FUTURA

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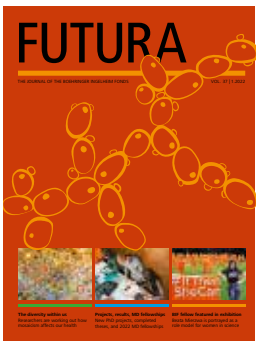
The diversity within us
Researchers are working out how mosaicism affects our health

Projects, results, MD fellowships
New PhD projects, completed theses, and 2022 MD fellowships

BIF fellow featured in exhibition
Beata Mierzwa is portrayed as a role model for women in science



The cover illustration shows a simplified model of *Saccharomyces cerevisiae*. The yeast is highly useful in the lab because it is easy to grow in enormous numbers and is a eukaryote with organelles and a nucleus. *S. cerevisiae* has become a mainstay of research into the basic operations of eukaryotic cells, including gene regulation, division, protein secretion, and RNA transcription.

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PHD RESULTS

MD PROJECTS

FOUNDATION

"IF SHE CAN SEE IT, THEN SHE CAN BE IT"

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55116 Mainz
Germany
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E-mail: secretariat@bifonds.de
www.bifonds.de

Editor-in-Chief Dr Stephan Formella

Editors Kirsten Achenbach (BIF, executive editor), Dagmar Puh (muehlhausmoers corporate communications gmbh)

Authors in this issue Kirsten Achenbach, Dr Mitchell Leslie

Translating, copy-editing, and proofreading Adam Blauhut, Dr Caroline Hadley

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CHANGING THE WATCH, NOT THE COURSE

In this interview, BIF's former director and her successors answer questions about how they see the transition and what will come next.

At the end of June, Claudia Walther handed over the helm at BIF. For 23 years, she not only worked at but lived BIF: first as one of its senior officers and since 2008 as BIF's third managing director. From 2009 on, she set up the rapidly growing Boehringer Ingelheim Foundation (BIS). In 2020, Dr Stephan Formella and Marc Wittstock came aboard the Boehringer Ingelheim Foundation family ship to jointly steer the BIS as Managing Director of Science and Research and Managing Director of Finance and Administration, respectively. During the past year they also learned the ropes at BIF in preparation for taking over the helm from Claudia Walther. Despite the changing of the watch, BIF will hold a steady course and remain dedicated to funding outstanding basic research, giving comprehensive support to its fellows and alumni, and helping them to achieve their best.

Stephan and Marc joined the foundations when Corona had just started. How did this impact the transition?

Stephan Formella: It gave us sufficient time to approach the new working environment and learn the routines in a non-for-profit organization – precious time that would have been hard to find in a business-as-usual scenario. At the same time, we also had to wait until we could experience the reality of BIF at full steam with all its events such as communication and progress seminars.

Claudia Walther: The foundations are not large in terms of personnel, which means the diversity and depth of the work is much greater for everyone, from funding strategy and staff decisions to website design, from counselling fellows to IT, to handling legal issues and BIF's budget, etc. The restrictions gave us more time to talk about the many aspects of BIF and BIS, which was at least one good thing about the pandemic. →

Photos: BIF/C. Costard

»Rest assured, BIF's core principle remains rock solid: selecting the right people and supporting scientific excellence.«

Stephan Formella

Claudia, what was your motivation to join BIF 23 years ago?

Claudia Walther: BIF offers the rare combination of truly exciting research across all disciplines of basic biomedical research, ample exchange with leading scientists, and the comprehensive promotion of outstanding young talent. I wanted to be part of an organization that strives for excellence as rigorously as does BIF. And as a BIF alumna, I knew how special BIF's support is and what a difference it can make.

Stephan and Marc, you were both part of the Boehringer Ingelheim company. What made you switch to the non-profit world?

Stephan Formella: A key driver of my entire professional career has been the desire to feel a purpose in my work and the excitement of exposing myself to new environments and circumstances. The prospect of being exposed to a huge number of outstanding figures in the world of the biomedical sciences, supporting them, and designing the right projects and programmes was something that intrigued me from the start.

Marc Wittstock: I see the move into the world of non-profit organizations as a continuation of my personal and professional development over the last 25 years. I started my professional career in the very dynamic area of investment banking. Ten years later, I switched to the Boehringer Ingelheim company, partly because their medical products have a positive impact on people's lives. The older I got, the more I appreciated this. Now, working for the foundations, I can make an even more direct contribution to humanity – which is a very good feeling.

Why have a Managing Director of Finance and Administration?

Marc Wittstock: Both foundations have grown over the past few years. Over time, the administrative issues and the bureaucratic and regulatory requirements have become much more complex. In addition, I have taken on a few topics that were outsourced for several years, such as managing the foundations' financial assets. The income generated by these assets provides the funds for our programmes and is an important responsibility.

With all the economic upheaval around the globe – is BIF's future secure?

Marc Wittstock: Regarding the future of BIF, we are very pleased to note that just recently BIS decided to financially support BIF for another 10 years. BIF can look forward to a very positive future indeed.

Unlike many other organizations, the three foundations[1] have a very secure financial basis because the Boehringer and von Baumbach families are unwaveringly dedicated to them and their aims. In these uncertain times, the families' support of basic research gives scientists the necessary freedom to further our knowledge for the good of all of us.

Where do you see the differences between BIF and BIS? Will they be merged now that you are managing both?

Stephan Formella: Starting with your second question, the answer is a clear "no". BIF and BIS are completely separate, independent legal entities and will remain so in future. While both fund research in the life sciences, their programmes focus on different stages – BIF predominantly takes care of PhD students and other junior researchers, while BIS focuses its programmes on individual mid-career researchers and larger institutional funding measures, such as its support for the Institute of Molecular Biology (IMB) in Mainz or the EMBL.

Claudia, you've achieved so many things with the foundations. What are you most proud of?

Claudia Walther: First and foremost, having been able to make a difference in the lives and careers of so many junior scientists with BIF. Seeing them thrive in many different walks of life – that's very special to me. Also, successfully steering BIF through very difficult times and furthering its excellence while preserving its spirit.

In regard to BIS – leading BIS together with the executive committee and develop-

»Unlike many other organizations, the three foundations have a very secure financial basis because the Boehringer and von Baumbach families are unwaveringly dedicated to them and their aims.«

Marc Wittstock

ing it from a small, rather unknown organization into one of the largest foundations in Germany with all that entails. And of course, supporting the IMB from its conception and foundation.

Stephan and Marc, what traits of yours do you think will be most beneficial to BIF?

Stephan Formella: I think being a careful and sensitive listener and a strong observer coupled with the right degree of intuition. These are not only important when it comes to personal interviews but also during the entire journey from applicant to seasoned alumnus.

Marc Wittstock: That's a difficult question. I act calmly and prudently. I'll try to manage BIF's financial and administrative issues as discreetly as possible behind the scenes.

Of course, the most pressing question for everyone: what will change?

Stephan Formella: Instead of change, I see a continuous development, an adaptation to demands, requirements, and constraints by our fellows and the academic landscape. BIF wants to remain on the cutting edge. So while we want to change as little as possible, whenever change is needed to provide the right level of support to our fellows, we will adapt. But rest assured, BIF's core principle remains rock solid: selecting the right people and supporting scientific excellence. We will do so by focusing on the individual human being and fostering the long-term network of the BIF community.

Will there still be hiking in Hirschegg?

Stephan Formella: Of course, how could we change this wonderful tradition?

Claudia, in addition to science, one of your passions is climbing. What mountains do you plan to climb next?

Claudia Walther: I am indeed looking forward to having more time for some of my other passions such as travelling, hiking, climbing, reading, and my garden. To begin with, I went to Japan to hike up the beautiful Mount Fuji. I'm currently deciding where my professional path will lead me.

What are your passions, the ways you relax?

Stephan Formella: I go for a run in the forest, jump on a bike, or go for a ride on a motorcycle. Being in motion in a natural environment and being on my own for a time are good ways for me to gain new energy.

Marc Wittstock: I have a passion for sports in general. Besides that, I spend as much time as possible with my family. Now that my boys are old enough, I can combine both.

One last question: as an alumna, you'll surely remain part of the BIF family, won't you?

Claudia Walther: With pleasure – as BIF has always said: once a BIFler, always a BIFler.

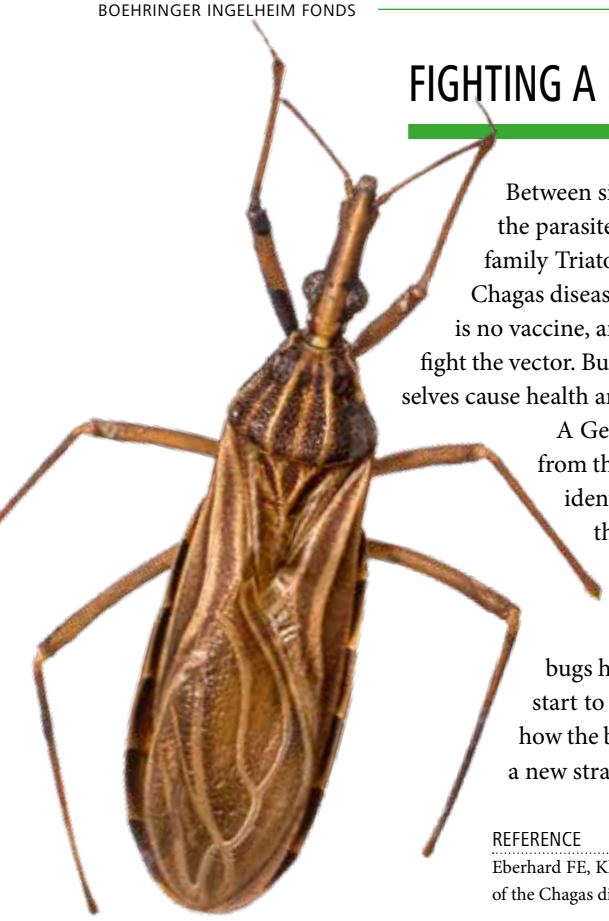
1 In addition to BIF and BIS, there is a third sister foundation, the Siblings Boehringer Ingelheim Foundation for the Humanities, which disburses printing grants for academic works in the humanities. Its management has also passed into the hands of Stephan Formella and Marc Wittstock.

