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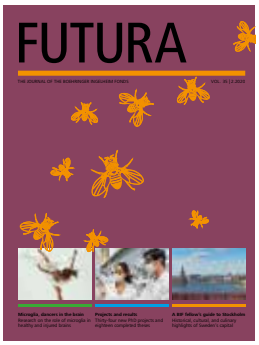
Microglia, dancers in the brain
Research on the role of microglia in healthy and injured brains

Projects and results
Thirty-four new PhD projects and eighteen completed theses

A BIF fellow's guide to Stockholm
Historical, cultural, and culinary highlights of Sweden's capital



The cover illustration shows a simplified model of *Drosophila melanogaster*, also known as the fruit fly, which originally found its way into laboratories thanks to Thomas Hunt Morgan, a biologist at Columbia University, New York. Due to its genetic toolkit, quick reproduction ability, and simple housing requirements, it is present in the laboratories worldwide and has helped several scientists to win Nobel Prizes.

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NEW PHD PROJECTS, SECOND ROUND 2020

PHD RESULTS

FOUNDATION

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RESEARCH AT WARP SPEED

»Let's preserve the revived spirit of sharing data and working collaboratively beyond the usual confines.«

In December 2019, the first cases of a new type of pneumonia were observed. Their cause – a new virus – was reported at the beginning of January 2020. Five days later, its genome sequence was published. By the end of February, the new virus, COVID-19, had infected people on all continents except Antarctica, and the death toll rose. Country borders were closed, businesses – and labs – were forced to shut down, and people were told to stay at home.

At the same time, many other boundaries fell as scientists started global collaborations far beyond their disciplines and usual communities. They freely shared their data on preprint servers and new open collaboration platforms; publishers tore down paywalls for corona-related research and offered free editing and translation services to researchers who were not native English speakers. Academia, biotech, and industry joined forces.

The results of this global effort are impressive: the virus and its effects are being deciphered in record time (see page 62 of this issue). We "flattened the curve" more than once and in November 2020, the first request for approval of a COVID-19 vaccine was submitted to the FDA, the responsible state agency in the USA. If it grants an "emergency use authorization", vaccinations could begin less than 12 months after the virus's identification. And this is only the first vaccine. At the time of writing, more than 50 further vaccine candidates are in clinical testing.

This impressively illustrates the power of today's life science research and technology. However, the pandemic also clearly showed that complex issues such as a pandemic cannot be fought by the natural sciences alone – the social sciences and the humanities are also essential. It taught us how crucial it is to have functioning government agencies and trust in public authorities. At the same time, it highlighted shortcomings that urgently need fixing.

Strong research systems that have been nurtured and developed over decades, if not centuries, are crucial to these successes. But these institutions and their members are also affected: in many areas, nearly all other research has ground to a halt and funding has or will run out before the original projects can be finished. Junior researchers are worrying about the next career step because they cannot finish and publish their projects, and hiring freezes are limiting their job options. Governments, administrators, and funders have responded with impressive flexibility. In Germany, for example, researchers on fixed-term contracts have been given more time through changed laws, and funding organizations have adapted and prolonged grant schemes and relaxed deadlines. We at BIF have helped by granting special COVID extensions to our fellows and have extended – in general and for all fellows – the maximum duration of our PhD fellowships from three to three and a half years.

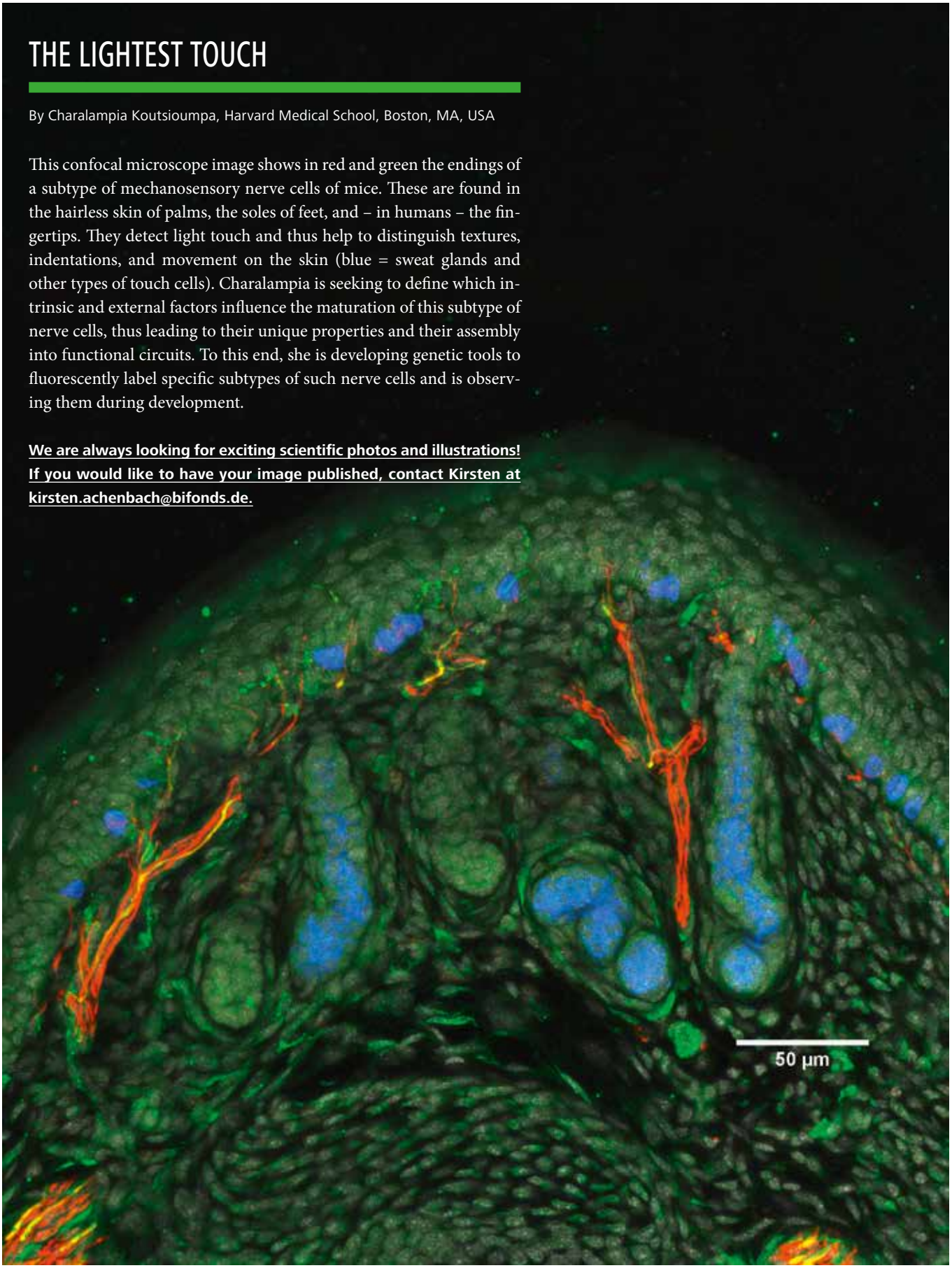
The pandemic has called into question and changed many things in the academic world and beyond. Let's hope that we will preserve the best of the changes, above all the revived spirit of sharing data and working collaboratively beyond the usual confines, in order to solve important problems.

THE LIGHTEST TOUCH

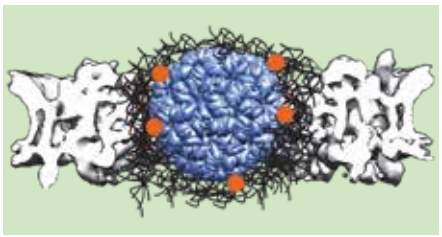
By Charalampia Koutsioumpa, Harvard Medical School, Boston, MA, USA

This confocal microscope image shows in red and green the endings of a subtype of mechanosensory nerve cells of mice. These are found in the hairless skin of palms, the soles of feet, and – in humans – the fingertips. They detect light touch and thus help to distinguish textures, indentations, and movement on the skin (blue = sweat glands and other types of touch cells). Charalampia is seeking to define which intrinsic and external factors influence the maturation of this subtype of nerve cells, thus leading to their unique properties and their assembly into functional circuits. To this end, she is developing genetic tools to fluorescently label specific subtypes of such nerve cells and is observing them during development.

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.



HOW DO LARGE CARGOES GET INTO THE NUCLEUS OF CELLS?



Model of a large molecule (blue), bound to multiple transporter proteins (orange dots).

The nuclei of mammalian cells have more than 2,000 nuclear pore complexes (NPCs) in their membrane that are used to transport a diverse range of molecules in and out of the nucleus. These NPCs are remarkable in terms of the diversity of sizes of cargoes they can transport, ranging from viruses and nuclear proteins (including histones and transcription factors) to essential components involved in protein manufacture (e.g. ribosome subunits). Yet exactly how cargoes greater than 15 nanometres are transported into the nucleus has remained a mystery. Now researchers at Johannes Gutenberg University in Mainz have provided some insights into this process. They created a model using cargoes based on viral capsids ranging from 17 to 36 nanometres in diameter. The cargoes were engineered to have different numbers of nuclear localization sequences (NLS) on their surface, which tell the NPC to allow access to the nucleus. The study found that the larger the capsid, the more NLSs were needed to enable efficient transport into the nucleus. There was also a much higher energetic cost associated with larger cargoes entering the nucleus, which the capsids overcame by binding to proteins inside the NPC.

REFERENCE

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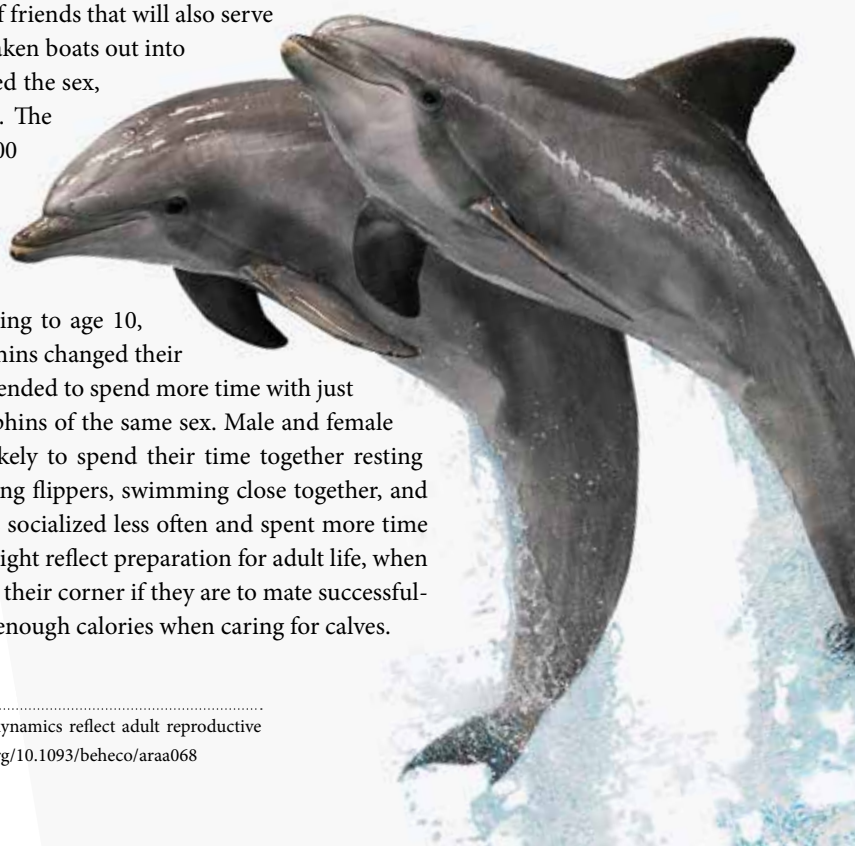
YOUNG DOLPHINS MAKE FRIENDS FOR LIFE TO HELP IN NEED

We all know the importance of a good network when you are growing up (and of course later). Well, imagine being a young dolphin, without any parental support, trying to navigate the world independently – as it turns out, choosing friends carefully is really important for dolphins as well. Researchers at Georgetown University and Duke University, USA, have shown that dolphins spend a lot of energy and time establishing the best network of friends that will also serve them well as adults. Since the 1980s, researchers have taken boats out into the remote Shark Bay in Western Australia and recorded the sex, age, and behaviour of the dolphins they encountered. The team analysed nearly 30 years of data for more than 1,700 wild bottlenose dolphins living in the bay. At around three or four years of age, dolphins leave the protection of their mothers to venture off on their own, living in constantly changing groups. The team analysed the behaviour of young dolphins from weaning to age 10, when there were no adults around. Incredibly, the dolphins changed their circle of friends every 10 minutes on average, but they tended to spend more time with just a few close friends and preferred to hang out with dolphins of the same sex. Male and female dolphins also behaved differently, with males more likely to spend their time together resting or engaging in friendly physical contact, such as rubbing flippers, swimming close together, and mirroring each other's movement. By contrast, females socialized less often and spent more time foraging for fish. The researchers think their findings might reflect preparation for adult life, when it is important for male dolphins to have other males in their corner if they are to mate successfully. Females, on the other hand, need to ensure they get enough calories when caring for calves.



