motif flanking the phosphorylation site that allows PP2A:B55 to recognize its substrates in a time-resolved manner. The more basic the recognition sequence of a substrate, the faster its dephosphorylation. We created mutants of “fast” substrates by reducing the basicity of the recognition motif, and observed the subsequent delayed dephosphorylation using live-cell imaging of HeLa cells. By uncovering the principle that establishes order at mitotic exit, my results help us to move beyond the passive observation of phosphatase regulation at the mitotic exit, towards active modulation.

PUBLICATIONS

Cundell MJ, Hutter LH*, Nunes Bastos R*, Poser E, Holder J, Mohammed S *et al* (2016) PP2A-B55 recognition signal controls substrate dephosphorylation kinetics during mitotic exit. *J Cell Biol* **214**: 539–554

THE ROLE OF DNA POL EPSILON IN DNA REPLICATION

cf. BIF FUTURA, VOL. 28 | 2.2013

AGNIESZKA JANSKA

Discipline: Biochemist, BSc

Institute: The Francis Crick Institute,

London, UK

Supervisor: Dr John Diffley



During replication, double-stranded DNA is copied by the replisome, a complex assembly of proteins that couples helicase and polymerase activities. The helicase unwinds the double helix, thus enabling the polymerases to access its individual strands. Eukaryotes have three main replicative polymerases, Pol α , ϵ , and δ , and one replicative helicase, the Cdc45-MCM-GINS (CMG) complex. MCM constitutes the motor, while Cdc45 and GINS are essential co-factors. Little was known about the essential function of Pol ϵ in DNA replication, which is performed by the non-catalytic part of the protein. Furthermore, the structure of Pol ϵ was not characterized extensively, and an understanding of the spatial relationship between Pol ϵ and CMG was lacking. In my PhD project, I investigated the role of Pol ϵ by looking at its interactions with key replication factors using biochemical and structural approaches and *Saccharomyces cerevisiae* as a model system. In collaboration with Alessandro Costa's group, I determined the structure of Pol ϵ by electron microscopy both alone and in complex with CMG. Pol ϵ was found to form an interaction network with key replication factors Sld2, Dpb11, Sld3, and GINS. My studies of interaction motifs suggested that Pol ϵ aids CMG assembly by recruiting GINS to MCM, and I identified the parts of Pol ϵ that are likely required. We showed that Pol ϵ consists of two lobes connected by a linker, which also separates them. The non-catalytic lobe is anchored at the front of CMG, while the catalytic lobe extends towards the side of the helicase. The catalytic lobe adopts one of two conformations, and this switch may allow the DNA to be handed off from one polymerase to the next. The architecture was unexpected, as polymerases trail behind the helicase in bacteria. My work sheds light onto CMG assembly and helps to explain why the non-catalytic part of Pol ϵ is essential for cell viability. It also provides insight into the spatial arrangement of the eukaryotic replisome, further adding to our understanding of DNA replication.

PUBLICATIONS

Yeeles JT, Janska A, Early A, Diffley JF (2016) How the eukaryotic replisome achieves rapid and efficient DNA replication. *Mol Cell* **65**: 105–116

Yeeles JT, Deegan TD, Janska A, Early A, Diffley JF (2015) Regulated eukaryotic DNA replication origin firing with purified proteins. *Nature* **519**: 431–435

PREVENTING PATHOGENIC DNA REPAIR AT DYSFUNCTIONAL TELOMERES

cf. BIF FUTURA, VOL. 28 | 1.2013

ROOS KARSEMEIJER

Discipline: Molecular Biologist, MSc

Institute: Laboratory of Cell Biology and Genetics,

The Rockefeller University, New York, NY, USA

Supervisor: Prof. Titia de Lange



During each round of cell division, the telomeres at the ends of chromosomes naturally shorten despite being protected by a protein complex called shelterin. When telomeres become too short for shelterin to function, they can fuse together, ultimately resulting in cell death. The activation of DNA damage signalling and repair pathways at critically short telomeres is an important tumour suppressor pathway. Despite its importance, little is known about the molecular mechanisms underlying DNA repair at telomeres. DNA repair protein 53BP1 regulates double-strand break (DSB) repair pathways by serving as a platform for the recruitment of other repair proteins. 53BP1 protects dysfunctional telomeres from resection and promotes their mobility, which stimulates telomere fusion, but the precise function of its interaction partners remained unclear. For my PhD project, I generated dissociation-of-function mutants of 53BP1 to better understand its role in DNA repair. By analysing the function of these mutant alleles at telomeres lacking the shelterin component TRF2, I identified a domain of 53BP1 that is responsible for promoting the mobility of dysfunctional telomeres, thereby increasing their chance of repair via the non-homologous end-joining pathway. These results are relevant not only to telomeres but also to DNA repair at DSBs elsewhere in the genome. As 53BP1 is also recruited to sites of replication stress, I examined its function at telomeres lacking the shelterin component TRF1, which results in replication fork stalling in the telomeric DNA. Unexpectedly, I identified a novel pathway of telomere fusion that occurs at the chromosome ends when TRF1 is missing. These results are important for our understanding of the development of cancerous cells, since telomere fusions occur early in tumorigenesis. Identifying the molecular mechanisms underlying these fusions will help us to understand how tumour cells prevent normally induced cell death.

PUBLICATIONS

Lotterberger F, Karssemeijer RA, Dimitrova N, de Lange T (2015) 53BP1 and the LINC complex promote microtubule-dependent DNA mobility and DNA repair. *Cell* **163**: 880–893

Scheiber HA, Loschki J, Karssemeijer RA, Meredith MM, Mucida D, Guernonprez P, Nussenzweig MC (2013) Intestinal monocytes and macrophages are required for T cell polarization in response against *Citrobacter rodentium*. *J Exp Med* **210**: 2025–2039

MECHANISTIC AND STRUCTURAL CHARACTERIZATION OF THE MOLECULAR CHAPERONE SPY

cf. BIF FUTURA, VOL. 27 | 1.2012

PHILIPP KOLDEWEY

Discipline: Biochemist, Diploma

Institute: Department for Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, USA

Supervisors: Prof. James Bardwell,
Prof. Johannes Buchner



Molecular chaperones are essential proteins found in all branches of life. They aid in cellular protein folding and thus are key players in protein folding related human diseases, such as Alzheimer's and Parkinson's disease. Despite their importance, how they function is not entirely clear. In my PhD project, I used the *Escherichia coli* chaperone Spy (Spheroplast protein γ) as a model system to identify and characterize key molecular interactions between chaperones and their protein substrates that promote folding. Spy is an ideal model because it is structurally simpler and smaller than most well-studied chaperones, yet it mediates substrate folding independently of co-factors and ATP. Using spectroscopic and calorimetric techniques combined with a new X-ray crystallographic method called READ (residual electron and anomalous density), I showed that Spy aids in protein folding by interacting with its unfolded substrate proteins in an amphiphilic and flexible fashion, forming many weak hydrophobic as well as electrostatic bonds. I examined Spy's interaction with the substrate protein Im7 (colicin-E7 immunity protein of *E. coli*), finding that Im7 can freely explore its folding energy landscape and hence fold to completeness while continuously bound to Spy. Surprisingly, the folding pathway of Im7 is relatively unaffected by the presence of the chaperone, suggesting that Spy acts as a passive folding aid that prevents protein misfolding and aggregation but does not actively alter the substrates folding pathway. Given that the amphiphilic substrate binding surface exhibited by Spy is a common feature of chaperones, this largely passive but protective mechanism may be central to chaperone function, and may answer the long-standing question of how chaperones facilitate the folding of various structurally unrelated proteins.