SELF-PORTRAIT ON THE GENE LEVEL ON 314,000 PAGES

By the artist Alicja Kwade, Germany

Do you know how large your genome is? Or how small the differences are between people? The answers are 1) about 3.1 billion base pairs, and 2) less than 0.01%. The image shows the work *Self-Portrait* by German artist Alicja Kwade at the Berlin Gallery. She had her genome sequenced, analyzed, and printed out – all 314,000 pages of it. Around 12,000 pages contain gene sequences unique to her genome, printed in bold. These pages were hung on the walls. The rest were stored in copper containers. Between two bold sequences there are sometimes several thousand letters, highlighting the fact that whatever our origins, our genomes are almost identical. Even the bioinformatician who identified these passages, Professor Sven Rahmann of the University of the Saarland, said that seeing the genome displayed in this way allowed him to grasp the genome on a wholly different level. The bronze columns are made to look like stacked smart phones forming the double helix of DNA.

https://berlinischegalerie.de/en/exhibition/alicja-kwade/



FIGHTING A BUG'S GUT BACTERIA TO PREVENT CHAGAS DISEASE

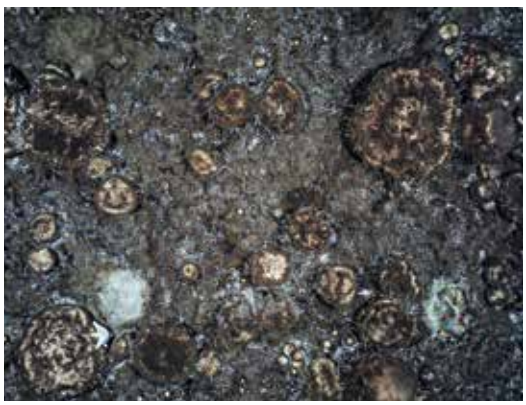
Between six and seven million people worldwide, mostly in Central and South America, carry the parasite *Trypanosoma cruzi*. The parasite is transmitted via blood-sucking bugs of the subfamily Triatominae and causes Chagas disease. While the acute infection is often mild, chronic Chagas disease can be deadly, leading to an enlarged heart and paralysis of the patient's gut. There is no vaccine, and treating advanced stages is difficult. So the main strategy against the disease is to fight the vector. But the bugs increasingly develop resistance against the insecticides used, which themselves cause health and environmental problems.

A German-Brazilian team is therefore taking a different tack: they want to heal the bugs from the parasitic infection by engineering probiotic bacteria for them. They first needed to identify suitable bacteria in the host's gut. Good targets are species that are essential for the bug's survival. To find such targets, they infected the bugs with the parasite and used metagenomic shotgun sequencing to study the bug's microbiome. This enabled them to identify four bacteria as suitable targets. These bacteria produce vitamin B, which the bugs do not get from their blood meals. This is why practically all bugs have them and why the bacteria stay in their guts across generations. Before they can start to engineer the probiotic bacteria, the researchers need further studies to understand how the bacteria interact with one another. But they have taken a very promising step towards a new strategy against a deadly disease by investigating which bacteria a bug has in its guts.

REFERENCE

Eberhard FE, Klimpel S, Guarneri AA, Tobias NJ (2022) Exposure to *Trypanosoma* parasites induces changes in the microbiome of the Chagas disease vector *Rhodnius prolixus*. *Microbiome* **10**: 45 https://doi.org/10.1186/s40168-022-01240-z

SPONGE GARDENS BENEATH THE ARCTIC ICE CAP



The dense sponge grounds represent an astonishingly rich ecosystem, demonstrating the ability of sponges and associated microorganisms to exploit a variety of refractory food sources.

There is little life in the dark and freezing depths beneath the permanent ice of the Arctic Ocean. Imagine the surprise, then, when researchers from Bremen and Kiel, Germany, found veritable gardens of giant sponges on the Langseth Ridge, an underwater mountain range near the North Pole. When it comes to nutrients, this ridge is one of the most barren stretches the ocean has to offer. So what feeds these hotspots of sponges, soft corals, small fish, and other animals? It turns out that they live off the remains of a once vibrant ecosystem. Thousands of years ago, nutrient-rich fluids seeped from a now extinct underwater volcano, feeding chemoautotrophic bacteria, the basis of a then diverse ecosystem. The fluid seeps eventually dried up and the bacteria and animals they nourished died, but the nutrients from their remains are currently feeding the sponge gardens. The sponges shelter a complex community of microorganisms that can convert the ancient remains into forms of carbon and nitrogen the sponges can take up. Such symbiotic relationships are typical for many sponges, the simplest of animals. Within their cups and on their sides, the sponges also offer protection and a habitat for crabs, feather stars, and other species. The biomass of these newly discovered gardens is similar to that of the sponge grounds in shallower waters with a constant nutrient input. The researchers assume that the sponge gardens under the arctic ice might, however, be only transient, despite sponges being able to live far longer than 100 years.

REFERENCE

Morganti TM, Slaby BM, de Kluijver A, Busch K, Hentschel U, Middelburg JJ, Grotheer H, Mollenhauer G, Dannheim J, Rapp HT, Purser A, Boetius A (2022) Giant sponge grounds of Central Arctic seamounts are associated with extinct seep life. *Nat Commun* **13**: 638 https://doi.org/10.1038/s41467-022-28129-7

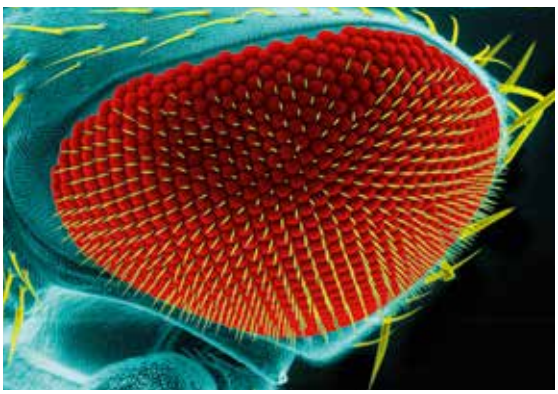
Photo: PS101 AWI OFOS system (bottom); alamy (top)

FLIES MOVE THE RETINA INSTEAD OF THE WHOLE EYE

When we try to catch a fly, the six muscles in each of our eyes work hard on tracking and focusing the fly. Evolution has found a different solution for the compound eyes of flies – and maybe other arthropods. Their eyes consist of a large number of fixed lenses, each with their own tiny retina. Although it was known that houseflies have two muscles attached to their retinas, their role in vision was unclear. Researchers from Germany and the United States have now shown that fruit flies move the retinas underneath each compound eye to track objects – such as a hand trying to catch them – and to give them depth vision. The movement of the fly retinas relative to the tracked object slows the perceived motion and stabilizes the image. However, as the retinas cannot move indefinitely in one direction, they jump back to a neutral position in fast movements at irregular intervals. This is the same strategy as in the vertebrate eye. The biggest difference: in flies the retina needs to move in the opposite direction of the object, as only the retina moves and not the retina and lens. Being able to move their retinas also gives the flies depth vision, which helps them to decide whether they can step across a gap. These striking similarities are an impressive example of convergent evolution and underscore the need to move at least part of your eyes. Next, the researchers want to study how the fly brain can distinguish whether a perceived movement is due to the retina or the object moving. As fruit flies are used to study vision in general, understanding their visual system better will help us understand our own visual processes better.

REFERENCE

Fenk LM, Avritzer SC, Weisman JL, Nair A, Randt LD, Mohren TL *et al* (2022) Muscles that move the retina augment compound-eye vision in *Drosophila*. *Nature*, doi: 10.1038/s41586-022-05317-5



The compound eye of a fruit fly seen up close.

BREATHING HELPS TO FORM MEMORIES AT NIGHT

Do you want to improve your memory? You might want to think about your breathing during sleep. At night, our brain is busy with sorting and storing all the day's impressions. Studies by neuroscientists from Munich, Germany, have now shown that breathing provides the necessary rhythm to coordinate the different brain regions of the limbic system that are involved in memory consolidation, including those in the hippocampus, medial prefrontal and visual cortex, thalamus, amygdala, and nucleus accumbens. Breathing, as the most essential body rhythm, was already known to modulate functions from attention to thought structure; however, it does not coordinate our activity across different brain regions while we are awake. During waking hours, this role is reserved for the sensory-motor input. During sleep, though, the brain is decoupled from the sensory-motor system. To test whether breathing takes on the role of pacemaker at night, the scientists monitored the activity of thousands of nerve cells in the limbic system of mice during sleep. They found that breathing rhythm modulates the excitability of the nerve cells within and between the studied areas, thereby coordinating their activity. The authors now hope that the newly discovered phenomenon, which they termed "respiratory corollary discharge", will lead to new theories and experiments that explain how breathing and memory consolidation are mechanistically connected. They also discovered that the link between breathing and brain activity during sleep is independent of olfactory inputs. This topples the current theory that breathing influences the limbic system only via olfactory cues.