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HIV EVADES DETECTION IN T-CELLS

Our cells have a sophisticated system for detecting DNA that is in the wrong place at the wrong time. An enzyme called cGMP-AMP synthetase (cGAS) senses DNA that is outside the nucleus, such as viral DNA, by looking at its length. It then binds the DNA and initiates a communication cascade that tells the cell to activate its adaptive immune response. Some viruses, such as herpesvirus, are known to activate this response; however, they have also developed ways to avoid it. For others, such as HIV, it has been difficult to determine whether cGAS is alerted. One of the challenges is that HIV is a retrovirus which only produces a single copy of DNA per infection, and even this is quickly imported into the cell nucleus. Also, previous studies of cGAS in HIV have used cell lines that do not accurately reflect the natural physiology of HIV infection. Researchers at the German Berlin Institute of Health and Charité – Universitätsmedizin Berlin used primary and immortalized human and mouse $CD4^+$ T-cells to study activation of the cGAS system. They confirmed that these immune cells have a fully operational cGAS sensing system. However, when they combined the cells with HIV, the cells failed to mount an immune response. This is even the case when they disrupted the HIV viral envelope, allowing more DNA to escape into the cell. The researchers suspect that the comparatively short length of HIV DNA and its low amount allow this virus to escape detection by the cGAS system.

REFERENCE

Elsner C, Ponnurangam A, Kazmierski J, *et al* (2020) Absence of cGAS-mediated type I IFN responses in HIV-1 infected cells. *PNAS* **117 (32)**: 19475–19486

GOOSE BUMPS PROVIDE LONG-TERM PROTECTION FROM THE COLD

It's always been assumed that goose bumps protect animals from very short cold periods by making their hairs stand on end. Researchers now found that they also have a longer-lasting effect. The process that results in goose bumps requires concerted actions between a tiny smooth muscle called the arrector pili muscle, the many hair follicle stem cells, and sympathetic nerves, which control our response to external stimuli. The purpose of the stem cells has not been fully understood, but now researchers from Harvard University, USA, have used electron microscopy in mice to reveal in extremely high resolution the sequence of events that happens when the cold triggers fur to stand on end. They found that the sympathetic nerve not only associates with the arrector pili muscle, but also wraps around hair follicle stem cells like a ribbon. This was surprising because epithelial stem cells are not usually targets for nerve cells. However, the researchers showed that the low level of nerve activity keeps the stem cells in a state where they are poised to proliferate. Under prolonged periods of cold, the nerve was much more active and released neurotransmitter molecules that causes the stem cells to proliferate, stimulating hair growth. When the team removed the arrector pili muscle in the mice, the connection between the nerve and the stem cell was lost. It turns out that goose bumps in mice not only provide short-term relief from the cold, but stimulate new hair growth to protect against longer cold spells, when a thicker coat of fur might be needed.

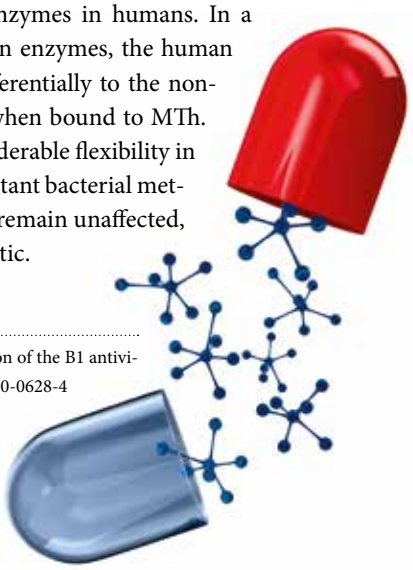
REFERENCE

Shwartz Y, Gonzalez-Celeiro M, Chen CL *et al* (2020) Cell types promoting goose bumps form a niche to regulate hair follicle stem cells. *Cell* **182**: 578–593

Goose bumps stimulate new hair growth to protect against longer cold periods.

ANTIVITAMINS SHOW PROMISE AS ANTIBIOTICS

In the urgent hunt for new antibiotics, the answer may lie with the natural defence mechanisms of bacteria themselves. Some bacteria are known to produce so-called antivitamins – toxic versions of vitamins – to compete with other bacterial species. One of these antivitamins – 2′-methoxy-thiamine (MTh) – is known to suppress bacterial growth, but until now its mechanism of action was unclear. The antivitamin has only a single addition – a 2′-methoxy group – to the naturally occurring vitamin B1, in what was thought to be an unimportant structural position. Now researchers at Göttingen University, the Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, and Texas A&M University, USA, have used X-ray crystallography to show how MTh inhibits an important metabolic enzyme in *E. coli* called transketolase. The unique 2′-methoxy group of MTh clashes with a glutamate that is required for the enzyme's activation by a co-factor. Fortunately, the antivitamin does not seem to affect the same enzymes in humans. In a computer simulation of other transketolases and related human enzymes, the human enzymes either did not bind the antivitamin at all, bound preferentially to the non-methylated version, or retained their enzymatic activity even when bound to MTh. These detailed structural models suggest this is because of considerable flexibility in the binding site of the enzyme's co-factor. The finding that important bacterial metabolic enzymes of bacteria are inhibited, while human proteins remain unaffected, shows promise for the use of this antivitamin as a future antibiotic.



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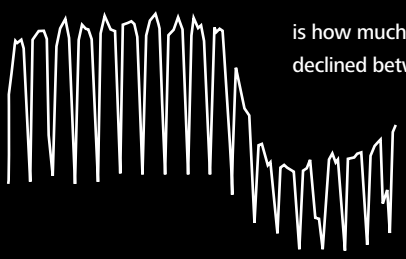
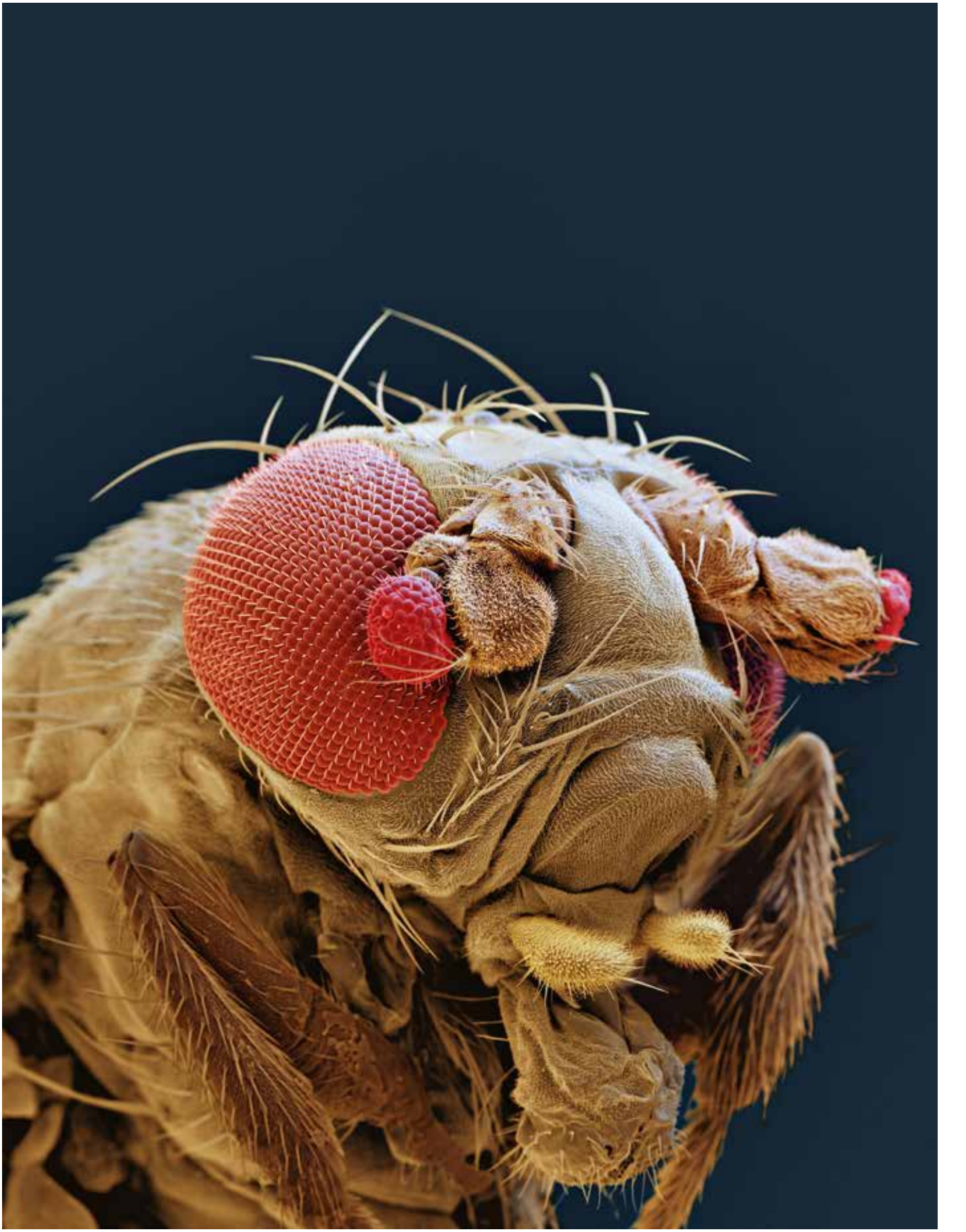


is how much human-linked vibrations in the earth declined between March and May 2020. The lack of human activity during the Covid-19 lockdown resulted in the longest and most pronounced quiet period of seismic noise in recorded history.

Source: Lecocq T, Hicks SP, Van Noten K *et al* (2020) Global quieting of high-frequency seismic noise due to COVID-19 pandemic lockdown measures. *Science* 23 July 2020: eabd2438 https://doi.org/10.1126/science.abd2438

Photos: istock/Andyworks (top); istock/lSerg (right)



Scanning electron microscope image of a mutant with small eyes on its antennae.

PROFILE OF **DROSOPHILA MELANOGASTER**

By Mitch Leslie

Drosophila melanogaster, also known as the fruit fly, found its way to laboratories due to its genetic toolkit, quick reproduction ability, and simple housing requirements. It remains one of the most widely used model organisms.

In the early 1900s, Thomas Hunt Morgan, a biologist at Columbia University in New York, needed a new model organism for his mutation studies, but he was short of space and research funding. A colleague suggested the fruit fly *Drosophila melanogaster*, which scientists were just beginning to study in the lab. The tiny insects did not need elaborate housing, reproduced quickly, and thrived on rotting bananas. Before long, Morgan and his colleagues were rearing them by the millions in milk bottles in what became known as the Fly Room.

The flies soon showed their value. Researchers had only recently rediscovered Mendel's work on genetics and were still dissecting how traits were inherited. Because Morgan could examine large numbers of flies, he was able to detect a rare mutation that causes the insects to have white eyes instead of red. By crossing flies with different eye colours and following the trait from generation to generation, he provided solid evidence that genes are located on chromosomes, a controversial idea at the time. For his work on fruit fly genetics, Morgan received the Nobel Prize in Physiology or Medicine in 1933.

Since then, the flies have proven essential for a variety of studies, as reflected in the diversity of Nobel Prize-winning research that has relied on the insects. For example, Morgan's student Hermann Muller, who performed much of his work at the University of Texas, used them to show that radiation causes mutations and then identified the phenotypes caused by them, earning the Nobel Prize in 1946. Developmental biologists capitalized on the flies' short generation time – they go from egg to adult in about 10 days – to probe how genes shape the body. In the 1970s and 1980s, Christiane Nüsslein-Volhard and Eric Wieschaus, then at the European Molecular Biology Laboratory in Germany, generated mutations in fruit fly embryos and pinpointed 15 genes as key for proper development. They shared the 1995 Nobel Prize with Edward Lewis of the California Institute of Technology, who uncovered a cluster of genes that determines the identities of certain segments in the fly.

The most recent Nobel laureates to ride the flies to success were the 2017 winners Jeffrey Hall of the University of Maine, Michael Rosbash of Brandeis University, and Michael Young of Rockefeller University. They used the insects to reveal the workings of the body's biological clock, discovering how a key gene helps cells tell time.

Drosophila melanogaster remains among the most widely used model organisms. Not only are scientists able to manipulate any gene within its genome with ease and cell-type specificity, but they have also expanded the genetic toolkit for an in-depth analysis of cellular function, including the study of the circuits of the (fly) brain. They can perform *in vivo* imaging and physiology experiments, and extensively charaterize *Drosophila* behaviour. Thus, *Drosophila* remains at the forefront of research in the life sciences, linking molecular mechanisms to cellular function and behaviour, both in health and disease.

WHO AM I? A FEW FACTS

- I first entered the lab around 1900.
- I am about 3 mm long.
- I live for an average of two to three months.
- I eat rotting fruit.
- I work mostly in genetics, developmental biology, and neuroscience.
- I've won six Nobel Prizes.

Photos: eye of science (left); istock/Jamesmcq24 (right)

Like dancers, microglia are rarely motionless, constantly changing partners and positions.

Photo: gettyimages/Christopher Peddecord 2009

MICROGLIA, DANCERS IN THE BRAIN

By Dr Liam Drew

If a microscopic film crew could make a movie of the cellular landscape of your brain, aside from the blood coursing through the brain's 650 km of blood vessels, most cells would be largely motionless except microglia. They would be the hyperkinetic dancing stars of this production.

There are three types of glia cells that together make up half of the brain's cells. Their smallest subtype, microglia owe their name to their tiny cell bodies, but from these diminutive stationary centres they extend an array of elaborate branches that are constantly in motion. "It's amazing. Every second, you see something different," says Professor Rosa Paolicelli, a microglia researcher at the University of Lausanne, France.