PUBLICATIONS

Horowitz S*, Salmon L*, Koldewey P*, Ahlstrom LS, Martin R, Quan S, Afonine P V, van den Bedem H, Wang L, Xu Q *et al* (2016) Visualizing chaperone-assisted protein folding. *Nat Struct Mol Biol* 23: 691–697

Koldewey P, Stull F, Horowitz S, Martin R, Bardwell JCA (2016) Forces driving chaperone action. *Cell* 166: 369–379

Stull F*, Koldewey P*, Humes JR, Radford SE, Bardwell JCA (2016) Substrate protein folds while it is bound to the ATP-independent chaperone Spy. *Nat Struct Mol Biol* 23: 53–58

VIGILIN – A LOCK KEEPER FOR FAT

cf. BIF FUTURA, VOL. 28 | 1.2013

MEHRPOUYA BALAGHY MOBIN

Discipline: Molecular Bioscientist, MSc

Institute: Institute of Molecular Health Sciences, ETH Zurich, Zurich, Switzerland

Supervisors: Prof. Markus Stoffel,
Prof. Thomas Tuschl



Cardiovascular diseases are a leading cause of death worldwide, with metabolic disorders such as fatty liver disease (hepatic steatosis) being a major contributing risk factor. To control this pandemic, it is important that we unravel the molecular mechanisms of fat regulation in the main hub of energy homeostasis – the liver. Despite major efforts, gene regulatory mechanisms that link a steatotic liver to the development of cardiovascular diseases remain elusive. For my PhD project, we therefore sought to identify post-transcriptional networks that are differentially active in healthy and disease states through the characterization of RNA-binding proteins. We identified a protein in liver cells known as vigilin that controls the release of fat into the bloodstream. We found that vigilin is upregulated in livers of obese mice and in patients suffering from fatty liver disease. Using a combination of *in vivo*, biochemical, and genome-wide approaches, we revealed that vigilin acts like a hepatic lock keeper and regulates the release of very low-density lipoproteins (VLDLs) from the liver into the bloodstream. VLDLs are classed as “bad” lipids as they carry high amounts of triglycerides and induce the formation of atherosclerotic plaques. A detached plaque often results in a heart attack or stroke. Consequently, hepatic knockdown of vigilin using RNAi *in vivo* decreased VLDL levels and the formation of atherosclerotic plaques in atherosclerosis-prone mice. We further found that vigilin does not bind directly to its targets but rather to distinct sites on their mRNA to promote the synthesis of the encoded protein. One such target protein of vigilin is apolipoprotein B, which is the core protein of VLDLs and responsible for exporting lipids from the liver. Given its high correlation with the degree of steatosis in patients with fatty liver disease, vigilin may represent a possible link between hepatic steatosis and cardiovascular diseases through the modulation of VLDL secretion. These studies therefore significantly advance our understanding of the breadth of translational regulation in metabolism and demonstrate the therapeutic potential of inhibiting vigilin in the fight against cardiovascular diseases.

PUBLICATIONS

Mobin MB, Gerstberger S, Teupser D, Campana B, Charisse K, Heim MH *et al* (2016) The RNA-binding protein vigilin regulates VLDL secretion through modulation of Apob mRNA translation. *Nat Commun* 7: 12848

STRUCTURAL MECHANISMS OF RNA POLYMERASE I TRANSCRIPTION INITIATION AND ELONGATION

cf. BIF FUTURA, VOL. 29 | 1.2014

SIMON NEYER

Discipline: Biochemist, MSc

Institute: Max Planck Institute of Biophysical

Chemistry, Göttingen, Germany

Supervisor: Prof. Patrick Cramer



RNA polymerase I (Pol I) synthesizes ribosomal RNA as the first step in eukaryotic ribosome biogenesis. During initiation, Pol I is recruited to the promoter by the transcription factors Rrn3 and core factor (CF). Well-defined initiation and accurate elongation are equally important for cell viability, but the mechanisms of both processes are poorly understood. In my PhD project, I determined the structure of elongating Pol I in *Saccharomyces cerevisiae* at 3.8 Å resolution using cryo-electron microscopy (cryo-EM). My findings suggest that the Pol I cleft state is coupled with activity: inactive Pol I has an expanded cleft, while binding an RNA-DNA hybrid contracts the cleft. To analyse Pol I on Miller spreads under *ex vivo* conditions, I collaborated with the laboratory of Achilleas Frangakis. We confirmed the contracted cleft state under near physiological conditions with cryo-electron tomography. I also reported a 3.4 Å resolution cryo-EM structure of initially transcribing Pol I bound to Rrn3 and CF. Using the crystal structures as guides, I generated a pseudo-atomic model of the initially transcribing complex. Other lab members obtained supporting results: Christoph Engel found a second CF-binding interface, which blocks the Pol I cleft for DNA loading, and Tobias Gubbey solved the CF structure. Our data show how upstream promoter DNA is bound by an interplay of Pol I and CF, which introduces a 30° bend in the DNA. Furthermore, I modelled a closed complex, which shows that DNA is loaded on Pol I in an expanded cleft state. For initial transcription, the cleft then contracts and allows RNA synthesis. We suggest that Pol I promoter recognition relies on DNA bendability and meltability rather than on sequence recognition. My work expands our mechanistic understanding of Pol I transcription for important regulatory steps, initiation, and elongation.

PUBLICATIONS

Engel C*, Gubbey T*, Neyer S*, Sainsbury S, Oberthuer C, Baejen C *et al* (2017) Structural basis of RNA polymerase I transcription initiation. *Cell* **169**: 120–131

Neyer S*, Kunz M*, Geiss C, Hantsche M, Hodirnau VV, Seybert A *et al* (2016) Structure of RNA polymerase I transcribing rDNA genes. *Nature* **540**: 607–610

Muehlbacher W*, Sainsbury S*, Hermann M, Hantsche M, Neyer S, Herzog F, Cramer P (2014) Conserved architecture of the core RNA polymerase II initiation complex. *Nat Commun* **5**: 4310

RNAI SCREEN FOR NOVEL REGULATORS OF MAMMALIAN MEIOSIS

cf. BIF FUTURA, VOL. 28 | 2.2013

MICHAŁ PASTERNAK

Discipline: Molecular Biologist, MSc

Institute: MRC Laboratory of Molecular Biology,

University of Cambridge, Cambridge, UK

Supervisor: Dr Melina Schuh



Oocytes develop into eggs through an important but highly error-prone cell division event known as meiosis. Defects in the egg – for example aneuploidy, which is characterized by an incorrect number of chromosomes – are a leading cause of pregnancy loss and several genetic disorders such as Down's syndrome. During my PhD, I investigated which genes control egg development and why human eggs are so frequently abnormal. Together with my colleagues, I performed the first high-content screen for genes regulating meiosis in mammals. We used RNA interference (RNAi) to target 774 genes that are highly expressed in the egg and followed the development of oocytes for 14–18 hours by high-resolution fluorescence microscopy. We then scored the recorded oocytes for 50 different phenotypes, including chromosome misalignment or lagging, to identify genes that prevent aneuploidy in the egg. In the second part of my PhD, I characterized one of these newly identified meiotic genes known as *Btg4*. During egg development, the oocyte undergoes two meiotic divisions and completes the second only after fertilization. We observed that *Btg4*-depleted oocytes underwent the two meiotic divisions without the presence of sperm, a phenomenon known as spontaneous egg activation. The second division was abnormal, with a high incidence of lagging chromosomes and fragmentation of the meiotic spindle. I showed that these defects arise from a perturbed translational reprogramming in the absence of the BTG4 protein. BTG4 is essential for a major mRNA decay event at the transition between the two meiotic divisions. When the function of *Btg4* is perturbed, it prevents the degradation of early-meiotic mRNAs, which saturates the translational machinery and prevents an efficient expression of late meiotic genes. My work explains why *Btg4*^{-/-} mice are infertile and points to *Btg4* as a potential target for the treatment of spontaneous egg activation. Together with the rest of the RNAi screen, these studies represent a milestone in understanding the causes of abnormalities in mammalian eggs.