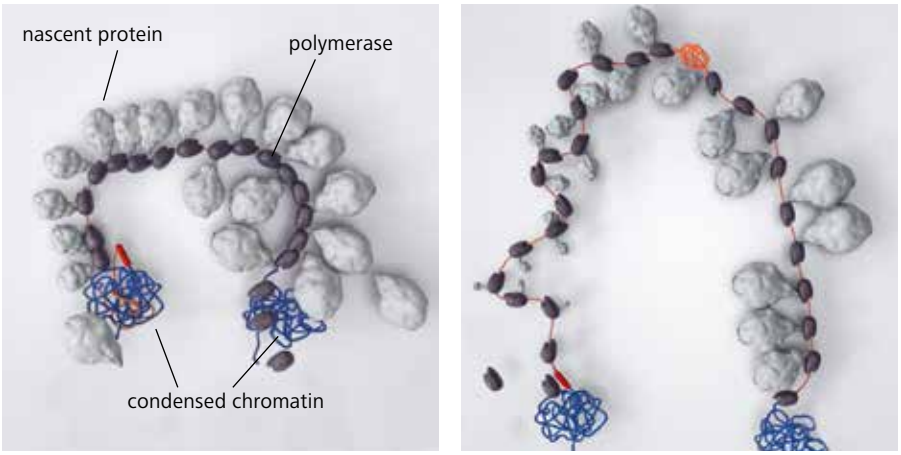
REFERENCE

Karalis N, Sirota A (2022) Breathing coordinates cortico-hippocampal dynamics in mice during offline states. *Nat Comm* **13**: 467

Photo: alamy (bottom left); GettyImages/DeanDrobot (top middle); Irina Solovei, Jack Bates, Susanne Leidescher, and artists Dimitar Kralev & Tamako Suzuki (animation top right)

Breathing coordinates our activities across different brain regions while we are asleep.

ARE TRANSCRIPTION LOOPS IN SMALL GENES JUST TOO SMALL TO BE SEEN?



Polyermases (dark grey) pack onto a gene (orange) leading to a transcription loop.

Up to now it was thought that there are two ways in which individual genes are organized in space during transcription: factories and loops. In transcription factories – considered the norm – genes move through static clusters of polymerases. In transcription loops – so far only seen in so-called lampbrush chromosomes – the polymerases travel along DNA loops extruded from the chromosome. We know this because these loops are visible under the microscope. An international team of researchers doubted that there are two different versions of a process as important as transcription. Most of our knowledge about transcription stems from smaller, highly expressed mammalian genes, while lampbrush chromosomes are only found in non-mammals. Perhaps the loops of mammalian genes are just too small to be visible under light microscopes? To test this idea, the researchers searched for very large, highly expressed genes in cultured mammalian cells – a rarity – and studied them during transcription. They found that polymerases attach to the gene one after the other and then travel along the strand. With more and more polymerases, the densely coiled gene straightens and extrudes as a loop from the chromosome. The many polymerases with their growing and maturing proteins cause the loop to stiffen, pushing the chromosome slightly apart. The researchers postulate that in short genes with few introns, the smaller nascent proteins take up much less room, which possibly explains why short genes do not form visible transcription loops. Based on their data and simulations, the researchers propose that the mechanism for transcription loops is a general aspect of transcription in eukaryotes.

REFERENCE

Leidescher S, Ribisel J, Ullrich S, Feodorova Y, Hildebrand E, Galitsyna A *et al* Spatial organization of transcribed eukaryotic genes. *Nat Cell Biol* **24**: 327–339

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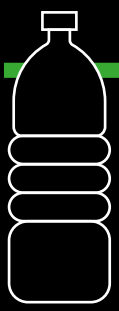

HOURS

is the time a newly discovered protein needs to enzymatically dissolve 90% of a bottle made from PET plastic. This is twice as fast as the former record holder. The protein also produces raw materials for new bottles.

Source: "Low Carbon Footprint Recycling of Post-Consumer PET Plastic with a Metagenomic Polyester Hydrolase"
https://doi.org/10.1002/cssc.202101062

PROFILE OF SACCHAROMYCES CEREVISIAE

By Mitch Leslie

Between 1999 and 2016, seven researchers who studied the yeast *Saccharomyces cerevisiae* won Nobel prizes. This run of successes illustrates how important the fungus has become for scientific research.

Saccharomyces cerevisiae is highly useful in the lab because it fills a gap. It shares many of the advantages of bacteria. Yeast cells are small and fast-growing, producing a new generation every 90 to 120 minutes. As such, they are easy to raise in enormous numbers. But like mice, fruit flies, and humans, *S. cerevisiae* is a eukaryote with organelles and a nucleus. The similarities extend beyond cellular structure. About one-third of *S. cerevisiae*'s roughly 6,000 genes have equivalents in humans. As a result, *S. cerevisiae* has become a mainstay of research into the basic operations of eukaryotic cells, including gene regulation, division, protein secretion, and RNA transcription.

Brewers, bakers, and vintners have been experimenting with *S. cerevisiae* for thousands of years, but scientists did not confirm it was alive until the early 1800s. Early on, the fungus proved its scientific value in studies of cell metabolism. In 1857, the French chemist Louis Pasteur showed that yeast cells generated alcohol through fermentation of glucose. Forty years later, the German chemist Eduard Buchner demonstrated that this process could occur even in the liquid from crushed yeast – no living cells were required. That revelation, which earned him the Nobel Prize in Chemistry in 1907, meant that scientists could study biochemical reactions outside of cells – a contentious idea at the time.

S. cerevisiae has also proven crucial for teasing out the functions of proteins and for determining how molecules work together to orchestrate cellular activities. By identifying mutant yeast with defects in the cell cycle, for instance, the biologist Leland Hartwell of the University of Washington tracked down more than 100 so-called CDC proteins that control a cell's progression through the cycle. Comparable proteins in humans are often defective in cancer. Hartwell shared the 2001 Nobel Prize in Physiology or Medicine for his discoveries.

The 2016 laureate in Physiology or Medicine, Yoshinori Ohsumi of the Tokyo Institute of Technology, also harnessed mutant yeast to uncover many of the proteins that control autophagy, a process that allows cells to remove and recycle worn-out organelles and proteins.

The main drawback of *S. cerevisiae* as a model organism is that it is single-celled. Because yeast cells do not congregate into tissues or organs, they can provide only limited information about how these complex structures form and function. Still, scientists continue to rely on the fungus to probe the mechanisms of ageing, develop new cancer treatments, and perform a range of other investigations.

Researchers have also turned to a distant relative of *S. cerevisiae*, the fission yeast *Schizosaccharomyces pombe*. One of *S. pombe*'s advantages is its simplicity – it has only three chromosomes versus *S. cerevisiae*'s sixteen. In addition, *S. pombe* shares key proteins with humans that its cousin lacks, including those that orchestrate RNA interference. However, *S. pombe* is haploid during most of its life cycle, whereas, like most multicellular eukaryotes, *S. cerevisiae* is frequently diploid.

CV OF *SACCHAROMYCES CEREVISIAE*

- I am about 5-10 µm in diameter.
- I can reproduce as often as once every 90 minutes.
- I feed on sugars and other organic molecules.
- I work mainly in molecular biology, genetics, and biotechnology.
- I have helped researchers win 11 Nobel Prizes.

Photos: eye of science (right); iStock / Getty Images Plus/ Claudio Rampinini (background)

The cells within each organism form a diverse and complex mosaic.

Photo: Adobe Stock/ktiannguen0128

THE **DIVERSITY** WITHIN US

Mitch Leslie

The girl born at Lucile Packard Children's Hospital in Stanford, California, in 2013 was only one hour old when her heart problems began. The upper and lower chambers of her heart were not beating in synchrony, and she suffered frequent episodes in which the ventricles raced. To stabilize her heartbeat, the doctors implanted a pacemaker and defibrillator; eventually the girl would need a heart transplant.

Determining what caused the newborn's heart to contract erratically took some detective work. Her doctors suspected long QT syndrome, a potentially fatal arrhythmia that can result from flaws in several genes. But tests for DNA alterations that can cause the syndrome were negative.

A more comprehensive and sensitive technique, whole genome sequencing, provided the answer. Sequencing DNA from the girl's blood and from cells in her saliva revealed an unusual mutation in the gene *SCN5A*, which codes for a protein that helps to control the heart's electrical activity. The surprise was that the mutation occurred in only about 8% of the girl's cells.

As doctors and researchers reported in 2016, the girl had somatic mosaicism, in which cells in the body of an individual differ genetically from one another. They calculated that the mutation in *SCN5A* probably arose in a single cell when the girl was still an embryo. When this original cell divided, it passed down the mutation to its offspring. They in turn transmitted the glitch to their descendants and so on until the mutation had spread to nearly one in ten of her cells.

Although the girl's heart condition was unusual, she has a lot in common with the rest of us. As researchers have discovered in the last couple of decades, all humans are mosaics. The cells within our bodies are genetically diverse, thanks to mutations that →

MOSAICS AND CHIMERAS

X chromosome inactivation gives calico cats their distinctive look

Mutations are not the only cause of mosaicism in the body. The size disparity between the sex chromosomes in mammals – about 90% of the genes on the X chromosome are missing from the Y – is another. To balance gene activity, each cell in females shuts down most of the genes on one of the two X chromosomes, a mechanism known as X chromosome inactivation. Which chromosome a cell silences is random, resulting in a mosaic distribution of the alleles of a given gene active throughout the body.