In a healthy brain, a microglia's processes constantly bend, probe, extend, and retract. Occasionally, they touch a synapse and attach for five or so minutes, before uncoupling and moving on. Other times, a branch touches a neuron's cell body, spending, on average, 25 minutes attached.

And this is only one aspect of the dynamic nature of microglia. When a brain is injured, microglia will retract their processes to assume an amoeboid form and migrate to the site of damage. They will also proliferate under certain conditions due to injury or disease. →

But then microglia come from a nomadic tribe of cell types. They are immune cells born in the periphery that flood into the central nervous system very early in development to take up lifelong residency there. In humans, they enter already in the second month of gestation – before true neurons even exist.

Later in development, a barricade forms around the central nervous system. The blood-brain barrier is created by a tight coupling between blood vessel cells and prevents bacteria and viruses – as well as circulating immune cells – from entering the brain. This means that microglia are the brain's resident immune system.

For much of the 101 years that scientists have known microglia, they were viewed as cells that become active only in a crisis – be it upon infection or in response to injury or degenerative illness. Their amoeboid form was seen as their active state. When assuming a branched form, they were thought to be "resting". Today, Paolicelli tells her students that "to call microglia resting is an offence – they are anything but resting."

Equipped with an array of new techniques, microglia research has been expanding rapidly in the 21st century. The aims are twofold: one, to work out exactly what these ceaselessly moving cells do in the healthy brain, and two, to resolve how they contribute to disease. With progress being made on both fronts – but with many mysteries remaining – "the field", says Paolicelli, "is exploding."

1919: GLIA'S BIG BANG

Microglia were conclusively described for the first time in 1919 by Spanish scientist Pio del Rio Hortega. However, if we look at Alois Alzheimer's drawings today, it is clear that, around a decade earlier, Alzheimer had unwittingly seen microglia when describing the pathology of the disease that now bears his name.

In those days, uncovering the brain's microscopic structure depended critically on developing stains that labelled only select populations of cells. Hortega's mentor Santiago Ramon y Cajal had won the Nobel Prize in 1906 for showing that brains are composed of networks of discrete neurons connected to one another by synapses. He could only do so because the co-recipient of that prize, Camillo Golgi, had developed a method for labelling neurons.

Cajal went on to characterize astrocytes – a previously known type of glia named for its star-like morphology – in great detail. But he could never satisfactorily label other non-neuronal cell types and referred to what was only glimpsed as the brain's "third element".

His mentee Hortega, though, found two new staining techniques and showed that the third element was not one element but two: the cells now called oligodendrocytes and microglia.

In four papers written in Spanish, Hortega in 1919 described the morphology of microglia in healthy, degenerating, and injured brain tissue, showing that microglia vary in different brain regions. He argued that the cells change form, from branched to amoeboid, and become migratory in pathological scenarios. He also described how they consume cellular debris by the process of phagocytosis, and inferred that they are not brain cells per se, but immune cells. Finally, he speculated that as neurons assemble into circuits during development, some neurons die and some neurons' processes are removed, and that microglia might clear the brain of these remains. Only recently, Professor Helmut Kettenmann of the Max Delbruck Center for Molecular Medicine, Berlin, Germany, helped translate Hortega's four papers into English, annotating them to highlight their contribution to many issues in microglia research. The translations were published in 2016 under the title "The Big Bang for Modern Glial Biology".

Kettenmann says that Hortega's correct assertion that microglia were related to phagocytic cells of the immune system "was kind of intuition". Debates raged about where exactly the cells came from until their origins were convincingly pinpointed as primitive macrophages in 2010 by Florent Ginhoux, then at Mount Sinai School of Medicine, USA, and colleagues.

For many decades after Hortega's discoveries, most microglia research – of which there was not much – focused on these cells being activated only by pathological events. Of note, says Kettenmann, is that in the 1960s and 1970s Georg Kreutzberg, then also working at the Max Planck Institute of Psychiatry, Munich, Germany, showed that if the facial nerve was injured, microglia digested the synapses associated with this nerve, introducing the concept of microglia as "synaptic strippers".

In the 1980s and 1990s, researchers found ways to grow microglia in cell culture and started to better understand these enigmatic cells, showing, for example, that they release and respond to many chemical messengers associated with the immune system and inflammation, such as cytokines.

Then, after the millennium, microglia research truly gathered pace and also turned back to the intact brain. This was aided by genetically modified mice. Microglia are the only cells in the brain that make the receptor for the cytokine fraktaline. In a modern-day equivalent of Hortega's staining techniques, researchers elegantly visualized microglia in the brain of living mice by replacing their fraktaline receptor gene with the gene for the green fluorescent protein (GFP).

These mice allowed scientists to make the first movies of microglia in the living brain. Using mice, two independent groups observed how incredibly dynamic microglia are. "Absolutely nobody had suspected that," says Kettenmann. The techniques allowed researchers to watch in real-time how microglia respond to disturbances – they reach out their branches towards trouble. However, it begged the question of what microglia were doing when they were supposedly resting?

A LIFETIME'S WORK

Paolicelli entered the field as a PhD student in 2007, when a side project suddenly took off. Cornelius Gross, her supervisor at the EMBL in Rome, Italy, had acquired the fraktaline receptor-GFP mice, but instead of viewing them as only a visualization tool, he pondered the actual function of the fraktaline receptor. When he looked at the abundance of fraktaline itself in the developing hippocampus – a brain region critical for memory formation – he saw a spike in fraktaline expression shortly after birth. What's more, fraktaline was made by neurons, suggesting a way that neurons might talk to microglia.

Because of how the GFP gene was inserted into these mice's genome, if a mouse had two copies of the GFP gene, it no longer made fraktaline receptors. Paolicelli therefore used the mice to see what happened if this line of neuron to microglia communication was cut. The result? Hippocampal neurons had more synapses. It appeared that microglia prune away excess synapses during normal development. Supporting this conclusion, fragments of synapses were seen engulfed inside microglia.

Another group published work soon after, in 2012, showing that another messenger network known to be used by the immune system – complement signalling – also mediated communication between neurons and microglia and controlled synaptic pruning in the developing visual system.

"At that time, that was something pretty new," Paolicelli says. "Most of the studies were just in pathology." Ever since, synaptic pruning by microglia – first observed by Kreutzberg under pathological conditions – has been studied as a vital aspect of normal development and a contributor to brain plasticity in adulthood.

Microglia sensing the fraktaline released by neurons is just one of a number of channels by which neurons and glia message one another. Current research seeks to identify the full range of signals that coordinate pruning, including signals that draw the microglia toward synapses. Once there, a synapse may be tagged with either "eat me" or "spare me" signals.

One signal that microglia are especially attuned to is ATP. ATP is a fundamental part of energy processing in all cells, but it is also released by neurons – when they are either damaged or highly active. And microglia – owing to a receptor called P2Y12 – move towards spillages of ATP.

"Neurons speak their language, and microglia can listen in and translate this to respond accordingly," says Professor Ukpong Eyo, a microglia researcher at the University of Virginia, USA. He says that a central concept that has emerged from watching microglia behaviour in the brain is that they are surveillance cells. The cells' motile processes respond to many different signals by constantly and fleetingly contacting neurons and gobbling up unneeded parts of neurons. All this seems to point to the cells' primary function: endlessly checking that the neurons in their vicinity are okay.

Eyo warns, however, that much remains unknown about what precisely microglia do most of the time. Imaging data, his own included, have revealed much about how microglia move but they have provided few insights into the consequences of these movements. "We can say they're spending five minutes over here, ten minutes over there," he says, "but what are they doing during those ten minutes?" It is often unclear if anything of substance has actually happened to a neuron or synapse after contact. What has been shown is that under certain circumstances, such as following a stroke, the processes of the microglia spend more time attached to neurons. Potentially, they respond to physical damage caused by the stroke, but nobody knows for sure.

Eyo also notes that there have been convincing studies showing that microglia – in development and during learning – help induce the formation of synapses, the opposite of pruning. How this occurs will require further study, and Eyo says that the field in general still needs more methods for interfering with specific aspects of microglia activities to unearth their full range of functions.

BACK TO PATHOLOGY

Eyo's interest in how neural activity signals to glia led him to study epilepsy, in which neurons become hyperactive. He showed that during seizures, excess neural firing attracts microglia via ATP release. Strikingly, when Eyo blocked P2Y12 receptors or removed microglia altogether, seizures became worse, indicating →

The techniques allowed researchers to watch in real-time how microglia respond to disturbances – they reach out their branches towards trouble. However, this begged the question of what microglia were doing when they were supposedly resting?

that normally microglia recruitment quells epileptic activity. "What we're trying to identify now – which is more difficult – is how they're doing that," he says.

In Berlin, Kettenmann is looking at how the masses of activated microglia present in gliomas affect the progression of this aggressive form of brain cancer. The microglia appear to help create space for the tumours to grow and also release substances that keep the cancer cells alive.

Epilepsy and glioma are, however, just two of the diseases in which microglia are being investigated today. Others include multiple sclerosis, chronic pain, motor neuron disease, Parkinson's disease, autism, schizophrenia, depression, and the disease in which microglia were first seen about 115 years ago – Alzheimer's.

Dr Aleksandra Deczkowska, a postdoc in the laboratory of Professor Ido Amit at the Weizmann Institute of Science, Israel, has been studying a subpopulation of microglia termed disease-associated microglia (DAM) that appear in brains affected by Alzheimer's. "DAM are a child of the fact that we have a new technology, single-cell sequencing, that lets us see the cells as they are for the first time," she says.

Taking thousands of microglia from a mouse model of Alzheimer's and profiling the genes that each individual one expressed allowed the researchers to identify a small group of cells that looked different from the majority. These cells were also present in mouse models of motor neuron disease and multiple sclerosis. And microglia expressing the same tell-tale genes were seen in dissected brains of people who had had Alzheimer's.

The transition to the DAM state depends on the activation of a particular receptor called TREM2. It is activated by various molecules found in degenerating brains, including amyloid-beta – the molecule that congregates into the plaques that define Alzheimer's disease. TREM2 activates microglia much like pattern recognition receptors on other immune cells, which respond to generic molecules on infectious agents such as bacteria.

Deciphering what DAMs do, however, is proving challenging. These cells engulf and clear amyloid plaques and may be protective, but new data from other laboratories suggest that these microglia might, in fact, also help seed the plaques.

The most compelling argument for microglia playing a protective role in Alzheimer's pathology, Deczkowska says, is that a number of gene variants identified as increasing the risk of developing the condition affect genes expressed highly or uniquely by microglia. This suggests that deficits in microglia biology might somehow take an aging brain down a path toward dementia.

Paolicelli has also pivoted to studying neurodegeneration, probing how the elimination of synapses by microglia might contribute to disease progression across a range of conditions – especially if those microglia contain problematic gene variants. Although the hallmark of Alzheimer's disease is amyloid deposition, amyloid levels actually correlate poorly with dementia severity, suggesting that for certain people its presence is not problematic. A much better predictor of cognitive decline is the amount of synaptic loss.

"For too long these genes have been studied with a biased focus on amyloid removal," Paolicelli says. But the relationship between microglia, plaques, and synaptic pruning is complex. In a study in which Paolicelli increased the phagocytic capabilities of microglia in mice, she saw that microglia cleared away more amyloid but also pruned more synapses.

To pin down the exact balance of protective and damaging microglia functions, the full diversity of their contributions at different disease stages is needed. Deczkowska is now moving on to study the effects of aging on microglia, hypothesizing that a decline in these cells' functioning may relate to the fact that Alzheimer's is a disease of old age. Paolicelli speculates that if certain factors lead microglia to react differently to potential disease-provoking events, it could tip the balance towards bad outcomes. "Specific dysfunction in microglia could be sufficient to originate neurodegeneration," she says.

This is a challenge for every disease microglia are implicated in – are they starting the pathology or merely responding? And does this matter for targeting them for potential treatments? What has become clear is that microglia activation is neither an all or nothing event nor a simple one size fits all response. Eyo highlights that the way microglia change in epilepsy has essential differences from the classically described activated state. And Kettenmann and many others are looking at microglial changes associated with schizophrenia and autism as potential triggering mechanisms for these conditions in early development.

"The big question is what comes first," says Kettenmann. "In the old days, people said the microglia respond to injury and the injury came first. Now the game is open."

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

Shared first authorship is indicated with an *.

CRYO-EM STUDIES OF EUKARYOTIC DNA REPLICATION INITIATION AND ACTIVATION

cf. BIF FUTURA, VOL. 30 | 2.2015

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Discipline: Structural Biologist, MRes
Institute: Imperial College London, The Francis Crick Institute, London, UK
Supervisor: Dr Alessandro Costa

During eukaryotic DNA replication, the core of the replicative helicase, minichromosome maintenance complex (MCM), is loaded onto origins as an inactive double hexamer. The two MCMs are then converted into active Cdc45-MCM-GINS (CMG) holo-helicases that unwind DNA bi-directionally. However, the molecular mechanism and order of CMG activation were poorly understood. In my PhD project, I determined cryo-electron microscopy structures of both the inactive and active helicase bound to different DNA substrates. I showed that the inactive double hexamer forms a stable complex of two tilted rings with the two non-catalytic domains facing each other. I also found that the MCM double hexamer encircles bent, double-stranded DNA. Upon activation, the MCM rings transition to two separate monomers that bind single-stranded DNA, excluding the complementary strand from the main channel. I proposed a model for how the helicase opens up the DNA: conserved proteins within the channel of the MCM ring stretch duplex DNA and untwist the double helix. With my collaborators, I showed that CMG assembly, rather than the recruitment of additional factors, causes the double hexamer to dissociate. Furthermore, at the replication fork, the active helicase is oriented with its non-catalytic domain first. This means that helicases have to pass each other upon activation to ensure that all DNA is unwound. This mechanism could act as a fail-safe to maintain genome stability. To explore the structural complexity at a replication fork, I solved the structure of the CMG bound to the leading-strand polymerase. My results help to explain how polymerase binding stabilizes the helicase motor for productive DNA fork engagement and suggest how the helicase is assembled. They also show that the flexibility of the MCM ring is integral to its roles in replication initiation, helicase assembly, and replication fork progression.