PUBLICATIONS

Pasternak M, Pfender S, Santhanam B, Schuh M (2016) The BTG4 and CAF1 complex prevents the spontaneous activation of eggs by deadenylation of maternal mRNAs. *Open Biol* **6**: 160184

Pfender S*, Kuznetsov V*, Pasternak M*, Tischer T, Santhanam B, Schuh M (2015) Live imaging RNAi screen reveals genes essential for meiosis in mammalian oocytes. *Nature* **524**: 239–242

ANALYSIS OF THE HSP90 CO-CHAPERONE NETWORK DYNAMICS

cf. BIF FUTURA, VOL. 27 | 3.2012

PRIYANKA SAHASRABUDHE

Discipline: Biochemist, MSc

Institute: Technical University of Munich,
Munich, Germany

Supervisor: Prof. Johannes Buchner



Heat shock protein (Hsp) 90 and its interactors form one of the most important and complex chaperone networks in the cell. The functional and structural diversity of Hsp90's target proteins – called clients – and their roles as key effectors in the cell have made Hsp90 a key target for therapeutic intervention in cancer and other diseases. By binding to a select group of client proteins in their near-native states, Hsp90 assists in these proteins' proper folding and brings them into their active conformations – a process termed maturation. My PhD project aimed to elucidate how Hsp90 and its co-chaperones influence the maturation of different client proteins. In performing its chaperone function, Hsp90 undergoes large conformational changes coupled to ATP hydrolysis, during which it associates with a cohort of co-chaperones that modulate its activity. Although the interactions between some of these co-chaperones and Hsp90 have been well studied, a comprehensive understanding of how the Hsp90 machinery modulates such a diverse selection of client proteins is still lacking. Because Hsp90 is well conserved from yeast to human, I expressed human client proteins in yeast *Saccharomyces cerevisiae* to create a simplified cellular model. Using yeast allowed for testing the effects of the Hsp90 system on a specific human client without the influence of additional components of the client's downstream and upstream pathways. I first performed a systematic screen that examined how maturation and activation of client proteins was affected by the deletion of specific co-chaperones, finding that even within a single protein family, different clients required specific co-chaperones. I then conducted biochemical studies on several client-co-chaperone pairs to determine how co-chaperones influence client maturation. I found that a client protein's conformation could change in the absence of a specific co-chaperone, and that some co-chaperones competed with clients to bind to Hsp90, thereby reducing the time the client spends with Hsp90. Taken together, my work proposes that co-chaperones modulate the activity of Hsp90, and that these interactors customize its ability to chaperone diverse client proteins.

PUBLICATIONS

The results of this project have not yet been published.

LOCAL REGULATION OF DNA METHYLATION BY THE TRANSCRIPTION FACTOR REST

cf. BIF FUTURA, VOL. 27 | 3.2012

JULIANE SCHMIDT

Discipline: Biologist, MSc

Institute: Friedrich Miescher Institute for Biomedical
Research, Basel, Switzerland

Supervisor: Prof. Dirk Schübeler



DNA methylation is a chromatin modification that is associated with transcriptional silencing. Although mammalian DNA methylation patterns have been described at high resolution, their establishment remains poorly understood. Several DNA-binding factors have been suggested to locally reduce cytosine methylation. For example, the RE1-silencing transcription factor (REST) is necessary for establishing and maintaining hypomethylation but the underlying mechanism is not yet clear. To address this, we studied the molecular components involved in REST-associated hypomethylation in mouse embryonic stem cells (mESCs). We performed amplicon bisulphite sequencing in wild-type and *Rest*-knockout mESCs. As expected, REST-binding sites were hypomethylated in wild-type cells and became methylated in *Rest*-knockout cells. Next, we expressed full-length REST, its DNA-binding domain (DBD), and different truncated REST mutants in *Rest*-knockout cells. As the DBD is common to all these expressed proteins, we detected genomic binding to REST motifs in all chromatin immunoprecipitation sequencing (ChIP-seq) samples. By contrast, we found different degrees of hypomethylation at REST motifs for proteins that harboured protein-interaction domains. These also remodelled chromatin, leading to an absence of nucleosomes at the sites of binding. The DBD itself, however, was not sufficient to reduce local DNA methylation levels. Finally, we investigated whether active demethylation is contributing to the methylation state of REST-binding sites. We determined the methylation levels in mESCs deficient for all three TET proteins, which are involved in active DNA demethylation. These *Tet* triple-knockout cells showed hypermethylation that was restricted to the direct vicinity of the REST motif, suggesting a localized TET dependency. These results indicate that not all mammalian transcription factors are likely to affect the methylation levels of their binding sites. The ability to reduce local DNA methylation seems to be rather a consequence of cofactor recruitment and chromatin remodelling. Thus, the mammalian transcription factor repertoire is functionally diverse with respect to its interactions with DNA methylation.

PUBLICATIONS

Baubec T, Colombo DE, Wirbelauer C, Schmidt J, Burger L, Krebs AR *et al* (2015) Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. *Nature* 520: 243–247

RAPID ROTATION OF THE RIBOSOMAL SUBUNITS

cf. BIF FUTURA, VOL. 28 | 2.2013

HEENA SHARMA

Discipline: Biochemist, MSc

Institute: Max Planck Institute for Biophysical

Chemistry, Göttingen, Germany

Supervisor: Prof. Marina V. Rodnina



The ribosome is a large molecular machine that ubiquitously synthesizes proteins in all cells. During protein synthesis, the ribosome moves along the mRNA one codon at a time, and the two transfer RNAs (tRNAs) that are bound at the aminoacyl (A) and peptidyl (P) sites move to the P and exit sites, respectively – a process promoted by elongation factor G (EF-G). Large-scale conformational changes of the ribosome orchestrate all stages of protein synthesis. The rotation of the small subunit (SSU) of the ribosome relative to the large subunit is important for translocation of the tRNA-mRNA complex but was poorly understood. In my PhD project, I monitored real-time kinetics of subunit rotation using Förster resonance energy transfer (FRET) of ribosomes. The counterclockwise (CCW) rotation of SSU decreased the FRET signal, whereas clockwise rotation increased the FRET signal. In principle, the peptidyl transfer reaction can drive spontaneous CCW rotation. This prompted me to measure the kinetics of spontaneous rotation with different tRNAs in either the P or the A site. I prepared ribosome complexes with P-site tRNAs and initiated peptide bond formation with A-site tRNA. Using global kinetic analysis, I found that the SSU rotated spontaneously about 40 times per second, independently of which tRNAs were tested. I also showed that during EF-G-promoted translocation, CCW subunit rotation accelerates to 200 times per second – one of the fastest events on the translocation pathway. My work demonstrates that although the ribosome is a supramolecular assembly, its large-scale conformational changes are thermally driven, intrinsically rapid, and governed by its ligands. Understanding ribosome dynamics will improve our knowledge of protein synthesis, which could aid the design of new antibiotics against the bacterial ribosome.