X chromosome inactivation produces the patchwork of black and orange blotches on calico cats. Another consequence of the shutdown is that some diseases caused by faulty genes on the X chromosome are less severe in women – the defective gene is switched on only in about half of a woman's cells. One example is a variety of the inherited eye disease retinitis pigmentosa, in which the retina of the eye deteriorates. Men with the condition are typically legally blind by their 30s or 40s, whereas women rarely lose their sight. X chromosome inactivation differs from mutation-induced mosaicism because it involves changes in the activity of genes, not their DNA sequence.

A person's body may also harbour divergent DNA because the cells come from different individuals, a phenomenon known as chimerism. One way this mixing can come about is when two zygotes developing in the uterus combine into a single embryo. After birth, the baby will contain two distinctive lineages of cells. If the zygotes were different genders, the baby might have some cells with an X and a Y chromosome and some cells with two Xs. Developmental chimeras like this are rare, but a more common form of chimerism occurs when some of the fetus's cells slip into the mother's body during pregnancy. These cells may account for about one in ten million cells in a woman's bloodstream, researchers have found, and they can persist for more than 30 years in her body. Unlike genomic mosaics, chimeras do not involve the accumulation of mutations.

begin to accumulate shortly after fertilization and continue to build up throughout life. "We don't have a single genome – we have thousands of different ones," says genome biologist Alexander Bick of Vanderbilt University Medical Center in Nashville, Tennessee.

Researchers are still working out how this cellular heterogeneity affects our health. They suspect that in some cases mosaicism is beneficial, helping cells to specialize or adapt. The alterations may expand the capabilities of our brains, for instance. The accumulation of mutations "could be a mechanism that further diversifies our brain cells," says neuroscientist Tracy Bedrosian of Nationwide Children's Hospital in Columbus, Ohio. But according to neuroscientist Joseph Gleeson of the University of California, San Diego, since these mutations can occur throughout the genome, "They are going to land on some genes that cause disease." Studies have implicated mosaicism in a range of illnesses, including cancer, heart disease, and diabetes. Mosaicism has also been linked to ageing, although whether it is a cause or a consequence is not clear, Gleeson notes.

For more than a century, scientists have known that somatic mosaicism occurs in a variety of organisms. Some people, such as former Soviet leader Mikhail Gorbachev, are born with a red or purple patch on their face or neck known as a port wine stain. In this section of skin, the capillaries are abnormally large, conferring the distinctive colour. Dogs with the striped pattern known as brindle are mosaics. In plants, the most famous instance of mosaicism is the differently coloured kernels in some ears of corn. Geneticist Barbara McClintock of the Cold Spring Harbor Laboratory in New York won the Nobel Prize in Physiology or Medicine in 1983 for dissecting how these patterns develop.

Despite these examples, scientists thought that mosaicism was rare. Until recently, the conventional wisdom held that almost all of the cells in our bodies harbour identical DNA. Researchers believed that the only exceptions were sperm and eggs – which only carry one copy of each chromosome – as well as B and T cells, both defensive cells of the immune system. As B and T cells mature, they reshuffle the genes that code for antibodies and pathogen-detecting receptors, removing some DNA segments and recombining the remaining ones. Because there are so many possible combinations of the gene segments – according to one estimate, our T cells can potentially make more than 10^{20} versions of their receptor – each person's immune cells are genomically diverse.

Still, simple calculations suggest that mosaicism was much more common, says evolutionary biologist Steve Frank of the University of California, Irvine. Each time a cell divides, on average it picks up one new mutation. So the 50 to 100 trillion cells in the

Photo: Adobe Stock/Vladimir Liverts

human body should sport a large number of mutations, he says. The problem is that "historically, people couldn't see into the body and identify these mutations."

In the last decade or so, however, new technologies have allowed scientists to detect these alterations. The two most important techniques for identifying mutations have been whole genome sequencing, which can scan all of a person's DNA for variants, and single-cell sequencing, which can reveal changes within individual cells.

When scientists applied these techniques, they found that mutations are plentiful and widespread in the body. "We used to believe we inherited our DNA from our parents and it was the strict blueprint for life. We now know that isn't true," says Bedrosian. Researchers have also discovered that DNA alterations accrue at a regular pace. "The mutation rate is roughly constant across all tissues and across ages," says computational biologist Alexej Abyzov of the Mayo Clinic in Rochester, Minnesota. At birth, each of a baby's cells already carries 200 to 1,000 mutations, he says. By the time that child reaches old age, each cell may sport 3,000 to 4,000.

Almost all of the mutations in our cells "are probably benign," says Abyzov. They are located in non-coding portions of the genome and do not affect a cell's fitness. However, some mutations alter cell functions and may have negative or positive effects on our health. Researchers are starting to uncover the mechanisms.

One type of mosaicism with broad impact on health, known as clonal haematopoiesis, develops in the blood. New blood cells form when stem cells in the bone marrow divide. Over time, the stem cells acquire mutations, and occasionally one of them picks up a mutation that enables it to divide faster than other stem cells or that provides another competitive advantage. As a result, over decades the mutated cell and its offspring, known as a clone, come to constitute a larger and larger share of the cells in the blood and bone marrow. Scientists have determined that in some people with clonal haematopoiesis a single clone can account for 10% or more of white blood cells. The condition becomes more common with age, occurring in less than 1% of people under 40 but more than 20% of people over 70.

Most people with clonal haematopoiesis suffer no ill effects. But there are "multiple lines of evidence" that it can cause disease, says Bick. People with the condition are ten times more likely to develop blood cancers such as acute myeloid leukemia. That connection makes sense – many of the genes mutated in clonal haematopoiesis are also faulty in these cancers. But clonal haematopoiesis also doubles the odds of developing heart disease, and researchers have linked it to other illnesses, including type II diabetes, kidney disease, and chronic obstructive pulmonary disease. The condition may contribute to such a wide range of illnesses because it promotes inflammation, a common factor in many chronic diseases.

What happens in the blood also happens elsewhere in the body, including the bladder, esophagus, and skin. Rather than being peaceful collections of cooperating cells, tissues become patchworks of different clones, generated by somatic mutations, that vie with one another. "It's the war of the clones," says statistical geneticist Carl Anderson of the Wellcome Sanger Institute in Hinxton, United Kingdom. In a 2020 study, he and his colleagues investigated this contest in the intestines, where stem cells live in deep pits known as crypts. The stem cells in each crypt belong to the same clone, and they typically do not stray from their home crypt. In patients with inflammatory bowel disease (IBD), however, bouts of inflammation wipe out most of these stem cells. The survivors then begin to reproduce, competing with one another to colonize the vacated space.

When the researchers sequenced DNA from the stem cells, they discovered that some of the winners of this competition carried mutations common in cancer, which presumably allow faster division. But some of the cells also showed alterations in genes that help to control inflammation. In this case, Anderson says, mosaicism seems to be beneficial: "We often think about somatic mutations as the evil villains, but here they could be advantageous." One reason is that they help the stem cells to withstand inflammation. If researchers can figure out how, he says, they might be able to develop drugs to protect the intestines of patients with IBD.

Scientists are now probing the effects of somatic mosaicism in other organs. Neuroscientist Jerold Chun of the Sanford Burnham Prebys Medical Discovery Institute in La Jolla, →

Researchers suspect that in some cases mosaicism is beneficial, helping cells to specialize or adapt. The alterations may expand the capabilities of our brains, for instance.

California, and colleagues were the first to show that mosaicism exists in the brain as well. In 2001, they revealed that cells in the brains of adult and embryonic mice can contain unusual numbers of chromosomes. Chun recalls that many neuroscientists at the time were sceptical. "They said 'no way'." However, research over the last two decades has confirmed that many other forms of somatic mutation are prevalent in the brain. On average, each neuron in an adult brain can contain thousands of these alterations.

Scientists have demonstrated that these changes cause some types of epilepsy and a rare condition in which one hemisphere of the brain grows too large. Some circumstantial evidence also suggests they contribute to more common brain diseases, including neurodegenerative illnesses like Alzheimer's disease. In a 2022 study, for instance, scientists performed whole genome sequencing on DNA from neurons of healthy people and patients with Alzheimer's disease. The neurons from the patients with Alzheimer's carried more mutations, which might harm the cells. So far, scientists have not confirmed that somatic mutations help drive neurodegenerative diseases. "The bar for causality is high," says Chun.

And some brain mutations could be beneficial. The brain contains a broad range of cells that can perform an assortment of functions, and researchers have proposed that somatic mutations could help produce this variety. For instance, Chun and his team revealed in 2018 that some neurons use a mechanism known as somatic recombination that enables them to alter the genes' DNA sequences and change the number of copies present in the genome. This mechanism could create numerous different versions of a gene and might be a way of "editing the cell's original genomic blueprint," he says. Transposons, or stretches of DNA that can move from place to place in the genome, might also increase brain cell diversity. When a transposon jumps to a new location, it changes the DNA sequence at its landing point – although these alterations could also be harmful and may promote ageing-related brain diseases and psychiatric disorders.

Scientists have long known that mosaicism is important in cancer. By definition, a tumour is a mosaic, harbouring mutations typically absent in healthy cells that unleash uncontrolled growth. However, sequencing studies have revealed new details about how mosaicism within tumours affects the success of treatments and about who is at risk for cancer.