PUBLICATIONS

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THE ROLE OF POLYCOMB GROUP PROTEINS IN MOUSE PRE-IMPLANTATION DEVELOPMENT

cf. BIF FUTURA, VOL. 30 | 2.2015

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Discipline: Geneticist, MSc
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Supervisor: Prof. Antoine Peters

At the onset of life, two highly specialized gametes fuse to create a totipotent embryo, which has the potential to differentiate into all tissues of the adult organism. Differentiation is governed by transcriptional networks that arise during pre-implantation development and is orchestrated by dynamic changes in DNA methylation, chromatin organization, and histone modification. Polycomb group (PcG) proteins are an evolutionarily conserved group of chromatin regulators that play a crucial role in gene regulation during differentiation and development. They regulate transcription by conferring a repressive chromatin state through their capacity to catalyse histone modifications and chromatin compaction. In my PhD project, I addressed the role of PcG proteins in embryonic development and gene regulation during mouse pre-implantation development. Using single-embryo RNA sequencing, I showed that Polycomb repressive complex 2 (PRC2), which is composed of several PcG proteins, represses genes involved in development and differentiation. I further identified a subset of genes that is regulated by PRC2 specifically on the maternal allele in early embryos. This allele-specific gene repression depends on the function of PRC2 in the oocyte, indicating that these genes inherit the repressive histone H3 lysine 27 trimethylation (H3K27me3) mark from oocytes to early embryos. I found that among the genes regulated in this fashion is the X inactivation regulator *Xist*. The long non-coding RNA *Xist* mediates the chromosome-wide inactivation process, which specifically silences the paternal X chromosome in pre-implantation embryos. My results suggest that H3K27me3 is the imprint that suppresses expression of *Xist* on the maternal chromosome in order to prevent its inactivation. I also showed that embryos that are both maternally and zygotically deficient for PRC2 exhibit aberrant expression of lineage markers, suggesting that PRC2 contributes to the correct specification of the first cell lineages. I further showed that maternal deletion of PRC2 impairs post-implantation development. In summary, my work demonstrates that PcG proteins function as dynamic regulators of gene expression and dosage compensation in pre-implantation embryos *in vivo*.

PUBLICATIONS

The results of this project have not yet been published.

ARCHITECTURE AND DYNAMICS OF CYTOSKELETAL NETWORKS OF FTSZ AND ITS CROSS-LINKERS

cf. BIF FUTURA, VOL. 32 | 2.2017

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Supervisor: Asst Prof. Martin Loose

During bacterial cell division, the tubulin homologue FtsZ forms a ring-like structure at the centre of the cell. This Z-ring recruits several proteins to mid-cell and plays a key role in distributing proteins at the division site via the treadmilling motion of FtsZ filaments around the septum. What regulates the architecture, dynamics, and stability of the Z-ring is poorly understood. In my PhD project, I used an *in vitro* reconstitution approach to study how FtsZ-associated proteins (Zaps) affect FtsZ filament dynamics and organization into large-scale patterns. I focused on the role of the highly conserved cross-linking protein ZapA. I combined high-resolution fluorescence microscopy with image analysis to study pattern organization and polymerization dynamics of active FtsZ filaments. My results provide a model for how Zaps can increase the precision and stability of the bacterial cell division machinery without compromising the vital dynamics of FtsZ treadmilling filaments. Furthermore, I present automated quantitative methods that can be used to analyse a large variety of dynamic cytoskeletal systems, using standard time-lapse movies of homogeneously labelled proteins obtained from experiments *in vitro* or even inside the living cell. These cytoskeletal networks of FtsZ and its cross-linkers represent a novel and distinct form of active biological matter characterized by the absence of mechanical stresses that are usually present in gels composed of eukaryotic filaments and molecular motors. My results are not only relevant to basic research into bacteria, but also may contribute to the development of new broad-spectrum antibiotics.

PUBLICATIONS

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SPATIAL-MEMORY CONTROL OF DEFENSIVE ACTIONS

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Discipline: Neurobiologist, MD
Institute: University of Cambridge, UK
Supervisor: Prof. Tiago Branco

Although defensive behaviours are driven by instinct, they are modulated by cognition, namely spatial memory. However, the dynamics of escape flexibility and their neural substrates were not known. The goal of my PhD project was to understand how the spatial environment controls anti-predator defensive actions. I designed behavioural assays which showed that mice, when confronted with predatory sensory cues, escape towards shelter, rather than away from threat. Mice learned the shelter location in under a minute, and this memory was rapidly updated upon changes in the environment. Escape was characterized by an initial head rotation followed by straight acceleration; therefore, orientation to shelter determined the spatial accuracy of escape. I found that this orientation strategy relied primarily on a path-integration mechanism, not on local sensory cues. To study the neural circuits controlling orientation to shelter upon escape, I recorded neural activity in retrosplenial cortex (RSP) and superior colliculus (SC), and showed that both regions contain neurons that continuously map the angular distance to shelter in egocentric coordinates. Projection-specific inactivations paired with recordings showed that SC inherits this spatial signal from RSP and uses it to implement orientation movements. Inactivation of the monosynaptic projection from RSP to SC resulted in spatially inaccurate escapes without perturbing escape vigour. This phenotype is the consequence of disrupting the memory signal that is conveyed from the RSP to the SC. I reached this conclusion based on the discovery that the RSP input to SC was not critical for SC's role in controlling head rotation. My work suggests a general model for translating memory signals into goal-directed actions that relies on neocortical computational power to process complex operations which are transmitted to subcortical effector circuits in readily usable formats.

PUBLICATIONS

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PROTEIN TRANSLATION USING A SIX-LETTER GENETIC ALPHABET IN A SEMISYNTHETIC ORGANISM

cf. BIF FUTURA, VOL. 33 | 1.2018

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All organisms use a four-letter genetic alphabet to store and retrieve genetic information. Selective base pairing – A·T/U and G·C – enables DNA replication and transcription, and ultimately guides protein synthesis via translation. Natural protein functionality has evolved using the proteogenic set of amino acids, which comprise a limited number of functional groups. The hydrophobic unnatural base pair dNaM·dTPT3 uses a different base-pairing mechanism. This allows semisynthetic organisms based on *Escherichia coli* to replicate the expanded genetic alphabet and thus transmit noncoding synthetic information to their progeny. Efforts to expand the genetic code with additional synthetic amino acids predominantly overwrite and reuse nonsense stop codons. During my PhD project, I showed that dNaM·dTPT3 could be used in new unnatural codons to direct the site-specific incorporation of a non-canonical amino acid into a protein. In this way, the unnatural base pair functionally stores information in DNA and retrieves information as RNA and protein. I showed that the unnatural base pair could be used to encode nine different codons and anticodons that incorporated non-canonical amino acids with very high fidelity. Finally, I showed that multiple codons can be used at the same time to incorporate different amino acids into the same protein without any cross-talk. Future availability of orthogonal transfer RNAs and aminoacyl-tRNA synthases can allow for a wide set of unnatural amino acids to be incorporated and used by living organisms with expanded genetic alphabets. This allows us to ask the question: would expanded genetic information have been beneficial to evolution?

PUBLICATIONS

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STRUCTURAL STUDIES OF ctTEL1, AN ATM KINASE ORTHOLOGUE FROM *CHAETOMIUM THERMOPHILUM*

cf. BIF FUTURA, VOL. 31 | 2.2016

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Supervisor: Prof. Karl-Peter Hopfner

Double-strand breaks (DSBs) are the most dangerous form of DNA damage. They can lead to chromosomal rearrangements, aneuploidy, and cell death. DSBs activate the central signalling kinase ataxia-telangiectasia mutated (ATM), which orchestrates the DNA damage response by activating the DNA repair machinery, inducing cell cycle arrest and apoptosis. A lack of structural knowledge of ATM hampers a full understanding of its activation mechanism. The goal of my PhD project was to solve the three-dimensional structure of ATM. I used telomere maintenance 1 (Tel1), an orthologue from the thermophilic fungus *Chaetomium thermophilum* that is more stable than ATM. As full-length ATM and its orthologues cannot be expressed recombinantly, I established a method to purify endogenous Tel1 from *C. thermophilum* (ctTel1). I used single-particle cryo-electron microscopy to determine its structure in high resolution. This structure enabled me to build the first complete atomic model of an ATM kinase, revealing a striking dimeric butterfly-shaped architecture. I showed that a large regulatory domain at the N-terminus is flexibly connected to the rest of the protein. Binding assays with a truncated construct revealed that this domain binds DNA and activating proteins, which regulate the kinase activity allosterically. The ctTel1 structure showed that the kinase domain is at the C-terminus and its active site is entirely structured, revealing in atomic detail how it coordinates ATP and Mg^{2+} ions. Although these features are normally hallmarks of an active kinase, ctTel1 is rendered inactive by a conserved regulatory α-helix blocking access to the active site. By overlaying the ctTel1 structure with structures of other protein kinases in complex with a peptide substrate, I found that such a peptide would indeed clash with this regulatory element. This indicates that ctTel1 is in an ATP-bound yet inactive state, and can only be activated by removing this helix. My work provides the first complete atomic model of an ATM kinase. By revealing its autoinhibitory circuitry, my work will aid further studies on the activation mechanism of ATM kinases.

PUBLICATIONS

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RECONSTRUCTING UNIQUELY HUMAN CORTICOGENESIS AND NEURAL DIVERSITY

cf. BIF FUTURA, VOL. 31 | 1.2016

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Supervisor: Prof. Barbara Treutlein

The human brain has undergone dramatic changes during evolution that represent the basis for our remarkable cognitive abilities. However, the molecular underpinnings of the differences between humans and our closest living relatives, the great apes, especially during early brain development, are not well understood. To illuminate these differences, I used a three-dimensional stem cell-based *in vitro* model system called cerebral organoids combined with single-cell mRNA sequencing (RNA-seq) to study molecular changes at high resolution and throughput. In an initial investigation using a valve-based microfluidic system for single-cell RNA-seq, I found that the gene expression patterns of human and chimpanzee progenitor cells and neurons were remarkably similar. However, genes related to integral membrane signalling were more highly expressed in human progenitor cells than in chimpanzee progenitor cells. In a more detailed survey comprising thousands of cells using droplet-based microfluidics, I reconstructed the differentiation of organoids over four months. This led to the discovery that cortex-like cells develop at a slower pace in humans than in chimpanzees and macaques. Moreover, I dissected human-specific gene expression differences for specific cell states, some of which were linked to changes in chromatin accessibility and overlapped human-specific sequence changes. This highlighted cadherin 7 (*CDH7*), a gene involved in intercellular signalling, as a promising candidate gene for further studies. By combining these early differences with single-nucleus RNA-seq data from adult brain tissue from human, chimpanzee, bonobo, and macaque, I discovered that some of these human-specific gene expression differences persisted even into adulthood. These studies show that cerebral organoids are a useful model to study early developmental dynamics and provide candidate genes for further investigation.

PUBLICATIONS

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MOLECULAR MECHANISMS OF piRNA BIOGENESIS IN *DROSOPHILA*

cf. BIF FUTURA, VOL. 32 | 2.2017

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A large fraction of eukaryotic genomes consists of transposons – repetitive elements whose uncontrolled mobilization can be harmful. A widely conserved mechanism to counteract transposon activity is the PIWI-interacting RNA (piRNA) pathway. In this gonad-specific system, piRNAs – small non-coding RNAs – form complexes with effector proteins, which recognize active transposons and initiate their silencing. Failure to do so often results in sterility, thus underscoring the essential role of the piRNA pathway for animal fitness. In my PhD project, I investigated the molecular mechanisms of piRNA biogenesis using *Drosophila melanogaster* as a model organism. Using a combination of genetic, biochemical, and imaging approaches, I investigated how piRNA precursor transcripts are chosen for processing and how they are transported to mitochondria, where piRNA production occurs. I explored the role of the uncharacterized gene *CG10880/Daedalus* (*Daed*), which had previously been linked to transposon regulation in the fly ovary. I discovered that Daed is required for piRNA production, as it recruits key proteins to mitochondria. I generated an interaction map of proteins involved in piRNA biogenesis and showed that some of them can shuttle between subcellular compartments during this process. I found that one RNA helicase, Armitage (Armi), is critical for delivering precursor transcripts to mitochondria, where it assists in their processing. I showed that Armi is first loaded with piRNA precursors in dedicated perinuclear structures and then translocates to mitochondria. This precise, multi-layered regulation ensures that only the correct RNA substrates are used for piRNA biogenesis. These insights into the piRNA pathway will aid our understanding of the molecular mechanisms counteracting transposon activity in *Drosophila*.