PUBLICATIONS

Sharma H, Adio S, Senyushkina T, Belardinelli R, Peske F, Rodnina MV (2016) Kinetics of spontaneous and EF-G-accelerated rotation of ribosomal subunits. *Cell Rep* 16: 2187–2196

Belardinelli R, Sharma H, Caliskan N, Cunha CE, Peske F, Wintermeyer W, Rodnina MV (2016) Choreography of molecular movements during ribosome progression along mRNA. *Nat Struct Mol Biol* 23: 342–348

Belardinelli R, Sharma H, Peske F, Wintermeyer W, Rodnina MV (2016) Translocation as continuous movement through the ribosome. *RNA Biol* 13: 1197–1200

ZYGOTIC GENOME ACTIVATION AT SINGLE-MOLECULE RESOLUTION

cf. BIF FUTURA, VOL. 28 | 2.2013

CARINE STAPEL

Discipline: Molecular Biologist, MSc

Institute: Max Planck Institute of Molecular Cell Biology

and Genetics (MPI-CBG), Dresden, Germany

Supervisor: Dr Nadine Vastenhouw



The development of many metazoans relies on maternally deposited proteins and RNAs until the zygotic genome is activated. In zebrafish embryos, transcription is first activated about 3 hours post-fertilization, when the embryo already consists of a thousand cells. RNA-sequencing studies have revealed when specific genes are activated, but little is known about the coordination of transcription activation between cells and how gene expression patterns that are important for embryonic development are established. To address this question, I developed a protocol for single-molecule fluorescence *in situ* hybridization (smFISH) on cryosections of zebrafish embryos as well as an analysis pipeline that combines automated transcript detection with cell segmentation. With this approach, I can detect single transcripts at subcellular resolution. I measured differences in transcript levels between cells at 5-minute intervals from the onset of transcription to the onset of gastrulation. I analysed eight different genes and found that for each of these, it takes more than one cell cycle until transcription is activated in all cells in their expression domain. I was able to show that this stochastic activation is a result of cell-cycle asynchrony between cells as well as random interactions of the transcription machinery at the promoter. Stochastic transcription activation initially leads to large differences in transcript levels between cells. However, transcript levels are low at this time and therefore these cell-to-cell differences might not have a major effect on cellular function. Over time, transcript levels strongly increase because degradation rates are low and multiple transcription events occur in each cell. This accumulation of transcription events leads to a decrease in transcript level variability between cells. Thus, precise expression patterns are established in zebrafish embryos, even though genes are stochastically activated. We hypothesize that a similar process occurs in other species to produce precise gene expression during early development.

PUBLICATIONS

Stapel LC, Lombardot B, Broadus C, Kainmueller D, Jug F, Myers EW *et al* (2016) Automated detection and quantification of single RNAs at cellular resolution in zebrafish embryos. *Development* 143: 540–546

Stapel LC, Vastenhouw NL (2014) Message control in developmental transitions; deciphering chromatin's role using zebrafish genomics. *Brief Funct Genomics* 13: 106–120

SPATIOTEMPORAL ANALYSIS OF GLUTATHIONE OXIDATION IN MURINE MACROPHAGES

cf. BIF FUTURA, VOL. 27 | 1.2012

SEBASTIAN VIRREIRA WINTER

Discipline: Immunologist, Diploma

Institute: Max Planck Institute for Infection Biology,

Berlin, Germany

Supervisor: Prof. Arturo Zychlinsky



Reactive oxygen and nitrogen species (ROS and RNS) have reputations as toxic molecules, as mammals use them to kill microbes. However, emerging data suggest that ROS and RNS are also second messengers that modulate intracellular signalling via redox modifications of cysteines. In my PhD project, I investigated how redox modifications regulate signalling by focusing on innate immunity. I began by analysing the dynamics and subcellular localization of ROS/RNS-dependent redox changes during activation of various immune receptors in phagocytes. I established an *in vitro* system to detect glutathione oxidation and pH changes with subcellular resolution in murine macrophages, using the protein-based sensors Grx1-roGFP2 (a fusion of glutaredoxin-1 and redox-sensitive green fluorescent protein) and SypHer, respectively. Using this system, I investigated redox changes during NLRP3 inflammasome activation and Toll-like receptor (TLR) signalling. ROS and RNS have been reported to contribute to the activation of the NLRP3 inflammasome. However, my high-resolution live-cell imaging revealed that glutathione was oxidized only after pyroptosis, an inflammasome-dependent type of cell death, suggesting that significant redox changes occur downstream of NLRP3 activation. In contrast, activation of most TLRs transiently oxidized mitochondrial glutathione. This oxidation coincided with a temporary increase in the pH of the cytosol and mitochondria, which predisposes cysteines to redox modifications. To study the regulatory potential of cysteine-based redox modifications in another setting, I generated glutathione reductase (Gsr)-deficient mice, which had a higher ratio of reduced to oxidized glutathione (GSH:GSSG) but developed normally. Although NLRP3 is inhibited in macrophages with an increased superoxide content and GSH:GSSG ratio when superoxide dismutase 1 (SOD1) is mutated, macrophages in Gsr-deficient mice released normal amounts of the pro-inflammatory cytokine IL-1 β upon NLRP3 activation. However, Gsr-deficient mice showed impaired immune cell recruitment into the peritoneal cavity during sterile inflammation. In addition to suggesting that ROS and RNS are most likely not the common trigger of NLRP3 activation, my work emphasizes that physiological redox changes may be highly compartmentalized and dynamic.

PUBLICATIONS

The results of this project have not yet been published.

ANALYSIS OF THE LYSOSOMAL TRANSPORT RECEPTOR LIMP-2 AND ITS ROLE IN PARKINSON'S DISEASE

cf. BIF FUTURA, VOL. 28 | 1.2013

FRIEDERIKE ZUNKE

Discipline: Biochemist, MRes

Institute: Institute of Biochemistry,

University of Kiel, Kiel, Germany

Supervisor: Prof. Paul Saftig



Most lysosomal enzymes are targeted to lysosomes through the mannose-6-phosphate receptor pathway. However, the lysosomal hydrolase β -glucocerebrosidase (GBA1) is transported to lysosomes by the lysosomal integral membrane protein type 2 (LIMP-2). Mutated *GBA1* causes Gaucher disease, a lysosomal storage disorder. *GBA1* mutations also represent the highest genetic risk factor for Parkinson's disease (PD). The first goal of my PhD project was to uncover the molecular details of the interaction between LIMP-2 and GBA1. Using site-directed mutagenesis, I was able to delineate the structural characteristics of this unique interaction. Furthermore, my cell-based and *in vitro* assays suggested that one function of LIMP-2 is to stabilize the GBA1 protein. This discovery enabled me to design a small LIMP-2-derived peptide that increased lysosomal GBA1 activity. My second goal was to address the role of LIMP-2 in the regulation of the cellular homeostasis of α -synuclein, the accumulating and neurotoxic protein in PD. Using *in vivo* and cell culture studies, I found reciprocal relationships between LIMP-2, GBA1 activity, and α -synuclein levels. LIMP-2 deficiency in mice caused α -synuclein accumulation in the brain, which was accompanied by neurological impairments. Increasing LIMP-2 expression and GBA1 activity (e.g. by the LIMP-2-derived peptide) led to a reduction of α -synuclein levels and could be a promising method for the treatment of Gaucher disease and PD.