By sequencing DNA from multiple sites in tumours and from individual tumour cells – as well as by tracking changes across time – researchers have discovered that different cells within a tumour can boast different somatic mutations. This heterogeneity is one of the main obstacles to cancer treatment. The mutations are fodder for natural selection, often allowing the tumour to evolve resistance to a particular therapy. This discovery is already reflected in cancer treatments – patients often receive multiple therapies to curb the development of resistance. But researchers are investigating novel approaches for thwarting mutations. For instance, inflammation promotes chromosome breakage that can result in mutations, so targeting inflammation could help rein in resistance.

New findings also suggest that hidden somatic mutations increase the risk that someone will develop cancer. Mutations that lead to specific types of cancer can be inherited, but some patients who develop these cancers do not have these mutations. Instead, these individuals may have mutations that appeared early in embryonic development and thus are only found in a fraction of the body's cells. Until recently, researchers did not know how common these alterations were. In 2022, a team led by Diana Mandelker, Fresia Pareja, and Jorge Reis-Filho of the Memorial Sloan Kettering Cancer Center in New York City performed the first study to determine their abundance. The team had access to DNA sequencing data from tumours and blood samples for more than 35,000 cancer patients. They found that about 1 in 1,000 of these people carried a somatic mutation that had likely occurred during embryonic development, most often in the anti-tumour gene *TP53*. Standard sequencing at hospitals does not uncover these patients, Reis-Filho says, but identifying them is important because they may be susceptible to multiple cancers and could transmit cancer-promoting mutations to their children.

Mosaicism has also become important as a research tool, with researchers harnessing it to investigate cell function, embryonic development, and other processes. A genetic engineering technique introduced in 2005, mosaic analysis with double markers (MADM), enables researchers to induce mosaicism in mouse tissues. MADM creates mutant and normal cells in close proximity. Both cell types carry fluorescent labels, so researchers can observe the effects of the mutation. Scientists have put the technique to use to study topics as diverse as what functions particular proteins perform, how brain tumours begin, and how the heart recovers from damage.

Although researchers have made progress in understanding mosaicism, some important questions remain, including how common this DNA diversity is in different organs and tissues. Scientists in the field are confident that they will soon have answers to some of these questions thanks to a new program, Somatic Mosaicism Across Human Tissues, launched by the US National Institutes of Health in 2021. The goal of the programme is to create a catalogue of mutations in different organs by analyzing DNA from 150 healthy people who donated their bodies for research. The work will provide a baseline so that scientists can better understand how mosaicism contributes to disease. Because of this and other research, Anderson says, in the next decade or so scientists' ability "to understand the consequences of these cellular mutations is going to skyrocket."

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

Therefore, this pdf continues with the section Results.

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

Shared first authorship is indicated with an *.

X-CHROMOSOME DOSAGE COMPENSATION IN HUMAN EARLY EMBRYONIC DEVELOPMENT

cf. BIF FUTURA, VOL. 33 | 1.2018

TSOTNE CHITIASHVILI
Discipline: Molecular Biologist, PhD
Institute: University of California, Los Angeles (UCLA), CA, USA
Supervisor: Prof. Kathrin Plath

X-chromosome dosage compensation in female mammalian cells is a critical epigenetic process that takes place during early embryonic development. The balance of X-linked gene levels between male and female cells is achieved by one of two mechanisms. In some species, such as mice, the long non-coding RNA (lncRNA) X-inactive specific transcript (*XIST*) mediates X-chromosome inactivation (XCI). While human somatic cells also use XCI, a different form of dosage compensation in preimplantation embryos results in X-chromosome dampening (XCD). In this mechanism, the expression of genes on both X chromosomes is decreased but not silenced. The localization of *XIST* to the dampened X chromosomes indicates that *XIST* can be expressed without inducing silencing, which has never been observed in the mouse. *XIST* is also expressed in female human primordial germ cells (hPGCs) that give rise to the oocytes. Using a combination of single-cell transcriptomics and microscopy, I demonstrated that in addition to occurring in preimplantation human blastocysts, XCD takes place in hPGCs *in vivo*. By performing single-cell RNA sequencing, I also showed that *XIST* expression in female hPGCs correlates with the downregulation of X-linked genes. This finding suggests that *XIST* might be engaged in both types of X-chromosome regulation during human embryonic development. Lastly, I used fluorescent *in situ* hybridization to demonstrate that the primate-specific lncRNA X-active coating transcript (*XACT*), which has been described as a pluripotency-specific lncRNA, is also explicitly expressed from both active X chromosomes in hPGCs. Taken together, my results provide insights into how epigenetic mechanisms differ between mouse and human. My work increases our knowledge of X-chromosome regulation and creates strong bases for understanding the transmission of X-linked diseases through the generations.

PUBLICATION

Chitiashvili T, Dror I, Kim R, Hsu F-M, Chaudhari R, Pandolfi E *et al* (2020) Female human primordial germ cells display X-chromosome dosage compensation despite the absence of X-inactivation. *Nat Cell Biol* **22**(12): 1436–1446

SPEN INTEGRATES TRANSCRIPTIONAL AND EPIGENETIC CONTROL OF X-CHROMOSOME INACTIVATION

cf. BIF FUTURA, VOL. 32 | 2.2017

FRANÇOIS DOSSIN
Discipline: Molecular Geneticist, MSc
Institute: European Molecular Biology Laboratory (EMBL), Heidelberg, Germany; Institut Curie, Paris, France
Supervisor: Prof. Edith Heard

In mammals, the presence of two X chromosomes in females and only one in males leads to dosage imbalances of X-linked genes. Dosage compensation is achieved in females through transcriptional inactivation of one of their two X chromosomes. X-chromosome inactivation (XCI) takes place early during embryonic development and is maintained epigenetically to ensure that female somatic cells, like their male counterparts, express a single X chromosome. XCI is mediated by *Xist* (X-inactive specific transcript), an X-chromosome-encoded long non-coding RNA that coats and silences the chromosome from which it is transcribed. How *Xist* triggers chromosome-wide transcriptional repression was not clear. During my PhD project, I revealed that SPEN, an *Xist* RNA-binding protein, is the central orchestrator of X-linked gene silencing. Using conditional loss-of-function approaches in mouse embryonic stem cells and preimplantation embryos, I showed that loss of SPEN results in the complete failure of gene silencing during XCI. Live-cell imaging and the CUT&RUN technique revealed that SPEN is recruited to the X chromosome immediately upon *Xist* coating. My analyses showed that active transcription provides a favourable context for SPEN binding, as SPEN specifically targets active promoters and enhancers and disengages from chromatin once gene silencing begins. I identified the SPOC (SPEN paralogue and orthologue C-terminal) domain as the core mediator of SPEN's gene silencing function and showed that tethering it to the X chromosome is sufficient for XCI. Furthermore, I showed that the SPOC domain interacts with several factors involved in transcription and chromatin regulation. My work has therefore unveiled SPEN as a molecular integrator linking *Xist* RNA with multiple layers of epigenetic and transcription regulation to promote efficient and robust gene silencing during XCI.

PUBLICATIONS

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INSIGHTS INTO A HUMAN CARGO ADAPTOR, BICD2

cf. BIF FUTURA, VOL. 33 | 1.2018

ROBERT FAGIEWICZ
Discipline: Structural Biologist, MSc
Institute: Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Strasbourg, France
Supervisor: Dr Helgo Schmidt

The motor protein dynein-1 is involved in cell division, transport of organelles and vesicles, and brain and muscle development. Pathogenic viruses can hijack dynein-1 to reach specific cellular locations. In neurons, it carries essential signals and organelles from distal axons to the cell body. To assemble into a functional and motile complex, dynein-1 requires its activating complex, dynactin, and a cargo adaptor. In my PhD work, I reconstituted and characterized a complex of dynein-1, dynactin, the cargo adaptor BicD2, and the nucleoporin RanBP2. This complex is essential in mammalian neocortex development, and its impairment leads to severe neuropathologies such as lissencephaly and microcephaly. One of the main challenges in my approach was producing the recombinant full-length BicD2. BicD2 is an elongated, dimeric, coiled-coil protein that under physiological conditions exists in an auto-inhibited conformation in which the protein interacts with itself. After completing an elaborate solubilization approach, I used cryo-electron microscopy and other techniques to show that full-length BicD2 displays multiple conformations in response to changes in its chemical environment. Variations in pH within a physiological range can severely alter the state of the protein. Slightly acidic conditions unfold the protein, potentially compromising its oligomeric state. Neutral pH yields the auto-inhibited state, in which the C terminus of the protein interacts with its N-terminal counterpart. In addition, my cryo-electron microscopy analysis of the BicD2 dimer revealed that the auto-inhibited state can adopt multiple self-interacting states. These auto-inhibited conformations can be released upon interaction with a cargo and – as I discovered in my work – by an increase in the pH of the solution. These results raise a number of questions about whether these conformational changes are relevant only *in vitro* or if intracellular pH changes can also alter the state of BicD2, which would open up a possible dynein-1 activation pathway. My findings lay the groundwork for further studies on BicD2 reactivity and regulation, and on its role in the formation of motor protein complexes.