PUBLICATIONS

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STUDYING DETERMINANTS OF FERROPTOSIS SENSITIVITY IN HUMAN MAMMARY EPITHELIAL CELLS

cf. BIF FUTURA, VOL. 30 | 1.2015

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Supervisor: Dr Christina Scheel

Metastatic disease constitutes a major challenge, because treatment options remain limited. Ferroptosis is a regulated form of necrotic cell death caused by iron-dependent lethal accumulation of peroxidized phospholipids. It can be induced by pharmacological or genetic deletion of the ferroptotic key enzyme glutathione peroxidase 4 (GPX4). Although ferroptosis has emerged as a promising approach for therapeutic intervention in specific cancers, the cellular and metabolic states that confer sensitivity to this process remain elusive. The goal of my PhD project was to study ferroptosis mechanisms in the context of human mammary epithelial cells (HMECs). Specifically, I set out to elucidate whether induction of the epithelial-mesenchymal transition (EMT), a process involved in metastasis that leads to the migration of individual mesenchymal cells, affects ferroptosis sensitivity. Pharmacological inhibition of GPX4 in epithelial and EMT-induced immortalized HMECs revealed an unexpected finding. Independently of EMT, both cell states were sensitive to GPX4 inhibition at low cell densities but resistant at high cell densities. I established ferroptosis as a cell death modality using ferroptosis inhibitors, lipophilic antioxidants, and iron chelators. My results were confirmed by genetic deletion of GPX4 by CRISPR-Cas9. When I studied ferroptosis in primary HMECs, I demonstrated that density-dependent ferroptosis sensitivity is already present in primary cells. By screening the proteome of both epithelial and EMT-induced immortalized HMECs, I identified several candidate proteins potentially involved in cell density-dependent ferroptosis. My first validation experiments indicated that low cell density induces triglyceride (TAG) hydrolysis, providing free fatty acids for mitochondrial fatty acid oxidation. Analysis of the lipid profile revealed that at low cell density, cells massively accumulated TAGs enriched with polyunsaturated fatty acids, which are particularly prone to peroxidation. Together, these results suggest that cell density leads to a metabolic switch correlating with ferroptosis sensitivity in HMECs. In addition to a potential role in mammary gland function, this process may have therapeutic potential in cellular contexts where cells are disseminated – such as during metastasis.

PUBLICATIONS

The results of this project have not yet been published.

USP48 IDENTIFIED AS A NOVEL GENE SUPPRESSOR IN THE FANCONI ANAEMIA PATHWAY

cf. BIF FUTURA, VOL. 32 | 1.2017

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Institute: Research Center for Molecular Medicine of the Austrian Academy of Science (CeMM), Vienna, Austria
Supervisor: Prof. Joanna I. Loizou

The Fanconi anaemia (FA) pathway repairs damaged DNA, particularly DNA with interstrand cross-links. Single gene defects in the FA pathway result in the rare disease Fanconi anaemia, which has a high mortality rate. The goal of my PhD was to understand the molecular mechanism underlying this pathway and identify novel components. I focused on generating a network of synthetic rescue interactions in the FA pathway. These interactions rescue cell viability through the simultaneous deletion or mutation of two genes. To generate the first deletion, I used CRISPR-Cas9 on the near-haploid cell line HAP1, resulting in five cell lines with defects in the FA pathway. All of these so-called FA cell lines were highly sensitive to cross-linking agents such as mitomycin C (MMC). To generate the second deletion, I performed high-throughput loss-of-function screens by gene-trap insertional mutagenesis. Then the FA cells, now harbouring a second mutation, were treated with MMC. After retrieving MMC-resistant clones, I used next-generation sequencing to identify the genes responsible for resistance. One of the top hits was ubiquitin specific peptidase 48 (USP48), a de-ubiquitin enzyme not previously described as part of the FA pathway. Using immunofluorescence assays, I found that the loss of USP48 improves the DNA damage response in the FA cell lines. Furthermore, loss of USP48 promotes the recruitment of proteins such as breast cancer associated gene 1 (BRCA1), a marker for error-free DNA repair. To confirm these findings, I performed metaphase spreads after treating the FA cells with MMC. This showed that loss of USP48 reduces the level of chromosomal abnormalities, a hallmark of Fanconi anaemia. My comprehensive map of synthetic rescue interactions furthers our understanding of the FA pathway. The identification of USP48 as a novel component of this pathway also presents a potential therapeutic target for Fanconi anaemia.

PUBLICATIONS

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MTN-PROJECTING FLRT3 RETINAL GANGLION CELLS THAT RESPOND TO DOWNWARD-MOVING STIMULI

cf. BIF FUTURA, VOL. 30 | 2.2015

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Supervisor: Prof. Rüdiger Klein

In mammals, the retina captures visual information about an individual's surroundings and transmits it to the brain. The transmitter cells, retinal ganglion cells (RGCs), comprise 30 subpopulations that each respond to the same set of visual inputs in a specific way. A subset of RGCs that project to nuclei of the accessory optic system are specialized to respond to objects moving upward, downward, or forward. These cells provide essential visual input to control image stabilization via the optokinetic reflex. However, genetic markers for each subpopulation within this group of RGCs are lacking, which hinders the study of their circuitry and function. During my MSc project, I confirmed fibronectin leucine-rich transmembrane protein 3 (Flrt3) as a marker of a subpopulation of cells in the mouse retina. Based on these findings, I generated a Flrt3-specific knock-in mouse model, which allowed me to specifically target a small population of Flrt3-positive neurons in the retina. The goal of my PhD project was to elucidate the identity of these cells and evaluate their functional responses. Molecular characterization revealed that Flrt3 labelled a small subpopulation of RGCs (~4.6% of all cells) in the ganglion cell layer. Using Cre-dependent intravitreal adeno-associated viral injections, I revealed the projection pattern of Flrt3-positive RGCs in the brain. I found that a subgroup of these cells specifically targets the medial terminal nucleus (MTN), part of the accessory optic system. Intracranial injections of a retrograde adeno-associated virus into the MTN enabled me to target only the MTN-projecting Flrt3-positive RGCs (hereafter MTN-Flrt3-RGCs). Patch-clamp recordings of the MTN-Flrt3-RGCs revealed that they respond specifically to downward-moving stimuli. In addition, the MTN-Flrt3-RGCs showed an on-off bi-stratifying pattern in the inner plexiform layer and were positive for the on-off direction-selective ganglion cell marker CART (cocaine and amphetamine-regulated transcript). These features distinguish MTN-Flrt3-RGCs from previously discovered MTN-projecting RGCs. MTN-Flrt3-RGCs might therefore represent a previously unknown functional type of RGCs. This finding underpins the importance of searching for new genetic markers to capture the full functional diversity of RGCs.

PUBLICATIONS

The results of this project have not yet been published.

THE EFFECTS OF CCL3 ON DENDRITIC CELL MIGRATION AND IMMUNE CELL ACTIVATION

cf. BIF FUTURA, VOL. 32 | 1.2017

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Supervisor: Prof. John H. Sampson

Recent advances in dendritic cell (DC) vaccines for cancer immunotherapy are showing encouraging results in clinical trials. To amplify potential immune responses, DCs can be matured *in vitro* and injected into the skin, where they migrate to lymph nodes and prime cytotoxic T lymphocytes. However, less than 5% of injected DCs reach the lymph nodes. Improving migration to the lymph nodes is therefore expected to elicit a more robust anti-tumour immune response. The Sampson laboratory discovered that inflammatory preconditioning of the vaccine injection site – using the tetanus/diphtheria toxoid (Td) recall response – increases DC migration and overall survival in patients. A key mediator of the increased immune response is C–C motif chemokine ligand 3 (CCL3). My PhD project aimed to investigate the effects of CCL3 on DC migration and immune cell activation. Using murine models, I found that while memory CD4+ T cells are responsible for secreting CCL3 systemically, another source of CCL3 is required to increase DC migration. I showed that Td-specific CD4+ memory effector T cells traffic to the preconditioning site and that CCL3 is produced at the skin – but not by these T cells. This highlights the complex interactions between inflammatory preconditioning and DC migration from the skin to the lymph nodes. My experiments also indicated that the chemokine receptor CCR5, which binds CCL3, is required to increase migration of DCs after inflammatory preconditioning. Finally, I found that injecting CCL3 intravenously can increase DC migration to levels comparable to those in mice that received inflammatory preconditioning, as well as significantly reducing tumour cell growth. Replacing inflammatory preconditioning with a simple injection of CCL3 has the potential to reduce the biological variability that is inherent to inflammatory preconditioning. Furthermore, as inflammatory preconditioning takes several weeks, using intravenous CCL3 instead would reduce the time patients have to wait before receiving a DC vaccine.

PUBLICATIONS

Schaller TH, Sampson JH (2017) Advances and challenges: dendritic cell vaccination strategies for glioblastoma. *Expert Rev Vaccines* **16**: 27–36

Mitchell DA, Batich KA, Gunn MD, Huang MN, Sanchez-Perez L, Nair SK *et al* (2015) Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. *Nature* **519**: 366–369

UNMASKING *IN VIVO* STEM CELL DYNAMICS IN MAMMARY TISSUE AND MAMMARY TUMOURS

cf. BIF FUTURA, VOL. 30 | 2.2015

COLINDA SCHEELE
Discipline: Molecular Biologist, MSc
Institute: Netherlands Cancer Institute, Amsterdam, The Netherlands
Supervisor: Prof. Jacco van Rheenen

The mammary gland (MG) is a dynamic organ that develops mainly after birth. During puberty, the MG develops into a branched network of ducts, which continuously grows and regresses during every oestrous cycle. Stem cell (SC) markers are lacking for the MG, and the identity of mammary SCs remains elusive. Understanding mammary SC dynamics is important, because the tumorigenic version of SCs – cancer stem cells – might drive tumour growth. To uncover the SC dynamics, I combined unbiased lineage tracing, modelling, and single-cell sequencing with intravital imaging to follow the fate of the same cells in a living mouse. With this approach, I defined the localization, identity, and dynamics of SCs in the pubertal MG. I found that most ductal ends consist of proliferative, lineage-committed SCs that are heterogeneous in their expression profile and short-term contribution to ductal growth. Through cell rearrangements, each mammary SC within this heterogeneous population contributes to ductal growth. In the adult MG, I showed that turnover is driven by three SC populations that are scattered throughout the ducts. Each SC drives tissue turnover in the immediate ductal compartment, leading to clonal fields that can span multiple ducts. This process might explain how oncogenic mutations are fixed and spread through the MG. Using the same approach in mouse models for mammary carcinoma, I found that tumour growth is driven by a small population of cancer stem cells. Within the progeny of one of these cells, the normal cellular hierarchy is still maintained. Most tumour cells are differentiated and do not contribute to tumour growth, whereas only a few cells proliferate long-term and follow the dynamics of the MG. This work identifies the mammary SCs and uncovers striking parallels between healthy MGs and tumour growth.

PUBLICATIONS

Corominas-Murtra B*, Scheele CLGJ*, Kishi K, Ellenbroek S, Simons BD, van Rheenen J *et al* (2020) Stem cell lineage survival as a noisy competition for niche access. *Proc Natl Acad Sci USA* **117**(29): 16969–16975

Scheele CLGJ*, Hannezo E*, Muraro MJ, Zomer A, Langedijk NSM, van Oudenaarden A *et al* (2017) Identity and dynamics of mammary stem cells during branching morphogenesis. *Nature* **542**: 313–317

Hannezo E*, Scheele CLGJ*, Moad M, Drogo N, Heer R, Sampogna RV *et al* (2017) A unifying theory of branching morphogenesis. *Cell* **171**: 242–255

IMPORTANCE OF RARE VERSUS COMMON GENETIC VARIANTS IN HUMAN TRAITS AND DISEASES

cf. BIF FUTURA, VOL. 31 | 2.2016

ARMIN SCHOECH
Discipline: Computational Biologist, MPhil
Institute: Departments of Biostatistics and of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA
Supervisor: Prof. Alkes Price

Many heritable human traits and diseases have a so-called complex genetic architecture – that is, they are caused by a large number of genetic factors as well as environmental influences. These genetic factors are loci in the genome that differ between individuals in the population, with variants at these loci increasing or decreasing the target trait or disease risk. A crucial question is the relative importance of rare versus common genetic variants. In other words, are particular traits or diseases affected mostly by genetic variants that occur in only a few individuals or those that are shared by a large fraction of the population? As part of my PhD research, I developed a statistical method to answer this question. The method assumes that genetic effect sizes follow a probability distribution that depends on the variants' frequencies in the population. It then calculates the frequency dependence of the effect size that best fits the data. I applied this method to analyse 25 complex traits – including height, body mass index, asthma, and hypertension – using genetic and trait data from 114,000 individuals. For all traits, I estimated that the average per-variant effects were larger for rare than for common genetic variants. This can be explained by the action of natural selection: if genetic variants that cause a trait are more likely to be under selection, they will be enriched in the lower-frequency spectrum, so lower-frequency genetic variants will on average have larger trait effects. I showed that the frequency dependence estimated from the data naturally arises in plausible evolutionary models and simulations. I also found that, despite larger effect sizes for rare variants, rare genetic variants (those with a variant frequency <1%) collectively explain less than 10% of total genome-wide genetic effects for most of the traits I analysed. This indicates that future studies aiming to predict complex traits based on genotypes should focus on common variants. Future studies on rare variants may still reveal interesting biology and potentially actionable drug targets, since they have larger average effects on a per-variant basis.