PUBLICATIONS

Zunke F, Andresen L, Wessler S, Groth J, Arnold P, Rothaug M *et al* (2016) Characterization of the complex formed by beta-glucocerebrosidase and the lysosomal integral membrane protein type-2. *Proc Natl Acad Sci USA* **113**: 3791–3796

Rothaug M*, Zunke F*, Mazzulli JR, Schweizer M, Altmepfen H, Lullmann-Rauch R *et al* (2014) LIMP-2 expression is critical for beta-glucocerebrosidase activity and alpha-synuclein clearance. *Proc Natl Acad Sci USA* **111**: 15573–15578

Neculai D, Schwake M, Ravichandran M, Zunke F, Collins RF, Peters J *et al* (2013) Structure of LIMP-2 provides functional insights with implications for SR-BI and CD36. *Nature* **504**: 172–176

MD FELLOWS 2017
 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 their workplace (institution and city) for at least ten months to join an internationally renowned laboratory. Here, we present the nine fellows who were granted an MD fellowship in 2017.

MAXIMILIAN BÖHM

Secondary bleeding during intracerebral haemorrhage as a possible mechanism for haematoma expansion

MARTIN DANIELS

Assessing the nucleus reticularis gigantocellularis and its governing role in generalized arousal of the CNS

KATJA DINKELBORG

Targeting translation of HIF2 α for treatment of renal cell carcinoma

RICARDO GRIESHABER BOUYER RODRIGUES

The role of CD177 in neutrophil biology

CLARA GROSSMANN

Determining mechanisms regulating B-cell tolerance which may be defective in patients with autoimmune diseases

KEVIN ALEXANDER KOCH

Signalling of specific oncogene mutations influences progression and malignancy of pancreatic ductal adenocarcinoma

MARCEL RAUER

The contribution of lysyl oxidase-like 2 to systemic vascular stiffening

SIMON RUFFING

Investigating the role of tumor endothelial cells in metastasis formation

SYLVIA VORECK

The role of direct macrophage-epithelial cell contact in the promotion of tubule repair after kidney injury

SECONDARY BLEEDING DURING INTRACEREBRAL HAEMORRHAGE AS A POSSIBLE MECHANISM FOR HAEMATOMA EXPANSION



MAXIMILIAN BÖHM

Duration: 08/16–08/17

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

Supervisor: Prof. Cenk Ayata, MD

Home University: Charité – Universitätsmedizin Berlin

ASSESSING THE NUCLEUS RETICULARIS GIGANTOCELLULARIS AND ITS GOVERNING ROLE IN GENERALIZED AROUSAL OF THE CNS



MARTIN DANIELS

Duration: 11/16–10/17

Project at: The Rockefeller University, New York, NY, USA

Supervisor: Prof. Donald W. Pfaff

Home University: Charité – Universitätsmedizin Berlin

TARGETING TRANSLATION OF HIF2A FOR TREATMENT OF RENAL CELL CARCINOMA



KATJA DINKELBORG

Duration: 08/16–07/17

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

Supervisor: Prof. Othon Iliopoulos, MD, PhD

Home University: University Medical Center Göttingen

THE ROLE OF CD177 IN NEUTROPHIL BIOLOGY



RICARDO GRIESHABER BOUYER RODRIGUES

Duration: 09/16–08/17

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

Supervisor: Prof. Peter A. Nigrovic, MD

Home University: Heidelberg University Hospital

DETERMINING MECHANISMS REGULATING B-CELL TOLERANCE WHICH MAY BE DEFECTIVE IN PATIENTS WITH AUTOIMMUNE DISEASES



CLARA GROSSMANN

Duration: 11/16–09/17

Project at: Yale University, Immunobiology Department, New Haven, CT, USA

Supervisor: Prof. Eric Meffre, PhD

Home University: University of Münster

SIGNALLING OF SPECIFIC ONCOGENE MUTATIONS INFLUENCES PROGRESSION AND MALIGNANCY OF PANCREATIC DUCTAL ADENOCARCINOMA



KEVIN ALEXANDER KOCH

Duration: 08/16–07/17

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

Supervisor: Prof. Dan Duda, DMD, PhD

Home University: Münster University Hospital

THE CONTRIBUTION OF LYSYL OXIDASE-LIKE 2 TO SYSTEMIC VASCULAR STIFFENING



MARCEL RAUHER

Duration: 08/16–08/17

Project at: The Johns Hopkins University School of Medicine, Department of Anesthesiology and Critical Care Medicine, Baltimore, MD, USA

Supervisor: Prof. Jochen Steppan, MD

Home University: Münster University Hospital

INVESTIGATING THE ROLE OF TUMOR ENDOTHELIAL CELLS IN METASTASIS FORMATION



SIMON RUFFING

Duration: 09/16–08/17

Project at: The Rockefeller University, Laboratory of Systems Cancer Biology, New York, NY, USA

Supervisor: Prof. Sohail Tavazoie, MD, PhD

Home University: University of Münster

THE ROLE OF DIRECT MACROPHAGE-EPITHELIAL CELL CONTACT IN THE PROMOTION OF TUBULE REPAIR AFTER KIDNEY INJURY



SYLVIA VORECK

Duration: 04/16–01/17

Project at: Yale University, School of Medicine, New Haven, CT, USA

Supervisor: Prof. Lloyd G. Cantley, MD

Home University: Universitätsklinikum Erlangen

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.

THE YEAR 2016 AT BIF

Facts and figures. 54

PAPERS IN THE SPOTLIGHT

Papers by Susanne Falkner, Michael Hauer, and Christian Schmidt. 56

PERSPECTIVES

How to become a medical writer: Dr Julia Frojanic-Klapproth. 58

PROFILES

Awards and more. 59

A BIF FELLOW'S GUIDE TO ... SZEGED

BIF fellow Akos Nyerges presents Szeged, Hungary's sunniest city. 60

PAY-OFF FOR TENACITY AND OUT-OF-THE-BOX-THINKING

How BIF alumni Peter Kohl proved a hypothesis he formulated 25 years ago. 61

UPCOMING EVENTS

Dates and locations. 61

THE YEAR 2016 AT BIF

ITC INTERNATIONAL TITISEE CONFERENCES

for leading scientists



No. **113**: Building Tools for Quantifying Brain and Behaviour

No. **114**: Molecules and Mechanisms in Magneto-, Thermo-, and Mechanosensation

120
participants



86
scientific talks



476
litres of water



BIF NETWORK

207+
professors
around **90**
principal investigators

55
nationalities

around **1,400**
fellows from the PhD, MD, and (until 1995) postdoc programmes

PHD FELLOWSHIPS:



50
approved



The ratio of female to male fellowship holders approved in the last five years is 52:48.

627
applicants

In 2016

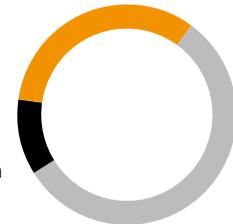
635
applicants

Average 2012–2016

110 current fellows

Their nationalities are:

33%
German
11%
non-European



56%
other European countries

They work in:

29%
Germany
16%
other



20%
UK
8%
Switzerland
27%
USA

90%
of fellows stay
in touch with
BIF

around
390
face-to-face contacts
between BIF staff
and fellows

92
travel allowances
for
fellows

9
meetings and
seminars

MD FELLOWS 2006 to 2016



86
funded



Fellows went to:

- 62** USA
- 15** Europe (without Germany)
- 4** Germany
- 5** other countries

TRAVEL GRANTS



146
approved



They went to
16
countries

The ratio of female to male travel grant recipients over the last five years is 67:33.