PUBLICATIONS

Fagiewicz R, Crucifix C, Deville C, Kieffer B, Nominee Y, Busselez J *et al* (2022) *In vitro* characterization of the full-length human dynein-1 cargo adaptor BicD2. *Structure*, doi: 10.1016/j.str.2022.08.009

A NEURAL CODE FOR PUP CALL REPRESENTATIONS IN THE MOUSE AUDITORY CORTEX

cf. BIF FUTURA, VOL. 32 | 1.2017

ISA-MARIA GROSS
Discipline: Neuroscientist, MSc
Institute: Max Planck Institute of Neurobiology, Planegg, Germany
Supervisor: Prof. Tobias Bonhoeffer

The ability to adapt to a dramatically changing environment is crucial for an animal's survival. When female mice give birth, their environment changes drastically and offspring-directed caring behaviours are induced. When isolated outside the nest, pups emit ultrasonic vocalizations (USVs), triggering retrieval behaviour in mothers. Virgin mice can also perform pup retrieval, provided that they either have experience with pups in their home cage (referred to as experienced virgins) or are repeatedly exposed to pups in a pup retrieval task (naïve virgins). During my PhD project, I established a framework in which mice retrieved pups in a semi-natural, freely behaving setting. Head-mounted miniscopes allowed me to image neural activity in the auditory cortex of adult mice during the task. Tracking mouse behaviour over several sessions showed that the three different pup exposure regimes had distinct behavioural profiles: mothers retrieved pups most efficiently, followed by experienced virgins and then naïve virgins. In addition, I found that not only pups but also adult females emitted USVs during pup retrieval. By training a neural network, I was able to separate these two call categories. I showed that adult females vocalized more in the pup retrieval context than when pups were absent. The biological purpose of these USVs is unknown, as pups are deaf at this age. The imaging in the auditory cortex revealed that the response of the neuronal cell population to pup USVs was sparse and unreliable. I measured strong modulation of neuronal activity during more biologically relevant events, such as pup retrievals and nest entries or exits. This finding shows that cortical activity is modulated not only by USVs but also by context and movement. I found that although single-cell responses to pup calls were sparse, the population of neurons carried information about pup calls in all three groups of adult mice. Finally, I found that the neural code – the pattern of neuronal activity – for pup call representations is sparse and dynamic. Together, my results show how different pup exposure regimes can affect the learning of this essential offspring caring behaviour, and that these different learning types differentially enhance the neural representations of auditory and task-related cues.

PUBLICATIONS

The results of this project have not yet been published.

CYCLIC AMP SIGNALLING IN PRIMARY CILIA DRIVES GENE EXPRESSION AND KIDNEY CYSTS

cf. BIF FUTURA, VOL. 33 | 2.2018

JAN NIKLAS HANSEN
Discipline: Biomedic, MSc
Institute: Institute of Innate Immunity, University of Bonn, Germany
Supervisor: Prof. Dagmar Wachten

Primary cilia are tiny membrane protrusions found in most vertebrate cell types. These organelles orchestrate signal transduction independently of the cell body. Their underlying signalling mechanisms and cellular functions are elusive, but components of cyclic AMP (cAMP) signalling have been identified in primary cilia. The goal of my PhD project was to investigate ciliary cAMP signalling. Common tools based on genetics, bulk assays, or pharmacology do not allow ciliary and cell body signalling to be clearly distinguished. To manipulate ciliary signalling specifically, I targeted optogenetic tools to the cilium. In addition, I developed the software CiliaQ, which quantifies cilia in fluorescence microscopy images and distinguishes ciliary from cell body signals. I applied these tools in murine kidney cells, a common model for primary cilia research. Combining optogenetics and RNA sequencing, I demonstrated that a ciliary cAMP stimulus induces a specific gene expression program that is distinct from the program evoked by a cytosolic cAMP stimulus. Based on these results and by using functional assays, I identified a new signalling pathway in primary cilia: ciliary cAMP evokes protein kinase A (PKA)-dependent phosphorylation of the transcription factor CREB (cAMP-responsive element binding protein) in the cilium, which in turn exits the cilium and regulates a cilium-specific gene expression program. In an optogenetic 3D culture model, I observed that chronic stimulation of this signalling pathway transforms a tubular kidney epithelium into cysts. My work enhances our understanding of the physiology of primary cilia and provides molecular insights into the pathogenesis of polycystic kidney disease, a common genetic disease caused by defective ciliary signalling.

PUBLICATIONS

Hansen JN, Kaiser F, Leyendecker P, Stüven B, Krause JH, Derakhshandeh F *et al* (2022) A cAMP signalosome in primary cilia drives gene expression and kidney cyst formation. *EMBO Rep* **23**(8): e54315

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Hansen JN*, Kaiser F*, Klausen C, Stüven B, Chong R, Bönigk W *et al* (2020) Nanobody-directed targeting of optogenetic tools to study signaling in the primary cilium. *Elife* **9**: e57907

HOW NONLINEAR PROCESSING SHAPES NATURAL STIMULUS ENCODING IN THE RETINA

cf. BIF FUTURA, VOL. 33 | 1.2018

DIMOKRATIS KARAMANLIS
Discipline: Neuroscientist, MSc
Institute: University Medical Center Göttingen, Germany
Supervisor: Prof. Tim Gollisch

Natural vision starts in the retina, where light is transformed into electrical signals that are carried down the optic nerve by retinal ganglion cells (RGCs). Our understanding of retinal processing is based on experiments using artificial visual stimuli, such as full-field illumination or spots of light. In these contexts, each RGC responds in a linear way to a particular region of visual space: cell activity is modulated by changes in average brightness within the region. In my PhD project, I used multi-electrode array recordings from mouse and marmoset retinas to show that this linear picture may be inaccurate for natural stimuli, such as pictures of foliage. By analysing the responses of hundreds of RGCs to natural images, I inferred that these stimuli can drive cell-type-specific nonlinear processes in the retina. Quantitative models based on linear processing fail to capture cell responses to natural images, because a large proportion of cells also respond to fine spatial structure in natural scenes, such as dark tree branches on a bright background. To explain this sensitivity to spatial structure, I designed a new class of models that incorporate nonlinear processing upstream of RGCs. These models have biophysically interpretable components that relate to the functional connectivity between RGCs and their presynaptic excitatory neurons. Compared to linear processing models, nonlinear models better capture cell responses not only to natural images but also to natural movies containing gaze traces of freely moving mice or fixating marmosets. My results suggest that natural stimuli drive nonlinearities in the retinal circuit, and that information about fine spatial structure is contained in the retinal output. My conclusions may help us to understand how these spatial structure signals are used by downstream brain regions – for example, for assisting in edge detection.

PUBLICATIONS

Karamanlis D, Schreyer HM, Gollisch T (2022) Retinal encoding of natural scenes. *Annu Rev Vis Sci* **8**: 171–193

Liu JK, Karamanlis D, Gollisch T (2022) Simple model for encoding natural images by retinal ganglion cells with nonlinear spatial integration. *PLoS Comput Biol* **18**(3): e1009925

Karamanlis D, Gollisch T (2021) Nonlinear spatial integration underlies the diversity of retinal ganglion cell responses to natural images. *J Neurosci* **41**(15): 3479–3498

MOLECULAR PROBES FOR LIVE-CELL IMAGING OF NEWLY SYNTHESIZED DNA

cf. BIF FUTURA, VOL. 34 | 2.2019

CARINA LÄMMLE
Discipline: Chemist, MSc
Institute: Max Planck Institute for Medical Research, Heidelberg, Germany
Supervisor: Prof. Kai Johnsson

Metabolic DNA labelling allows newly synthesized DNA to be visualized. This method is useful for studying biological processes in the cell, such as DNA replication during cell division, DNA repair, or retroviral DNA synthesis. The method relies on the cell activating nucleotide precursors and incorporating them into the nascent DNA chain. In a subsequent labelling reaction, the precursors are marked with a fluorophore, which was previously only possible in fixed cells. The goal of my PhD project was to develop different approaches for metabolic DNA labelling for applications in living cells. Being able to visualize newly synthesized DNA in living cells would enable a better understanding of the time course of dynamic processes. First, I developed a fluorescent probe for a proximity-enhanced reaction with previously reported nucleoside building blocks. The probe was based on a DNA binding dye that was equipped with a reactive moiety. Although I achieved efficient labelling in fixed cells, labelling in living cells was slow. To overcome this limitation, I investigated the incorporation of adenosine derivatives with higher reactive groups and fluorescent nucleosides into genomic, cellular DNA. To improve incorporation yields by skipping the first intracellular phosphorylation step, I also used the fluorescent nucleosides as protected monophosphates. All adenosine derivatives that I developed showed great potential as proliferation reagents – a possible application for metabolic labelling and an assay in cell biology. However, when using the nucleosides in imaging experiments to visualize newly synthesized DNA in proliferating cells, the spatial resolution was poor and the time scale on which the tool could be used was too long for relevant biological processes. The nucleosides also had cytotoxicity effects. If these drawbacks could be overcome, this probe could improve our ability to observe processes involving DNA synthesis in living cells. In future, it might also be possible to combine the probe with proximity labelling to allow spatio-temporal control technology using a photocaged dye.