PUBLICATIONS

Schoech AP, Jordan DM, Loh PR, Gazal S, O'Connor LJ, Balick DJ *et al* (2019) Quantification of frequency-dependent genetic architectures in 25 UK Biobank traits reveals action of negative selection. *Nat Commun* **10**: 790

AN RNA DEGRADATION COMPLEX REQUIRED FOR EPIGENETIC INHERITANCE OF HETEROCHROMATIN

cf. BIF FUTURA, VOL. 29 | 2.2014

GERGANA SHIPKOVENSKA
Discipline: Geneticist, MBiochem
Institute: Harvard Medical School, Boston, MA, USA
Supervisor: Prof. Danesh Moazed

Heterochromatin is a highly conserved feature of eukaryotic chromosomes. This silent chromatin domain represses gene expression, silences transposons, and maintains genome integrity. Heterochromatic domains can be inherited stably over many cell generations. Heterochromatin inheritance maintains cell identity throughout development, and failures in this mechanism manifest in developmental defects and tumorigenesis. Given the importance of heterochromatin inheritance for cell function, the goal of my PhD project was to interrogate the mechanisms that maintain heritable chromatin states. I used a novel reporter of inducible heterochromatin formation in the fission yeast *Schizosaccharomyces pombe* to conduct the first genome-wide screen for factors required for heterochromatin inheritance. This screen isolated mutations in a conserved and essential RNA processing complex, which my colleagues and I named the rixosome. The rixosome fulfills a well-characterized, essential function during ribosome biogenesis, but it has not previously been shown to play a role in heterochromatin inheritance. I found that the rixosome is recruited to heterochromatin by an interaction with the heterochromatic protein HP1Swi6. Using a separation of function allele, which specifically disrupts this interaction, I showed that the function of the rixosome in heterochromatin inheritance is independent of its role in ribosome biogenesis. Furthermore, I found that in rixosome mutants carrying this allele, heterochromatic transcripts are targeted for degradation via 5′–3′ exoribonuclease 2 (Dhp1) and that the accumulating transcripts disrupt the stability and inheritance of heterochromatic domains. Following this work, my colleagues and I showed that the human homologue of the rixosome is recruited to heterochromatic domains and that knockdown of rixosome subunits leads to loss of gene silencing at those domains. These results reveal the rixosome as a new and unexpected determinant of heterochromatin inheritance, with conserved functions in yeast and humans.

PUBLICATIONS

Shipkovenska G, Durango A, Kalocsay M, Gygi SP, Moazed D (2020) A conserved RNA degradation complex required for spreading and epigenetic inheritance of heterochromatin. *Elife* **9**: e54341

REGULATION OF α-KETOGLUTARATE-DEPENDENT DIOXYGENASE TET3 IN NEURONS

cf. BIF FUTURA, VOL. 31 | 1.2016

FRANZISKA TRAUBE
Discipline: Organic Biochemist, MSc
Institute: Department of Chemistry, University of Munich (LMU), Germany
Supervisor: Prof. Thomas Carell

Neural activity induces dynamic changes in DNA methylation patterns. α-ketoglutarate (αKG)-dependent ten-eleven translocation enzyme 3 (TET3) contributes to these changes by oxidizing 5′-methyl-2′-deoxycytidine (5mdC). TET3 deficiency results in a neurodevelopmental disorder reminiscent of Rett syndrome, a rare neurological disorder caused mostly by mutations to methyl-CpG-binding protein (MeCP2). In my PhD project, I studied TET3 interaction partners in neurons to learn more about how TET3 activity is regulated and how the enzyme is targeted to specific genomic loci. I found that TET3 and MeCP2 interact in neurons during synaptogenesis. MeCP2, but not two mutated versions of this protein that are common in Rett syndrome, slows down TET3 activity in an HEK293T cell-based assay. In addition, I showed that genomic loci that are hypermethylated in TET3 knockout neurons are also hypermethylated in MeCP2 knockout neurons. Similarly, hypomethylated loci in TET3 knockout neurons were also hypomethylated in MeCP2 knockout neurons. I found the same at the transcriptome and protein levels: upregulation and downregulation in TET3 knockout neurons were linked to upregulation and downregulation in MeCP2 knockout neurons, respectively. These results suggest that TET3 plays an important role in the clinical progression of Rett syndrome. Furthermore, I showed that in neurons, TET3 interacts with glutamate dehydrogenase 1 (GDH), which converts ubiquitous glutamate into αKG. GDH is normally a mitochondrial enzyme, but in hippocampal neurons it is transported into the nucleus to supply TET3 with αKG. When hippocampal neurons were depolarized and a GDH inhibitor was applied, I observed a sharp drop in αKG levels and less 5mdC oxidation, along with a decrease in the expression levels of the neural activity-dependent genes *Npas4* and *Bdnf* compared to the depolarized, but untreated, control. My results link neuronal metabolism to epigenetic plasticity on the DNA level. As αKG is critical for energy metabolism and is a co-substrate for more than 60 αKG-dependent oxygenases, the increase in the effective αKG molarity only in the environment of TET3 is an elegant way to avoid globally changing αKG levels, which would produce various side effects.

PUBLICATIONS

Mulholland CB, Traube FR, Ugur E, Parsa E, Eckl EM, Schönung M *et al* (2020) Distinct and stage-specific contributions of TET1 and TET2 to stepwise cytosine oxidation in the transition from naive to primed pluripotency. *Sci Rep.* **10**: 12066

STRUCTURE OF THE SWI/SNF FAMILY CHROMATIN REMODELLER RSC BOUND TO A NUCLEOSOME

cf. BIF FUTURA, VOL. 31 | 2.2016

FELIX WAGNER
Discipline: Biochemist, MSc
Institute: Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
Supervisor: Prof. Patrick Cramer

Transcription depends on the accessibility of free DNA, which is enabled by the formation of nucleosome-depleted promoter regions (NDRs). The 16-subunit RSC (remodels the structure of chromatin) complex, a member of the SWI/SNF family of chromatin remodellers, uses the energy of ATP to clear nucleosomes from promoter regions. However, the mechanistic basis of remodelling and the role of the non-enzymatic, auxiliary RSC subunits are poorly understood. In my PhD project, I used single-particle cryo-electron microscopy to determine the structure of *Saccharomyces cerevisiae* RSC bound to a nucleosome. The structure revealed that RSC is formed by five modules – the ATPase, actin-related protein (ARP), arm, body, and DNA-interaction modules – four of which are interconnected by two subunits. I discovered the relative orientation of the modules towards each other and the nucleosome. The body module is the assembly platform for most of the other RSC subunits. The arm module binds to the nucleosome via the Sfh1 subunit. The highly dynamic ARP module forms a bridge between the body and ATPase modules. The ATPase module binds to the nucleosome and sandwiches it together with the arm module. The DNA-interaction module is highly flexible and binds the exiting DNA 20–40 base pairs upstream of the nucleosome. Based on these discoveries, I suggest a simple model for NDR formation in which one RSC molecule binds to either the flanking +1 or –1 nucleosome of the NDR and starts remodelling, moving the nucleosome away from the NDR and thereby increasing the length of the NDR. A second RSC complex then binds to the other nucleosome and works in the opposite direction. In this way, RSC would free the DNA and enable transcription initiation. My results provide a basis for a mechanistic understanding of chromatin remodelling by SWI/SNF family complexes and offer a starting point for further investigating their role in chromatin organization and transcription. In addition, the structure of RSC could be used to find protein-binding sites in PBAF, a homologous SWI/SNF chromatin remodelling complex that is mutated in cancers in humans.

PUBLICATIONS

Wagner FR, Dienemann C, Wang H, Stützer A, Tegunov D, Urlaub H *et al* (2020) Structure of SWI/SNF chromatin remodeller RSC bound to a nucleosome. *Nature* **579**: 448–451

VISUALIZING AND INTERFERING WITH UBIQUITIN-DEPENDENT DNA DAMAGE BYPASS

cf. BIF FUTURA, VOL. 31 | 1.2016

GEORGE YAKOUB
Discipline: Molecular Biologist, MSc
Institute: Institute of Molecular Biology (IMB), Mainz, Germany
Supervisor: Prof. Helle Ulrich

Timely resolution of replication problems is essential for genome stability. In eukaryotes, DNA damage bypass safeguards cells against replication stress. This pathway is regulated by ubiquitylation of the replicative clamp, proliferation cell nuclear antigen (PCNA). Monoubiquitylation of PCNA activates error-prone translesion synthesis, which relies on damage-tolerant DNA polymerases. Alternatively, modification of PCNA with K63-linked polyubiquitin chains promotes error-free template switching, a mechanism that uses information provided by the sister chromatid. In my PhD project, I aimed to discover previously unknown readers of K63-polyubiquitylated PCNA and gain insight into the spatial and temporal regulation of DNA damage bypass. I developed *in vivo* inhibitors and fluorescent sensors to distinguish between different species of ubiquitylated PCNA in *Saccharomyces cerevisiae*. In a genome-wide overexpression screen, I found that the DNA repair factor Rad5 could rescue the DNA damage sensitivity caused by one of the inhibitors. Further characterization revealed that Rad5's rescue capability depends mainly on its ubiquitin ligase and helicase domains. Using the fluorescent sensors in live-cell imaging, I investigated the kinetics of PCNA ubiquitylation in space and time. I showed that these sensors form distinct nuclear foci after drug-induced replication stress and act in a pathway-specific manner. Foci of ubiquitylated PCNA emerge at the onset of replication and resolve later in the G2/M phase. Analysing the association of ubiquitylated PCNA with replisomes, repair factors, and known DNA repair centres revealed that ubiquitylation is activated mainly behind replication forks and close to post-replicative repair territories. Unlike DNA double-strand breaks and collapsed replication forks, damaged DNA marked by ubiquitylated PCNA does not reside at the nuclear pores or the nuclear periphery. Overall, my work highlights the critical role of K63-linked ubiquitin chains on PCNA in maintaining the stability of newly synthesized DNA. Moreover, the sensors provided an independent view of the pathway by enabling the direct visualization of bypass events. They also allowed me to quantitatively study the relative contribution of fork-associated and post-replicative modes of damage bypass.

PUBLICATIONS

Lockhart A, Pires VB, Bento F, Kellner V, Luke-Glaser S, Yakoub G *et al* (2019) RNase H1 and H2 Are Differentially Regulated to Process RNA-DNA Hybrids. *Cell Rep* **29**: 2890–2900

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.

TEAMWORK, SERENDIPITY, AND KEEN OBSERVATION TO FIGHT SARS-COV-2

By Kirsten Achenbach

When the pandemic hit and researchers in many countries were told to stay at home and away from the lab, many used their expertise to unravel the new virus as fast as possible.

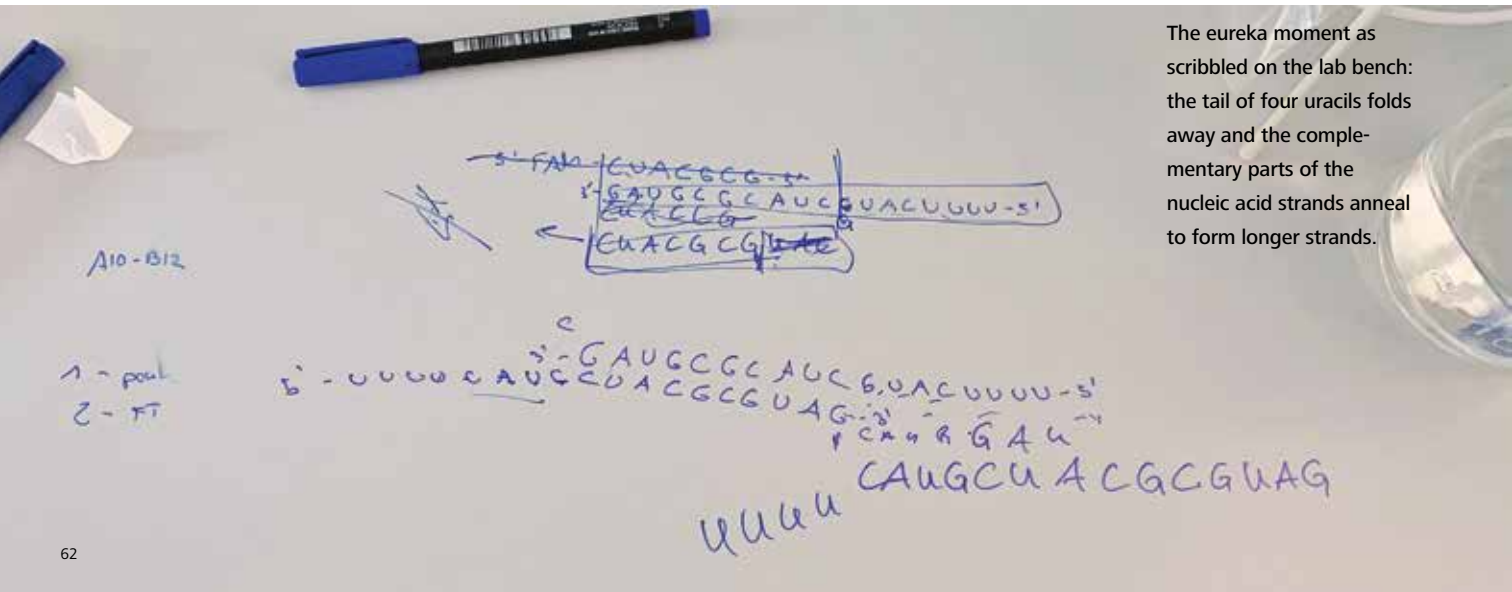
In this article, we highlight three projects by BIF alumni who looked at how SARS-CoV-2 replicates its RNA genome, how it synthesizes its proteins, and how it evades the human immune system. All three aspects are relevant for developing drugs to fight the pandemic.