28% Germany

28% USA



23% others



11% UK



10% France



273
applicants



56
nationalities

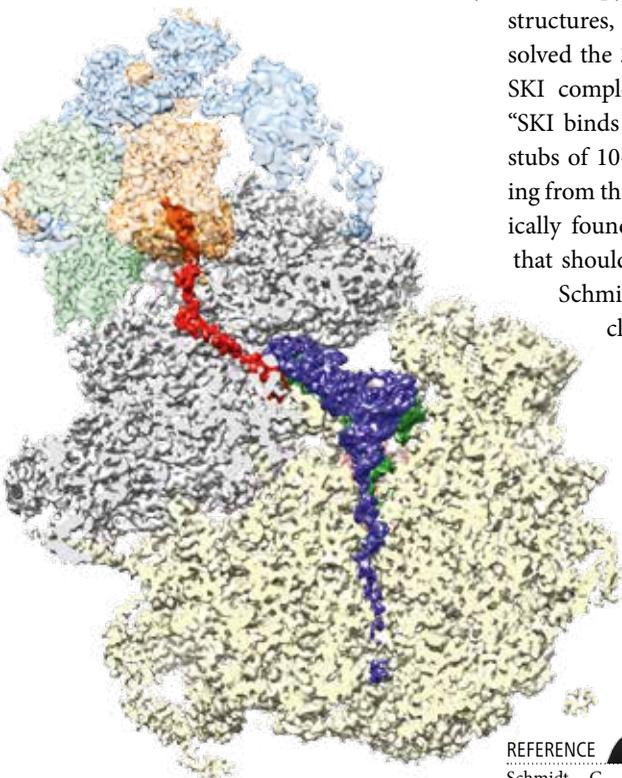
PAPERS IN THE SPOTLIGHT

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.

SKI LINKS RIBOSOME TO EXOSOME

It's textbook knowledge: proteins are built by ribosomes from their blueprints, the mRNAs. And as a part of housekeeping, a cell needs to shred blueprints that are faulty or have reached the end of their life cycle. This process can start either at the front, the 5' end, or the tail, the 3' end, of the mRNA. Overturning previous models, Christian Schmidt and Katharina Braunger from Roland Beckmann's group at the University of Munich (LMU) found the first evidence that the ribosome is directly

involved in the so-called 3'-5' decay of both faulty and expired mRNAs through the SKI complex (SKI). Already in 2013, BIF fellow Felix Halbach showed that SKI unwinds RNAs and feeds them – 3' end first – directly into the cell's RNA shredder, the exosome. Christian now showed that the ribosome binds directly to SKIs opposite side, triggering a shape shift in ribosome and SKI, thereby activating SKI's unwinding activity. Using cryo-electron microscopy and making use of existing structures, the team around Christian resolved the 3D structure of the ribosome-SKI complex at near-atomic resolution. “SKI binds almost instantly to 3' mRNA stubs of 10–20 nucleotide length projecting from the ribosome. Such stubs are typically found on ribosomes with mRNAs that should be degraded,” says Christian Schmidt. “We started out wanting to clarify how faulty mRNAs are degraded from their 3' end and ended up finding a physical link between translation and degradation which we think is involved in all 3'-5' mRNA degradation.”



REFERENCE

Schmidt C, Kowalinski E, Shanmuganathan V, Defenouillère Q, Braunger K, Heuer A *et al* (2016) The cryo-EM structure of a ribosome-Ski2-Ski3-Ski8 helicase complex. *Science* 354: 1431–1433

Christian Schmidt, fellowship 2014–2016

Katharina Braunger, fellowship 2015–2017

Cryo-electron microscopy resolved the ribosome-SKI complex to near atomic resolution.



PROTEASOME UNCHAINS

Imagine a library book with a page torn out. To restore it, you need to find a duplicate of the book and copy the missing information. Difficult to do if the books are chained to their shelves. The nucleosome around which the cell's DNA is wrapped act like such chains: they control access to it and keep it in place. Thus, they also hinder repair, especially of double strand breaks by homologous recombination, which matches damaged DNA with another copy. We already knew that damaged DNA is more mobile, making it more accessible and enabling it to search the whole nucleus for a copy. Michael Hauer from the laboratory of Susan Gasser at the Friedrich Miescher Institute for Biomedical Research in Basel, Switzerland, has recently discovered the mechanism: DNA damage triggers the proteasome to degrade, across the genome, up to 30% of all nucleosome building blocks, the core histones. This allows the DNA to uncoil from the histones and roam through the nucleus. Michael also showed that these effects depend on the DNA damage checkpoint, which senses damage, as well as the

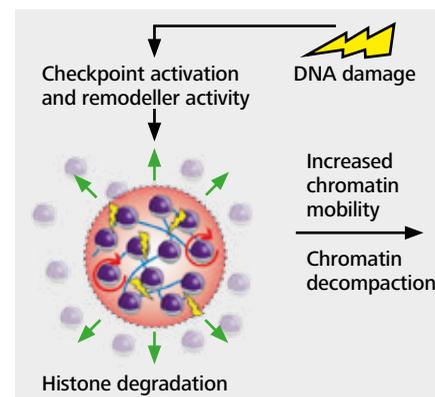


Photo: Christian Schmidt

DNA FOR REPAIR

chromatin remodelling complex INO80-C, which releases the DNA from the histones. “When we tricked undamaged cells into reducing histone levels, we got the same effect. It also increased recombination efficiency – probably through rendering the genome more accessible,” says Michael. This might be interesting for gene editing with CRISPR/Cas9: “In contrast to mammalian cells, yeast cells repair almost all double strand breaks using homologous recombination, making it easy to modify their genes. Decreasing histone levels and thus making DNA more accessible in mammalian cells might increase homologous recombination, needed for gene editing with CRISPR/Cas9.”

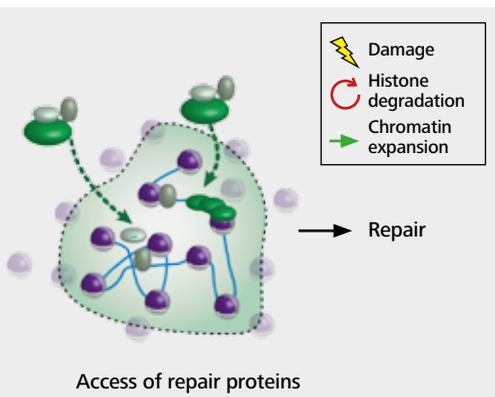


REFERENCE

Hauer MH, Seeber A, Singh V, Thierry R, Sack R, Amitai A *et al* (2017) Histone degradation in response to DNA damage enhances chromatin dynamics and recombination rates. *Nat. Struct. Mol. Biol.* **24**: 99–107

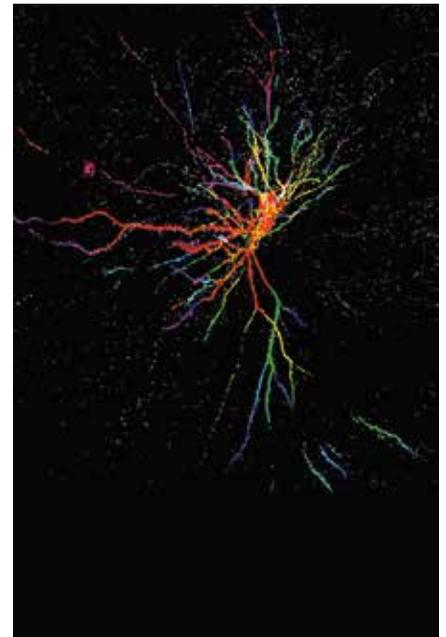
Michael Hauer, fellowship 2014–2015

Photos: Michael Hauer (bottom), Susanne Falkner (top right)