PUBLICATION

Lämmle CA*, Varady A*, Müller TG, Sturtzel C, Riepl M, Mathes B *et al* (2021) Photocaged Hoechst enables subnuclear visualization and cell selective staining of DNA *in vivo*. *ChemBioChem* **22**: 548–556

MOLECULAR MECHANISMS OF SMALL RNA-GUIDED DNA METHYLATION IN THE MAMMALIAN GERMLINE

cf. BIF FUTURA, VOL. 32 | 1.2017

MATEUSZ MENDEL
Discipline: Molecular Biologist, MSc
Institute: University of Geneva, Switzerland
Supervisor: Prof. Ramesh Pillai

The integrity of the eukaryotic genome is under constant threat from mobile genetic elements called transposons. These sequences, if not controlled, can copy themselves into new sites in the genome, disrupting genes or regulatory sequences. To protect the genome, short non-coding RNAs called PIWI-interacting RNAs (piRNAs) in animal gonads recognize transposons and mediate their silencing based on sequence similarity. The piRNAs serve as guides for their associated PIWI proteins, which either cleave transposons in the cytoplasm or recruit a transcriptional silencing complex to inhibit transposon transcription in the nucleus. Mutations affecting the piRNA pathway can cause sterility in mice and humans, highlighting the importance of this pathway for animal germline fitness. In my PhD project, I investigated the molecular mechanism of transcriptional silencing of transposons in the mouse male germline. Using multiple mouse models combined with biochemistry and next-generation sequencing, I explored how the nuclear PIWI protein MIWI2 localizes to transposon loci and what components of the transcriptional silencing complex are recruited by MIWI2. To identify components of the silencing complex, I immunoprecipitated MIWI2 and two other factors involved in piRNA-driven transcriptional silencing from mouse fetal testis and found potential new interaction partners. Next, I introduced a single amino-acid mutation in the MIWI2 catalytic tetrad with the intention of making the protein an active endonuclease capable of cleaving its targets. The lack of endonuclease activity is believed to allow MIWI2 to remain bound to the transposon target and to recruit the silencing machinery. However, I found that this mutation did not affect fertility or transposon expression. Finally, by artificially tethering N-peptide-tagged MIWI2 to a nascent transcript using *boxB* RNA hairpin motifs, I showed that MIWI2 binding to pre-mRNA is sufficient to establish DNA methylation of the particular locus. This finding experimentally confirms the model in which MIWI2 binding to the nascent transcript is sufficient for transcriptional silencing. My results expand our understanding of the mammalian piRNA pathway and MIWI2 mechanism of action, as well as opening new research avenues by identifying potential MIWI2 interaction partners.

PUBLICATIONS

The results of this project have not yet been published.

SEMISYNTHETIC CALCIUM INDICATORS FOR SUBCELLULAR CALCIUM IMAGING

cf. BIF FUTURA, VOL. XX| X.20XX

NICOLE MERTES
Discipline: Chemist, MSc
Institute: Max Planck Institute for Medical Research, Heidelberg, Germany
Supervisor: Prof. Kai Johnsson

The vast majority of signalling pathways rely on calcium ions (Ca^{2+}) as a second messenger. To control such a large number of processes via Ca^{2+}, the cell tightly regulates its Ca^{2+} concentration. This is achieved using Ca^{2+} channels that produce Ca^{2+} fluxes with unique profiles. Very small and transient Ca^{2+} fluxes, called micro-domains, have recently become the focus of research. However, they are difficult to observe using the two main classes of available Ca^{2+} indicators. Synthetic indicators bind Ca^{2+} quickly and are bright, but they cannot localize to subcellular domains. Genetically encoded Ca^{2+} indicators are localizable, but they are often too slow in Ca^{2+} binding kinetics or too dim to visualize events within such a small area. The aim of my PhD project was to develop and apply novel Ca^{2+} indicators that have the localization ability of genetically encoded Ca^{2+} indicators and the response speed of small organic dye indicators. To achieve this, I developed double-quenched semisynthetic Ca^{2+} indicators – synthetic indicators that are localizable via the HaloTag system and are fluorescent only when localized. In this way, I combined the advantages of synthetic indicators (speed, brightness) with those of genetically encoded indicators (localizability and low background fluorescence). My system, which I called Max Planck calcium (MaPCa) dyes, is based on several rhodamine dyes and Ca^{2+} chelators, which allowed me to tune the indicator's colour and affinity, respectively. With MaPCa dyes, I was able to image not only single action potentials in neurons but also subcellular Ca^{2+} fluxes from the endoplasmic reticulum to the cytosol. The endoplasmic reticulum is an important Ca^{2+} store within the cell, and its high Ca^{2+} concentration requires the application of specialized low-affinity indicators. MaPCa dyes are an important extension of the Ca^{2+} indicator toolbox, as they allow small domains to be visualized with unprecedented speed and brightness. This will hopefully help us better understand subcellular Ca^{2+} signalling in the future.

PUBLICATION

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ASYMMETRIC INHERITANCE OF CENTROSOMES IN HUMAN FOREBRAIN ORGANOIDS

cf. BIF FUTURA, VOL. 32 | 2.2017

LARS ROYALL
Discipline: Neuroscientist, MSc
Institute: Brain Research Institute, University of Zurich, Switzerland
Supervisor: Prof. Sebastian Jessberger

Centrosomes are the organelles responsible for providing the contractile forces required for chromosome separation and proper cell division in animal cells. Centrosomes duplicate once per cell cycle to produce an older and a younger centrosome, each of which is inherited by a different daughter cell after cell division. Studies in mice, fruit flies, and budding yeast have shown that non-random inheritance of the older and younger centrosomes is important for cellular behaviour. During brain development in mice, neural stem progenitor cells (NSPCs) must inherit the older centrosome to maintain their stemness; aberrant inheritance causes premature depletion of the NSPC pool. In my PhD project, I set out to understand whether this phenomenon is present in the developing brain in humans. I developed a novel genetic tool based on the endogenous expression of centrosomal proteins that stably and irreversibly marks centrosomes by their age using different protein tags. I used this tool to modify human embryonic stem cells and then used the modified cells to create forebrain organoids that mimic the early steps of human brain development. By comparing the age of centrosomes in NSPCs and in their differentiating progeny, I revealed that centrosome inheritance in human forebrain organoids is non-random. NSPCs preferentially retain the older centrosome, whereas the differentiating daughter cells inherit the younger centrosome. Final experiments are underway to probe the functional relevance of this non-random inheritance in humans. My results provide insights into the subcellular dynamics during proper development of the human brain. They also lay the groundwork for understanding the role that this phenomenon has in human diseases that affect brain development and maturation.

PUBLICATIONS

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Denoth-Lippuner A, Jaeger B, Liang T, Royall LN, Chie S, Buthey K *et al* (2021) Visualization of individual cell division history in complex tissues using iCOUNT. *Cell Stem Cell* **28**: 2020–2034.e12

Royall LN, Jessberger S (2021) How stem cells remember their past. *Curr Opin Cell Biol* **69**: 17–22

UNDERSTANDING CHROMOSOME FORMATION DURING EUKARYOTIC CELL DIVISION

cf. BIF FUTURA, VOL. 33 | 1.2018

MAXIMILIAN WOLFGANG GEORG SCHNEIDER
Discipline: Biochemist, MSc
Institute: Institute of Molecular Biotechnology (IMBA), Vienna, Austria
Supervisor: Dr Daniel W. Gerlich

During mitosis, chromosomes are faithfully transported to two daughter cells by tubulin microtubules of the mitotic spindle. Accurate genome segregation – a key prerequisite for successful cell division – involves chromatin compaction, although its precise role is unknown. A key structural component of chromosomes, the condensin complex, is dispensable for chromatin compaction. In my PhD project, I explored the mechanism of condensin-independent compaction. Using live-cell microscopy following genetic perturbations of condensin proteins, micromanipulation of chromosomes inside cells, and reconstitution of chromatin *in vitro*, I showed that chromatin compaction is driven by decreased chromatin solubility in mitosis. I found that fragmentation of chromosomes in live mitotic cells did not lead to dissolution of the compact chromatin. Based on this finding, I integrated chromatin solubility and condensin-mediated structural rearrangement into a chromosome-hydrogel model. The long polymer chain of each chromosome is cross-linked by condensins and can undergo compaction and swelling in response to solubility changes. I showed that the compact chromatin resulting from this phase transition resists microtubule perforation and excludes the microtubules from the mitotic spindle. This generates a force at the chromatin surface, which explains one aspect of the polar ejection of chromosomes. Microtubule exclusion thus stabilizes chromosomes mechanically against pushing forces and counteracts the entanglement of chromatin fibres with microtubules. My work shows the importance of chromatin for regulating chromosome mechanics during mitosis.

PUBLICATIONS

Schneider MWG, Gibson BA, Otsuka S, Spicer MFD, Petrovic M, Blaukopf C *et al* (2022) A mitotic chromatin phase transition prevents perforation by microtubules. *Nature*, **609**(7925): 183–190

Cuylen-Haering S, Petrovic M, Hernandez-Armendariz A, Schneider MWG, Samwer M, Blaukopf C *et al* (2020) Chromosome clustering by Ki-67 excludes cytoplasm during nuclear assembly. *Nature* **587**(7833): 285–290

Gibson BA, Doolittle LK, Schneider MWG, Jensen LE, Gamarra N, Henry L *et al.* (2019) Organization of chromatin by intrinsic and regulated phase separation. Cell **179**(2): 470–484.e21

Samwer M, Schneider MWG, Hoefler R, Schmalhorst PS, Jude JG, Zuber J, Gerlich DW (2017) DNA cross-bridging shapes a single nucleus from a set of mitotic chromosomes. *Cell* **170**(5): 956–2972.e23

EPIGENETIC REPRESSION OF INTRONLESS MOBILE ELEMENTS BY THE HUSH COMPLEX

cf. BIF FUTURA, VOL. 33 | 1.2018

MARTA SECZYNSKA
Discipline: Molecular Biologist, MSc
Institute: Cambridge Institute for Medical Research, University of Cambridge, UK
Supervisor: Prof. Paul Lehner