Let's start with the polymerase and with BIF alumni Hauke Hillen and Goran Kokic from Professor Patrick Cramer's group at the MPI for Biophysical Chemistry in Göttingen, Germany. They were the first to determine the structure of the SARS-CoV-2 polymerase in the act of replication, with important viral factors attached and bound to a nucleic acid strand. It was a mix of serendipity, perseverance, and keen observation that allowed the team to succeed.

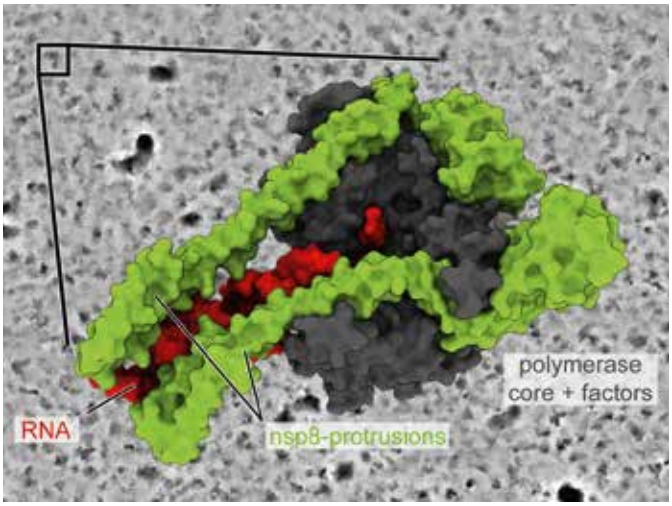
After taking cryo-EM images for ten days and nights, the team, which also included Lucas Farnung, still did not have an image that gave a clear view of the structure. So they went back to one they had almost discarded because it looked so strange. In the end, it provided the key: "After Goran, Lucas, and I had stared and puzzled over what it could mean for about an hour while scribbling on the bench, it hit us." It was a moment of dismay and celebration at the same time, Hauke recalls: "Contrary to our initial design, the nucleic acid strands we had fed the polymerase held complementary sections that had formed longer strands. This was really a case of serendipity, because it allowed us to see a unique feature of the SARS-CoV-2 polymerase." On top of the largely conserved body of the polymerase, there are two copies of the non-structural protein, Nsp8, forming a crane-like protrusion that binds to the backbone of the already copied RNA strand. These protrusions are only stable – and can therefore be imaged with cryo-EM – when the polymerase is bound to a longer nucleic acid strand. "We believe that this protrusion helps the coronavirus to stabilize its unusually long RNA genome while copying it," explains Goran.

The structure the team managed to resolve also shows the active site of the polymerase ready to accept a new nucleotide and incorporate it into the newly synthesized RNA strand. Remdesivir, the only antiviral drug known today to work to some extent against SARS-CoV-2, is a so-called nucleotide analogue. These drugs mimic nucleotides since their structure is similar and – depending on the drug – can be incorporated into the copy of the virus RNA. Most viruses are vulnerable to these drugs because, unlike us, they do not have proofreading mechanisms removing such wrong building blocks in their RNA. However,

Photos: H.Hillen, MPI-BC (top); R. Buschauer, LMU (bottom)



The eureka moment as scribbled on the lab bench: the tail of four uracils folds away and the complementary parts of the nucleic acid strands anneal to form longer strands.



Model of the SARS-CoV-2 RNA polymerase with RNA (red) and the crane-like protusions of Nsp8 (green) shown in front of a cryo-EM image.

coronaviruses seem to be an exception, making them harder to treat. The reason why remdesivir seems to work against this new virus is that it manages to evade the proofreading process. "With our structure, we can now start to study exactly how remdesivir works and maybe design more effective drugs," says Goran.

Knowing how the virus copies its genome is one step, but it is also important to learn how it evades the immune system. This aspect was studied by BIF alumnus Robert Buschauer, a member of Roland Beckmann's group at the University of Munich, Germany. Together with his colleagues Matthias Thoms and Michael Ameismeier, Robert looked at the structure of another viral protein, Nsp1. During the outbreak of another coronavirus, SARS-CoV-1, more than ten years ago, it was already known that this protein associates with the 40S subunit of the human ribosome and supresses the host's protein production. However, it was unclear how. In contrast to the research by Hauke and Goran, Robert reports that everything was "more straightforward than ever before". Using cryo-EM images as well, the team very quickly saw that Nsp1 not only attaches to the host's ribosome, but sticks in the entry channel of the ribosome, blocking it for mRNA and therefore preventing it from making proteins. The team also cooperated with members of Konstantin Sparrer's group at Ulm University, who showed that by stopping the protein production in human cells, Nsp1 also shuts down our innate immune response almost completely. "Blocking the ribosome channel should also prevent the host cell from transcribing viral proteins. But there are indications that the mRNAs of SARS-CoV-2 have a unique structure at their 5' end that might pry Nsp1 out of the channel, allowing viral proteins to be translated." The team achieved a very high resolution with its cryo-EM structure of 2.6 Å. Together with mutation studies, this allowed them to identify the exact residues of Nsp1 that bind to the ribosome and might be targeted by drugs. "This is especially interesting as many beta-coronaviruses have similar Nsp1 proteins and most likely block the ribosome through the same mechanism."

The research results obtained by an additional BIF alumnus, Professor Ivan Dikic at the University of Frankfurt, Germany, tie in directly with the immune suppressive abilities of the virus: he found that it entices the host cell to produce the viral protein PLpro (papain-like protease), which has two consequences: PLpro helps to build new viral particles, but also suppresses the production of the type 1 interferons that are part of the innate immune response, attracting killer cells to get rid of intruders. Ivan therefore sees blocking the production of PLpro as "a very promising double hit" against the virus as it curbs its replication and strengthens the immune response against it.

Asked about their experience with these projects, Hauke, Goran, and Robert say that the cooperation was exceptional and they were impressed by how quickly science can move: "During your main project, you want to do it all. If you prepare the sample, you also want to take the data and build the model. But in this case, everybody on the team did what they were best at," says Robert. "That way, we were very efficient and quick."

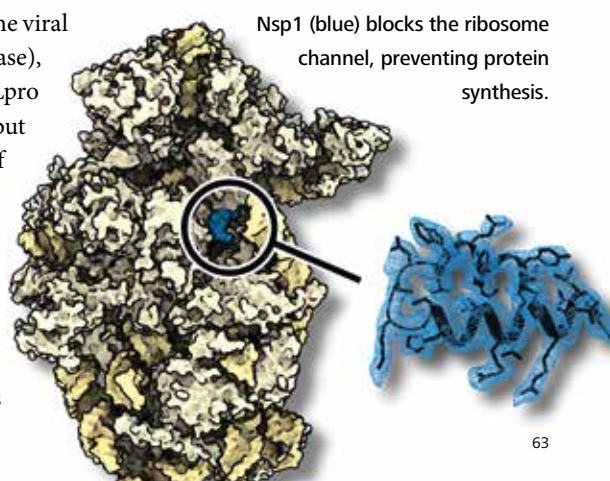
Hauke and Goran add that they have never found the term "equal contribution" to be as true as in this case – with five researchers involved, all sharing first authorship. They were also reminded never to discard or disregard a sample that does not fit expectations, because that just might be the most interesting one. And it was a great relief to be able to do something when the lockdown hit. As Robert puts it, "It felt good to return to the lab and try to do our part."

Hillen HS*, Kokic G*, Farnung L*, Dienemann C*, Tegunon D*, Cramer P (2020) Structure of replicating SARS-CoV-2 polymerase. *Nature* **584**: 154–156, DOI: 10.1038/s41586-020-2368

Kokic G, Hauke HS, Tegunov D, Dienemann C, Seitz F, Schmitzova J *et al* (2020) Mechanism of SARS-CoV-2 polymerase inhibition by remdesivir. *BioRxiv*, DOI: 10.1101/2020.10.28.358481

Thoms M*, Buschauer R*, Ameismeier M*, Koepke M, Denk T, Hirschenberger M *et al* (2020) Structural basis for translational shutdown and immune evasion by the Nsp1 protein of SARS-CoV-2. *Science* **369**: 1249–1255, DOI: 10.1126/science.abc8665

Shin D, Mukherjee R, Grewe D, Bojkova D, Baek K, Bhattacharya A *et al* (2020) Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity. *Nature*, DOI: 10.1038/s41586-020-2601-5



Nsp1 (blue) blocks the ribosome channel, preventing protein synthesis.

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.

SYNTHETIC GENETIC LANGUAGE GROWS

Emil Fischer and his colleagues in the lab of Floyd E. Romesberg at the Scripps Research Institute in La Jolla, CA, USA, have for the first time created a "semisynthetic" organism that can write "new words" using an expanded genetic alphabet and synthetic amino acids. They did so by altering a cell in such a way that it used a synthetic base pair to incorporate three synthetic amino acids into functioning proteins. All organisms depend on the genetic code of AGTC. Before the present study, the Romesberg group had already developed two new letters for the genetic alphabet, X and Y, which follow the same rules as the natural base pairs.

They now showed that out of the three-letter combinations, the codons, that encode amino acids many new unnatural combinations can lead to faithfully incorporated synthetic amino acid in a protein. They also showed that the new combinations are orthogonal, meaning that they work independently of each other: their new codons were faithfully translated encoding three different synthetic amino acids in the same protein. They used the green fluorescent protein GFP and could thus verify their success visually and via high-resolution mass spectrometry. If their cell glowed green and the mass of the protein was right, it meant GFP had been translated using their synthetic codon, which had incorporated their synthetic amino acid.

Thus, for the first time, the authors succeeded in completing the whole process from coding to protein synthesis using a synthetic code and artificial amino acids in living cells. These synthetic building blocks can be interpreted as new words in the genetic language that will make it possible to express new ideas – i.e. biological functions from sensing to chemical synthesis.

Semisynthetic organism using the new genetic code for protein translation.

REFERENCE

Fischer EC, Hashimoto K, Zhang Y, Feldman AW, Dien VT, Karadeema RJ *et al* (2020) New codons for efficient production of unnatural proteins in a semisynthetic organism *Nat Chem Biol* **16**: 570–576

Emil Fischer, fellow 2018–2020

UROMODULIN ROPES IN CYSTITIS-CAUSING PATHOGENS

Artistic illustration of bacterial pili binding to the sugar chains of uromodulin.

How the protein uromodulin protects against the urinary tract infection cystitis, usually caused by uropathogenic *E. coli*, has now been uncovered by Gregor Weiss from the group of Martin Pilhofer at the ETH Zurich in Switzerland. It was already known that bacteria bind with their hair-like pili to sugar chains on the surface of the cells of the urinary tract. It was also known that the 70% of people who have larger amounts of the protein uromodulin in their urine are better protected against cystitis, but not exactly why. Using cryo-electron tomography, Gregor and the other researchers now found that uromodulin forms long zigzag-shaped filaments with protruding arms on which it carries a large number of certain sugar chains. Further investigations showed that the filaments of uromodulin offer so many sweet binding sites for the bacteria in the urine that the bacterial pili tend to bind uromodulin instead of attaching to the bladder cell wall, ureter, or urethra. Furthermore, the uromodulin filaments entangle and literally rope together up to several hundred bacteria, preventing the pathogens from reaching the cells lining the urinary tract and thus from causing cystitis. The resulting clumps are then presumably removed via urination. The authors could show that uromodulin catches the bacteria not only in the petri dish, but also in patients with acute cystitis. Besides indicating that uromodulin's protective mechanism might also work against other pathogens, such as *Klebsiella or Pseudomonas*, the results have implications for finding new therapies for urinary tract infections. Targeting the binding mechanism of the pili to uromodulin to treat cystitis was thought to negate the proteins protective function. However, the authors found that once formed, the bonds between uromodulin and the bacteria's pili are so strong that they cannot be broken by active substances. Therefore, drugs targeting the same sugar binding mechanism can work in tandem with uromodulin.

REFERENCE

Weiss GL, Stanisich JJ, Sauer MM, Lin C-W, Eras J, Zyla DS *et al* (2020) Architecture and function of human uromodulin filaments in urinary tract infections. *Science* **369**: 1005–1010, doi:10.1126/science.aaz9866

Gregor Weiss, fellow 2016–2019

INTRODUCING TEAM MEMBER JAN KULLMANN

The neurobiologist Dr Jan Kullmann, born in Trier in 1981, joined the Boehringer Ingelheim Fonds in September 2019, taking over from Dr Carsten Lambert. His responsibilities include the selection process for BIF's PhD Fellowship Programme and the evaluation of applications for its Travel Grant Programme. This work entails interviewing candidates, selecting external referees, and – together with Vera Schlick – organizing BIF's Board of Trustees meetings. He has a background in developmental neurobiology and after completing his PhD in Germany, he pursued postdoctoral research in Memphis, TN, USA, and in Marburg, Germany. He worked predominantly on developmental processes such as the proliferation, polarization, and migration of neurons in the cerebellum.

"I enjoy meeting the talented PhD students applying for BIF grants and discussing the latest research endeavours with them. The wide variety of biomedical research disciplines I encounter in my work is fascinating." In his spare time, Jan loves to cook for his wife and two young daughters, play board games with them, and explore the forests around his home.