TRANSPLANTING BRAIN CELLS

If an adult mammal brain loses cells – for instance due to trauma, stroke, or diseases like Alzheimer’s – it has a very limited capacity to replace them. Thanks to Susanne Falkner from the department of Tobias Bonhoeffer at the Max Planck Institute for Neurobiology in Munich, we now know that transplanted embryonic neurons can functionally integrate into an existing brain – at least in mice. Susanne damaged a region of the visual cortex in these mammals and then injected nerve cells taken from the cortex of late-stage mice embryos. Using two-photon microscopy, she followed three approaches to analyse the development, connection, and function of individual transplanted cells. Each approach included several tens of cells, some of which she followed up to 11 months. “To collect this kind of data, we had to combine the expertise of Magdalena Götz’s group at the Helmholtz Center in Munich on how embryonic neurons develop and that of our own group on the structure and function of the visual cortex,” Susanne says. As it turned out, the transplanted cells developed like normal neurons and underwent the same kind of pruning. Analysing slices of the whole brain, the team also showed that the new cells established connections with the correct brain areas in similar numbers. “We think they may have followed gaps left by deceased cells or reacted either to growth factors produced by neurons that were bereft of their connections, or to developmental factors still present in the adult brain.” After a few weeks, the transplanted cells



Two mature, integrated donor neurons 45 days after transplantation, view from top.

had also learned to tune their response to the right kind of stimuli. This means that the brain can fully integrate new cells, raising hope that it may one day be possible to replace lost brain cells.



REFERENCE

Falkner S*, Grade S*, Dimou L, Conzelmann KK, Bonhoeffer T, Götz M, Hübener M (2016) Transplanted embryonic neurons integrate into adult neocortical circuits. *Nature* **539**: 248–253

Susanne Falkner, fellowship 2010–2012

Shared first authorship is denoted with an *.

PERSPECTIVES

FROM NEUROBIOLOGIST TO MEDICAL WRITER

In this section, we introduce BIF alumni from various scientific backgrounds and professional contexts to give you an overview of possible career options.

INTERVIEW WITH DR JULIA FROJANIC-KLAPPROTH, SENIOR PARTNER AT TRILOGY WRITING & CONSULTING GMBH



Julia, a Canadian native from Vancouver, always loved the part many other scientists struggle with – writing up the results. In 1997, while finishing her PhD thesis on the molecular genetics of neurons in fruit flies at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, she saw an ad for a medical writer and was instantly intrigued. For five years, she learned the craft at what was then Hoechst's in-house medical writing group. In 2002, she co-founded Trilogy Writing & Consulting, a Frankfurt-based company for medical writing.

What does a medical writer do?

Medical writing has two main areas: regulatory writing and medical communications. I do the first kind, writing texts for regulatory purposes which will be read and reviewed by other scientists and doctors at the regulatory authorities (e.g. EMEA or FDA) to assess if a medicine should be allowed on the market. As a regulatory writer you need to understand what these assessors are looking for and speak to them about the detailed science. Medical communications is writing about the products

for public use. This includes scientific manuscripts, but also texts directed at the general public, everything from educational and marketing material to websites.

Which skills from your scientific career are most helpful to you?

The ability to dive into mountains of data and decide what the messages are. The experience I bring as a scientist to reading and interpreting these data gives me peer status in the teams I work with. They include key opinion leaders, clinicians, statisticians, CEOs of pharmaceutical companies, and regulatory specialists. You need confidence to hold your own in these teams. You also need excellent interpersonal skills to ease tension, resolve differences of opinion, and deal with conflicts of priority often exacerbated by intercultural differences and tight timelines.

How does a medical writer differ from a scientific editor or a science journalist?

A medical writer is given thousands of pages of data analyses and has to communicate the story of those data to the regulatory agencies. He or she writes texts from scratch, working closely with the specialists. A scientific editor organizes the peer review process for a paper and/or improves the language and flow of thought in a text someone else has written. And a journalist is there to find stories and to write these up for the public.

What do you most enjoy about your work?

The constant intellectual challenge and the huge variety. I feel that I get to use my brain much more now on a daily basis than I did as a researcher. I have all the fun of working with the data, interacting with other scien-

tists, and finding the story without the drudgery of data collection and the some long hours in the laboratory.

What would you recommend to fellows interested in the field?

Try and get a job at a pharmaceutical company – they invest in the necessary training. It takes about five years to really come into your own as a medical writer. You spend the first year just learning about the world of clinical development with all its acronyms! The next three to four years are spent understanding how to write the different document types such as study protocols and reports, investigator brochures, etc. A single document takes about six to ten months to write. Other employers are clinical research organizations or other small writing companies. Find out how much they invest in training and do not accept a sink-or-swim approach. At Trilogy Writing, we hire about 50% of our writers straight out of university and invest heavily in training.

When you come home, do you still write?

I am actually a published poet! I love to play with nuances of meaning in language through poems.

What is it like running your own company?

I think either you are an entrepreneur by nature or you are not. Some people cringe at the thought of having to constantly ensure there is a flow of work and to negotiate contracts with a room full of lawyers. Others thrive on the thrill of it all. That said, many people don't know that they might enjoy being an entrepreneur. My advice to everyone is to try things and find out.

PROFILES

Professor Ulrich auf dem Keller

Technical University of Denmark (DTU), Copenhagen, Denmark
Fellowship: 2001–2003



Ulrich auf dem Keller has been granted a Young Investigator Award by the Novo Nordisk Foundation, which entails his move to the Department of Biotechnology and Biomedicine at the Technical University of Denmark as associate professor. The seven-year grant of 20 million Danish kroner will enable Ulrich to employ extensive technologies to examine the network of proteases in normal, inflamed, and damaged skin. The grants go to independent early- to mid-career researchers outside Denmark who are ready to conduct more ambitious studies. The goal is to help these researchers establish a laboratory in Denmark.

Professor Ivan Dikic

Goethe University Frankfurt, Germany
Postdoctoral fellow: 1997



Professor Rainer Friedrich

Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland
Fellowship: 1995–1997



Two BIF fellows have been awarded a prestigious ERC Advanced Grant of up to 3.5 million euros each. The project of alumnus Ivan Dikic is entitled “Dissecting and Targeting Ubiquitin Networks in the Course of Bacterial Infections”. Rainer Friedrich’s study is called “Connectivity, Plasticity and Function of an Olfactory Memory Circuit”. In the latest round, the ERC awarded 231 grants worth 540 million euros which, according to its website, “will not only allow top research-

ers to realize their best ideas at the scientific frontiers but will also lead to job creation as an estimated 2,000 postdocs, PhD students, and other staff can be employed in the grantees’ research teams”.

Professor Volker Haucke

Leibniz Institute for Molecular Pharmacology (FMP), Berlin, Germany
Fellowship: 2011–2013



Volker Haucke has been granted a Reinhard Koselleck Project worth 0.9 million euros over five years by the German Research Foundation (DFG) to unravel the mechanisms of quality control that allow nerve cells and the synapses formed between them to function properly. Since 2008, this programme has enabled outstanding researchers with a proven scientific track record to pursue exceptionally innovative, higher-risk projects. He has also been awarded the 2017 Avanti Award in Lipids from the American Society for Biochemistry and Molecular Biology for defining the roles of membrane lipid homeostasis in cellular transport. His findings were “of key importance for cell physiology and pathophysiology”, according to the nomination.

Professor Michael Krieg

Institute of Photonic Sciences, Barcelona, Spain
Fellowship: 2006–2008



Michael Krieg has been appointed group leader and ICFO professor at the Institute of Photonic Sciences in Barcelona. His group “Neurophotonics And Mechanical Systems Biology” will develop and deploy new optogenetic tools (FRET, synthetic biology, and genetic code expansion) to measure piconewton forces and their consequences inside cells. With these tools, they want to bet-

ter understand the importance of cell mechanical properties for health and disease on the molecular and systems level. He has also secured an ERC Starting Grant for his project “How to Build a Brain? Engineering Molecular Systems for Mechanosensation and -protection in Neurons”.

Professor Alexander Meissner

Max Planck Institute for Molecular Genetics, Berlin, Germany
Fellowship: 2003–2005



Alexander Meissner has been selected as director of the Max Planck Institute for Molecular Genetics in Berlin, Germany, and scientific member of the Max Planck Society. In June, he moved from the Broad Institute in Cambridge, MA, USA, to head the Department of Genome Regulation. His research focuses on developmental and stem cell biology with a particular interest in the role of epigenetic regulation.

Marion Silies

University Medical Center Göttingen, Germany
Fellowship: 2006–2008



Marion Silies has been awarded the most important award for early career researchers in Germany, the Heinz Maier Leibnitz Award, for her outstanding achievements. The recipients of the 20,000 euro prize are chosen by a selection committee in Bonn appointed by the DFG and the Federal Ministry of Education and Research. This year’s ten prizewinners were presented with the prize money on 3 May in Berlin, followed by a celebration of the 40th anniversary of the award.

A BIF FELLOW'S GUIDE TO ...

SZEGED



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 Ákos Nyerges. He reports from Szeged, Hungary, the country's sunniest city with around 2,000 hours of sunshine a year.

FACTS & FIGURES

Country: Hungary

Population: Approximately 162,000

Area: 280.9 km²

Students: About 20,000

Famous for: Sunshine, slippers, paprika, Pick salami, and fisherman's soup.

Website: www.szegedvaros.hu

WHERE TO STAY

Science Hotel: This new design hotel reflects Szeged's vibrant science life and is awaiting guests with a science-themed bar.

Hotel Tiszavirág: Wonderfully renovated historical townhouse, famous for its fine-dining restaurant with regional cuisine.

Hotel Forrás: Family and traveller-centric hotel on the riverside.

NIGHTLIFE

Maláta Pub: Craft beer and grill garden just a step away from Dóm square.

Nyugi, Campus, and Pivo Pub: Young milieu right in front of Szeged University's Klebelsberg Library, ranked as one of the "most majestic libraries in the world".

Kárász Street: Heart of Szeged with restaurants, bars, and music clubs. **1**

RESTAURANTS

Kiskörösy Fish Restaurant: Hungarian cuisine at its best! Here you can enjoy the rightly famous fisherman's soup with a picturesque view of Tisza and the riverside.

Bistorant: This bistro-style, centrally-located restaurant has a youthful atmosphere and combines tradition and modernity.

ACTIVITIES

Open-air theatre: Szeged's open-air theatre has been the region's main cultural event, attracting more than 75,000 attendees every year.

Wine festival **4:** Held twice a year, the festival invites you to taste the quintessence of Hungarian wine and cuisine.

Tisza riverside: From spring to late autumn, Tisza provides a hub for outdoor activities, for example, listening to the concerts that are held on many bar ships.

BEST SIGHTS

Dóm Square and the Dóm: The Dóm church, built after a flood destroyed most of Szeged, has been the proud symbol of the town since 1930. **3**

Old Bridge: Admire the view over the Great Plain and the art nouveau architecture of the riverside buildings.

St. Stephen's Square: Enjoy the best view of Szeged from the balcony of the square's century-old water tower, dubbed the "Old Lady". **2**

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

Name Ákos Nyerges
Nationality Hungarian
Age 27
University Biological Research Centre
of the Hungarian Academy of Sciences
Supervisor Csaba Pal, PhD



Ákos Nyerges

PAY-OFF FOR TENACITY AND OUT-OF-THE-BOX-THINKING

Twenty-five years ago, Peter Kohl took up a BIF postdoc fellowship at the University of Oxford, UK, to test his theory that non-muscle cells in the heart can conduct electricity from one muscle cell to another. This theory went against current belief but could explain unorthodox results he and other researchers had seen. The BIF board judged the proposal to be highly original, fascinating, and quite likely to fail. They took the risk because – if correct – it could change canonical thinking and potentially enable new therapies. Last November, Peter proudly sent BIF the published paper in which he and his team confirmed the original hypothesis – using techniques that were not available back then. “We used a protein that changes its fluorescence when exposed to electricity,” explains Peter,

“and selectively introduced it into either muscle or non-muscle cells of mouse hearts.” The results showed clearly that in the border zone of heart scars non-muscle cells can electrically couple to muscle cells. This coupling may involve so-called connexins, ion channels that link neighbouring cells. Electron tomography further showed nanotubes between the different cell types in the scar, which had been implicated in conductivity *in vitro*, but are very hard to detect *in vivo*, due to their tiny size. “Scar tissue is made up mostly of electrically passive non-muscle cells. Therefore, it is seen as something that stops electrical conduction in heart tissue – but that is not the whole story.” Thus, atrial fibrillation, caused by runaway electrical signals, is treated by creating scar lines to interrupt aberrant

electrical pathways and establish a regular beat. In roughly two-thirds of patients, the effect of the operation wears off within weeks or months. One of the reasons might be that non-muscle cells passively conduct the electricity across the new scar. “If we could influence this process, we might learn to make better scars to treat atrial fibrilla-



Cardiac myocyte, surrounded by non-myocytes in the heart.

tion, or to reduce the negative effect of heart attack scars. A key may be to control electrical connections between non-muscle cells and surviving muscle cells within the scar tissue,” says Peter. He goes on: “I am most grateful to the board for funding what seemed a crazy idea at the time, and I am very happy to have turned in my ‘homework’ before returning to Germany.” Last July, Peter left his chair at the Imperial College London to set up a new cardiovascular research institute at the University of Freiburg, Germany.

REFERENCE

Quinn TA, Camelliti P, Rog-Zielinska EA, Siedlecka U, Poggioli T, O’Toole ET, Knöpfel T, Kohl P (2016) Electrotonic coupling of excitable and nonexcitable cells in the heart revealed by optogenetics. *PNAS* 113: 14852–14857

UPCOMING EVENTS

26 AUGUST–1 SEPTEMBER 2017

Seminar for current PhD fellows working in Europe in Hirscheegg, Austria

On the agenda: project presentations of all participants, discussion of career topics, and guided hiking tours in the surrounding Alps. Further details with the invitation.

25–29 OCTOBER 2017

116th International Titisee Conference

“From Pathogen Evolution to Microbiome Dynamics” is the title of the 116th ITC at Lake Titisee, Germany. It will be chaired by Roy Kishony, Technion – Israel Institute of Technology, Haifa, Israel, and David A. Relman, Stanford University Medical Center, Stanford, CA, USA. This conference will bring together scientists from diverse fields, including synthetic biologists building multi-species communities, evolutionary biologists exploring the micro-evolution of individual species, and ecologists and computational biologists analysing the dynamics of multi-species communities. It hopes to identify new opportunities for major advances, and generate synergistic interactions between fields and investigators.

Participation is by invitation only.

4–5 NOVEMBER 2017

Meeting of BIF’s Board of Trustees

The trustees will decide upon the allocation of fellowships, review the proposals for the International Titisee Conferences, and settle all the Foundation’s matters of fundamental importance.

Need an update on upcoming events?

Check our website at www.bifonds.de



Boehringer Ingelheim Fonds
Stiftung für medizinische
Grundlagenforschung

Schusterstr. 46–48
55116 Mainz
Germany
Tel. +49 6131 27508-0
Fax +49 6131 27508-11
E-mail: secretariat@bifonds.de
www.bifonds.de

ISSN 0179-6372