Photos: istock/polesnoy (left); Marzia Munafò (right); Kullmann: privat

WHO'S WHO AT BIF?

PROFESSOR THOMAS BRAUN, MEMBER OF BIF'S BOARD OF TRUSTEES

Thomas Braun studied philosophy and medicine in Göttingen and Hamburg, Germany, and in 1987 was issued his medical licence. In 1993, he earned his PhD and habilitation in cellular biochemistry in Braunschweig, Germany. After working in Oxford, UK, and in both Cambridges (UK and USA), Thomas started his own laboratory. In 1997, he accepted a position as associate professor in Würzburg and shortly thereafter as full professor in Halle-Wittenberg. In 2004, he became the founding director of the Max Planck Institute for Heart and Lung Research in Bad Nauheim and professor at nearby Giessen University (both Germany). He has published some 300 papers in leading journals. His research focuses on the mechanisms of heart development, the diseases and repair processes of the heart, as well as on organ remodelling and regeneration in general.

What is your most remarkable BIF experience?
The dedication and enthusiasm of my colleagues on the board when reviewing applications. I serve on several boards, but the BIF spirit is unique and results in highly interesting discussions when we evaluate proposals.

Why did you choose a science-based career?
Clinical work is not my ballgame – it's too repetitive and leaves very little room for creativity. Science-based careers provide the opportunity for freedom and autonomy that can rarely be found in other professions – provided you find the right position.

What is your favourite activity?
In the lab, it's talking to smart and intelligent students, postdocs, and colleagues. At home, it's reading a good book.

What is your remedy for stressful situations?
Don't take the situation too seriously. In 100 years, most of us will be forgotten.

What fault in others can you tolerate best?
I think I can tolerate most everything, but when it comes to ignorance and particularly to arrogance, I start to decompensate. If I sense that someone is sticking to an opinion despite overwhelming arguments, I tend to walk away.

Your advice for fellowship holders?
You may not believe it, but it's the best time of your life. Take any opportunity that suits you, take advantage of your privileged situation, and follow your interests. If you only think about your career but lose your curiosity, you'll fail.

What scientific achievement do you admire most?
We stand on the shoulders of many giants who made possible what we're doing today. I do see scientific progress as a collective achievement, enabled by the virtues of many. There are a lot of myths about solitary geniuses, but most of them aren't true. In my field I admire the work of Harold Weintraub, who demonstrated in the late 1980s that a single molecule is able to reprogramme most cells into muscle cells.

Name one thing you couldn't live without.
I like travelling, but I can easily cope with the current travel restrictions. If I didn't have enough time to read or interact with colleagues, I'd miss these activities, but I'd probably manage. Humans can adapt to all kinds of conditions, even if it does sometimes feel unbearable.

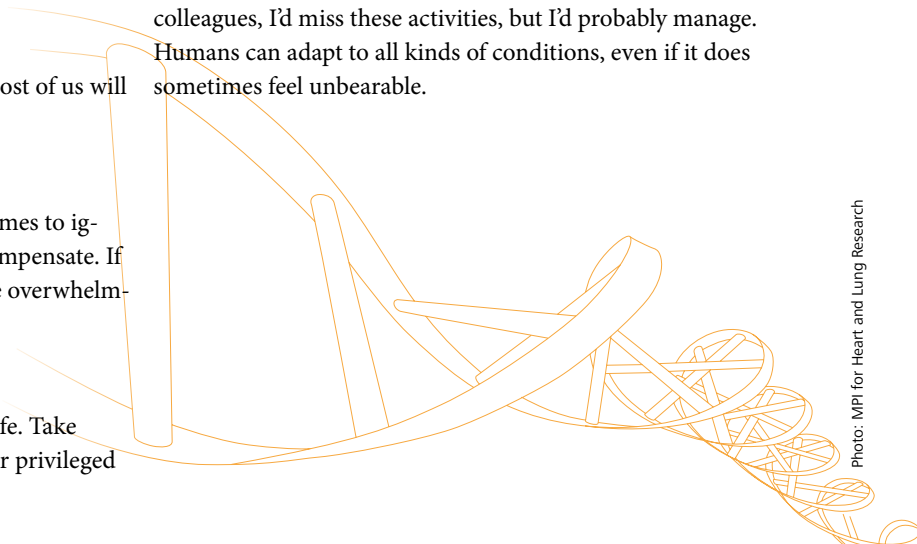


Photo: MPI for Heart and Lung Research

BIS WITH TWO NEW MANAGING DIRECTORS

The Boehringer Ingelheim Foundation (BIS) has two new managing directors. The Foundation's Executive Committee has appointed Dr Stephan Formella as Managing Director Science & Research and Mr Marc Wittstock as Managing Director Finance & Administration. BIS created both positions not only because it has grown substantially over the past 11 years under the stewardship of Dr Claudia Walther, but also because it plans to expand into additional areas of activity. Dr Claudia Walther will now focus on her role as Managing Director of the Boehringer Ingelheim Fonds (BIF) and will continue to run the Siblings Boehringer Ingelheim Foundation for the Humanities.

Over the past 16 years, Dr Stephan Formella has served with great success in various positions in the field of translational medicine at the Boehringer Ingelheim Group, most recently as Head of Early Clinical Operations. Marc Wittstock has spent the last 12 years working in different roles in the Department of Finance and Controlling at the Boehringer Ingelheim Group, most recently as Head of Corporate Treasury.

New and former managing directors of the Boehringer Ingelheim Foundation (l to r): Marc Wittstock, Dr Claudia Walther, Dr Stephan Formella

BIF's SISTER FOUNDATIONS

THE HEINRICH WIELAND PRIZE GOES VIRTUAL

Professor Craig M. Crews of Yale University, New Haven, CT, USA, has been awarded the 2020 Heinrich Wieland Prize, worth 100,000 euros, in recognition of having pioneered targeted protein degradation as a new principle in pharmacology. His research has opened the way for entirely new treatment options for numerous diseases, including certain types of cancer. In addition, he has founded two companies to chaperone his research from the lab bench to patients. The COVID-19 pandemic has made it necessary to postpone the festive award ceremony and scientific symposium until next year, but on 2 November, the Boehringer Ingelheim Foundation (BIS), which endows the prize, partnered with Berlin Science Week to host a virtual event with Professor Crews titled "Starting up Science: From Lab to Therapy". It featured a panel discussion on how ideas spawned from curiosity-driven research can pave the way for new therapeutic approaches and enable the successful founding of science-based start-ups in the United States and Germany.

From top left: laureate Craig M. Crews, science journalist Monika Seynsche, Andrea Tüttenberg, and Christian Hackenberger during the digital event.

Joining the award winner on the panel were Boehringer Ingelheim Prize Laureate Professor Andrea Tüttenberger (Mainz) and Professor Christian Hackenberger (Berlin), the first grantee of the Foundation's Plus 3 programme. At the end of the event, the audience of round 120 people engaged in a lively exchange with the panellists. A video can be found on the homepage of the Boehringer Ingelheim Foundation at: bistiftung.de/wissenschaftspreise/heinrich-wieland-preis-2020.html.

Photos: BIS (bottom);. BIS/Costard (top)

A BIF FELLOW'S GUIDE TO ...

STOCKHOLM

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 Dörte Schlesinger. She reports from Stockholm, Sweden, the city best known for hosting the Nobel Prize and the *Vasa* viking ship.

FACTS & FIGURES

Country: Sweden
Population: around 975,000
Area: around 190 km²
Students: around 90,000 (studyinstockholm.se/universities/)
Popular for fika, archipelagos, the Nobel Prize, midsummer celebrations, meatballs
Website: visitstockholm.com

BEST SIGHTS

Gamla Stan 4**:** narrow streets and colourful houses in Stockholm's Old Town.
Djugården: island park with the open-air Skansen Museum and the *Vasa* warship.
Södermalm 1**:** hip cafés, second-hand shops, nightlife, great views of the city.

RESTAURANTS

Hermans: a delicious, home-cooked, vegetarian buffet and stunning views of town.
Grillska Huset: typical, affordable fika (cosy coffee with cake) with roof terrace.
Bakfickan: small (slightly pricey) counter restaurant, great for Swedish meatballs.
Meno Male: excellent pizza, also for a take-away picnic on Norr Mälarstrand.

WHERE TO STAY

City Backpackers: cheap, close to the central station, Vasastan district, Old Town.
Den Röda Båten: rustic hotel and hostel on a boat in the heart of Stockholm.
Grand Hôtel: where the Nobel Prize laureates stay.

ACTIVITIES

Winter: ice-skate in Hellasgården, sweat in a sauna, take a brisk dip in one of the lakes, visit the many museums.
Spring: walk below the cherry blossoms of Kungsträdgården, visit Drottningholm Palace, and travel back in time at Skansen.
Summer: enjoy the free cultural festival, celebrate midsommar, spend the day in the archipelago 3, kayak in the waters around the city, explore the swimming spots.
Autumn: stroll through autumn foliage in Humlegården 2 or Tyresta National Park and enjoy the view through the sculptures in the Millesgården.

NIGHTLIFE

Trädgården: an outdoor venue where you can grab dinner and dance below the stars.
Södra Teatern: enjoy the outdoor concerts and beer garden at Mosebacketerrassen, dance in the 19th-century theatre, or sip a quiet drink on Champagnebaren balcony while looking out over the town.
Omnipollos hatt: a quirky popular bar with rotating experimental draft beers.
SoFo and Rörstrandsgatan: visit the pedestrian zones with their many restaurants, bars, and cafés.

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

Dörte Schlesinger is 27 years old and comes from Germany. She is studying at Karolinksa Institutet and her supervisor is BIF alumnus Professor Simon Elsässer.

Photos: Dörte Schlesinger

PROFILES

PROFESSOR DETLEF WEIGEL
Institute: MPI for Developmental Biology, Tübingen, Germany
Fellowship: 1987–1988

Detlef Weigel leads a team from Germany, France, and the USA that has been awarded one of the prestigious and highly competitive ERC Synergy Grants for the project PATHOCOM. They will use the 10 million euros to discover how pathogens team up to cause disease. They will study how frequently different types of interactions between microbes occur and how they are altered by ecology and genetics.

Detlef has also been awarded the 2020 Novozymes Prize, endowed with 3 million Danish kroner (approx. 400,000 euros) by the Novo Nordisk Foundation. The prize recognizes the outstanding research he has undertaken throughout his career, which has advanced plant research and spurred the development of innovative biotechnological solutions.

ASSIST. PROF. JULIA KAMENZ
Institute: University of Groningen, The Netherlands
Fellowship: 2009–2011

Since September, Julia has headed the Cell Cycle Dynamics Group at the Dutch University of Groningen as Rosalind Franklin Assistant Professor. Her team is studying the intricate regulation of the kinases and phosphatases involved in cell division processes, the impact of post-translational modifications in the regulation of cell division, and the complex signalling networks surveying and controlling cell cycle progression.

PROFESSOR MARIA HONDELE
Institute: University of Geneva, Switzerland
Fellowship: 2008–2011

ASSIST. PROF. OLEG SIMAKOV
Institute: University of Vienna, Austria
Fellowship: 2008–2011

Two BIF fellows have received prestigious ERC Starting Grants worth 1.5 million euros: **Maria Hondele** will use hers to investigate how certain non-membrane bound organelles assemble and function, selectively accumulate macromolecules, and control the fate of messenger RNAs. **Oleg Simakov** will investigate and characterize modes of genome evolution during major transitions in animal evolution on multiple levels of genome organization. He will focus particularly on the enigmatic clades of cephalopods using the emerging model organism *Euprymna scolopes* (the Hawaiian bobtail squid).

PROFESSOR ANDREAS BARNER
Shareholders' Committee, C.H. Boehringer Sohn AG & Co. KG, Ingelheim, Germany, Chairman of BIF's Board of Trustees

The chairman of our board Andreas Barner has received the highest distinction bestowed by the German government for services to society: the Federal Cross of Merit, First Class. It honours his outstanding contributions to the business community and science. When presenting the award, Malu Dreyer, minister president of Rhineland-Palatinate, emphasized Barner's energy and unwavering support for young researchers.

PROFESSOR SIMON ELSÄSSER
Institute: Karolinska Institutet, Stockholm, Sweden
Fellowship: 2008–2010

Simon Elsässer has received a Proof of Concept Grant worth 150,000 euros from the ERC for his project "Highly Multiplexed, Quantitative Epigenetic Profiling". Simon has also been accepted into the 7th Generation of Future Research Leaders, a prestigious programme funded by the Swedish Foundation for Strategic Research.

ASSIST. PROF. TORSTEN MEISSNER
Institute: Beth Israel Deaconess Medical Center, Boston, MA, USA
Fellowship: 2002–2004

Torsten Meissner has been promoted to assistant professor of surgery at the Beth Israel Deaconess Medical Center at Harvard Medical School. His research focuses on the generation of immune-silent blood vessels from stem cells for disease modelling and vascular reconstruction.

UPCOMING EVENTS

To protect our fellows and do our share in preventing the further spread of SARS-CoV-2, we have cancelled or postponed all in-person meetings until March 2021. As soon as dates for upcoming meetings are set, we will invite all participants personally and publish the information on our website: https://www.bifonds.de/news-network/seminars-events.html

Photos: Weigel: MPI DB; B; Kamenz: University of Stanford; Hondele: University of Basel; Simakov: University of Vienna; Barner: BI; Elsässer: private; Meissner: BIDMC

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