Mobile genetic elements such as transposons contribute to genome evolution, but their uncontrolled spread threatens host genome integrity. As the only autonomous and currently mobile transposons in humans, long interspersed elements-1 (L1s) pose the greatest threat. To control L1 activity, the human silencing hub (HUSH) complex promotes repressive chromatin at L1 loci and restricts L1 expression. The aim of my PhD project was to understand how HUSH recognizes L1s and initiates their repression. Using fluorescent L1 reporters, I mimicked genome invasion by L1s in human cell lines and found that HUSH recognizes incoming L1s independently of their integration site. By studying sequence determinants of L1 repression by HUSH, I discovered that in addition to L1s, HUSH can recognize and transcriptionally repress a broad range of transcribed, sequence-diverse invading DNAs, despite no prior exposure to them. These DNAs were longer than 1 kb and were characterized by a lack of introns and high adenine content in the sense strand. By deleting the promoter from HUSH targets, I showed that transcription is required for HUSH to initiate and maintain repression. I found that HUSH binds nascent RNA from the target loci, before initiating repressive chromatin, which explains how it recognizes invading elements. Whereas diverse intronless transgenes were susceptible to HUSH, introns protected transgenes from HUSH-mediated repression. By profiling HUSH-bound transcripts genome-wide, I showed that HUSH specifically binds transcripts from endogenous intronless genes. Long, intronless DNA is characteristic of mobile genetic elements that replicate through an RNA intermediate and are reverse transcribed before integrating into the host genome. As most mammalian genes have an exon-intron structure, the recognition of long, intronless DNA enables HUSH to distinguish 'self' from 'non-self' DNA. By silencing intronless DNA, HUSH controls the reverse flow of genetic information (i.e. from RNA to DNA) in the human genome.

PUBLICATIONS

Seczynska M, Bloor S, Cuesta SM, Lehner PJ (2021) Genome surveillance by HUSH-mediated silencing of intronless mobile elements. *Nature* **601**: 440–445

Douse CH*, Tchasovnikarova IA*, Timms RT*, Protasio AV, Seczynska M, Prigozhin D *et al* (2020) TASOR is a pseudo-PARP that directs HUSH complex assembly and epigenetic transposon control. *Nat Commun* **11**(1): 4940

MD FELLOWS 2022

With its MD fellowships, the Boehringer Ingelheim Fonds helps outstanding medical students to pursue an ambitious experimental project in basic biomedical research. Candidates study in Germany and change their workplace (institution and city) for at least ten months to join an internationally renowned laboratory. Here, we present the nine fellows who were granted an MD fellowship in 2022.

JONAS BÖHNLEIN

Functionally relevant lncRNA-protein interactions in adipocyte browning

TOBIAS FUNCK

Characterizing the role of efflux pumps in Mycobacterium abscessus drug tolerance

PAUL HEINRICH

Generation of genome-edited hypoimmunogenic human-induced pluripotent stem cell-derived cardiomyocytes using CRISPR-Cas9

ISABEL KELLER

Mechanisms of neuronal regulation of breast cancer metastasis

JIHEE KIM

Restoring intestinal barrier function in obesity and diabetes

DANIEL MARKETT

Characterization of ZZEF1, ZFP36, and other candidates as master regulators of tRNA expression

VICTORIA PRADLER

Elucidating the role of Ankef1 loss-of-function mutations in the development of viral encephalitis

PAUL STÜMPGES

Shared gene regulatory networks in copy number variation models for schizophrenia

CHARLOTTE SUBKLEWE

The role of intestinal microbiota in CAR T-cell therapy

FUNCTIONALLY RELEVANT LNCRNA-PROTEIN INTERACTIONS IN ADIPOCYTE BROWNING

JONAS BÖHNLEIN

Duration: 08/21–10/22

Project at: Dana Farber Cancer Institute, Department of Cancer Immunology and Virology, Boston, MA, USA

Supervisor: Prof. Carl Novina

Home University: German Cancer Research Center (DKFZ)

CHARACTERIZING THE ROLE OF EFFLUX PUMPS IN MYCOBACTERIUM ABSCESSUS DRUG TOLERANCE

TOBIAS FUNCK

Duration: 09/21–10/21

Project at: Harvard University, Boston, MA, USA

Supervisor: Prof. Eric J. Rubin.

Home University: Universitätsklinikum Heidelberg

GENERATION OF GENOME-EDITED HYPOIMMUNOGENIC HUMAN-INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES USING CRISPR/CAS9

PAUL HEINRICH

Duration: 11/21–8/22

Project at: Stanford University School of Medicine, Stanford, CA, USA

Supervisor: Prof. Sean M. Wu

Home University: Deutsches Herzzentrum München (TUM)

MECHANISMS OF NEURONAL REGULATION OF BREAST CANCER METASTASIS

ISABEL KELLER

Duration: 04/21–08/22

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

Supervisor: Prof. Sohail Tavazoie

Home University: Universitätsklinikum Münster

RESTORING INTESTINAL BARRIER FUNCTION IN OBESITY AND DIABETES

JIHEE KIM

Duration: 04/21–09/22

Project at: University of Pennsylvania, Philadelphia, PA, USA

Supervisor: Prof. Christoph Thaiss

Home University: Klinikum der Universität München (LMU)

CHARACTERIZATION OF ZZEF1, ZFP36, AND OTHER CANDIDATES AS MASTER REGULATORS OF TRNA EXPRESSION

DANIEL MARKETT

Duration: 03/21–02/22

Project at: University of California, San Francisco (VCSF), San Francisco, CA, USA

Supervisor: Prof. Hani Goodarzi

Home University: Universitätsklinikum Münster

ELUCIDATING THE ROLE OF ANKEF1 LOSS-OF-FUNCTION MUTATIONS IN THE DEVELOPMENT OF VIRAL ENCEPHALITIS

VICTORIA PRADLER

Duration: 07/21–05/22

Project at: Icahn School of Medicine at Mount Sinai, New York, NY, USA

Supervisor: Prof. Adolfo Garcia Sastre

Home University: Universitätsklinikum Münster

SHARED GENE REGULATORY NETWORKS IN COPY NUMBER VARIATION MODELS FOR SCHIZOPHRENIA

PAUL STÜMPGES

Duration: 08/21–06/22

Project at: Karolinska Institutet, Stockholm, Sweden

Supervisor: Associate Prof. Hjerling-Leffler

Home University: Universitätsklinikum Heidelberg

THE ROLE OF INTESTINAL MICROBIOTA IN CAR T-CELL THERAPY

CHARLOTTE SUBKLEWE

Duration: 10/21–07/22

Project at: Memorial Sloan Kettering Cancer Center, New York, NY, USA

Supervisor: Prof. Marcel van den Brink

Home University: Universitätsklinikum Heidelberg

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

1

BIF FELLOW BEATA MIERZWA ON SCIENCE, ART, AND ORANGE STATUES

In 2022, the Smithsonian unveiled more than 120 life-sized bright orange statues of women who work in STEM fields – the largest collection of women statues ever assembled 2. The exhibition is part of the AAAS IF/THEN Initiative to showcase a wide diversity of female role models excelling in STEM-related fields. Its central idea is "IF she can see it, THEN she can be it". The programme is a collaboration between the American Association for the Advancement of Science (AAAS) and Lyda Hill Philanthropies, an organization committed to funding transformational advances in science and nature. The statues include one of our BIF fellows, Beata Mierzwa, University of California, San Diego 3. The travelling exhibition has already been seen by more than three million people.

What does the AAAS/IF THEN Ambassador programme mean to you?

I've always had two passions: art and science. The first time I combined them was for a BIF seminar in Hirschegg, when we fellows each created a small picture of our research as a thank you to BIF and its staff 4. People remembered me and my science better because of my art. Before then, I had never really seen myself as a science communicator, but after discovering the combination of science and art, as well as the IF/THEN Initiative, I started to. For me, that was life-changing. I now create drawings and artwork for other people and science-themed fashion to highlight the beauty of science.

What does the programme offer?

The statues are just a small part of it. All 125 selected women were invited to a three-day

Photos: Matthew Cooney (1, 3); IF/THEN Collection (2); Beata Mierzwa (4)

summit where we were trained in science communication, STEM education, and diversity and inclusion. The programme offers many opportunities for media appearances. For example, I was on the Saturday morning TV show "Mission Unstoppable", which aired nationwide on CBS. It highlights female scientists and targets younger audiences. I was also part of the international San Diego Comic-Con, the largest event of its kind 1. I took part in a panel discussing where the future is headed: think super-resolution microscopy and CRISPR gene editing. I also showcased my art and a video game I co-developed with programme funding. It's called Microscopya and uses art and music to make complex cellular processes accessible to a wider audience. It's already been awarded several prizes in the educational games category.

When would you have liked to have a role model like the person you are now?

When I started doing art and science, I didn't know there were so many scientists and non-scientists who appreciated this combination. It's hard if you don't devote every minute of your time to working at the bench. Now I try to show that you can combine art and science, and I give advice and encouragement to others. The idea is catching on: in March 2023, I will be chairing the art and biology session at the VIZBI conference on visualizing biological data, organized by the EMBL. This visualization is important for science communication both inside and outside the scientific community.

Can you still separate science and art and science communication?

My art has really enriched my science and the other way around. To make a scientific image, you need to figure out the story, find the most important aspect, and highlight that. We all get so excited and want to talk about everything. Every time I make a drawing, I practice highlighting the most important thing. I've always believed that creativity and science go hand in hand. You need creativity to ask the right questions.

So, how did they make the statues?

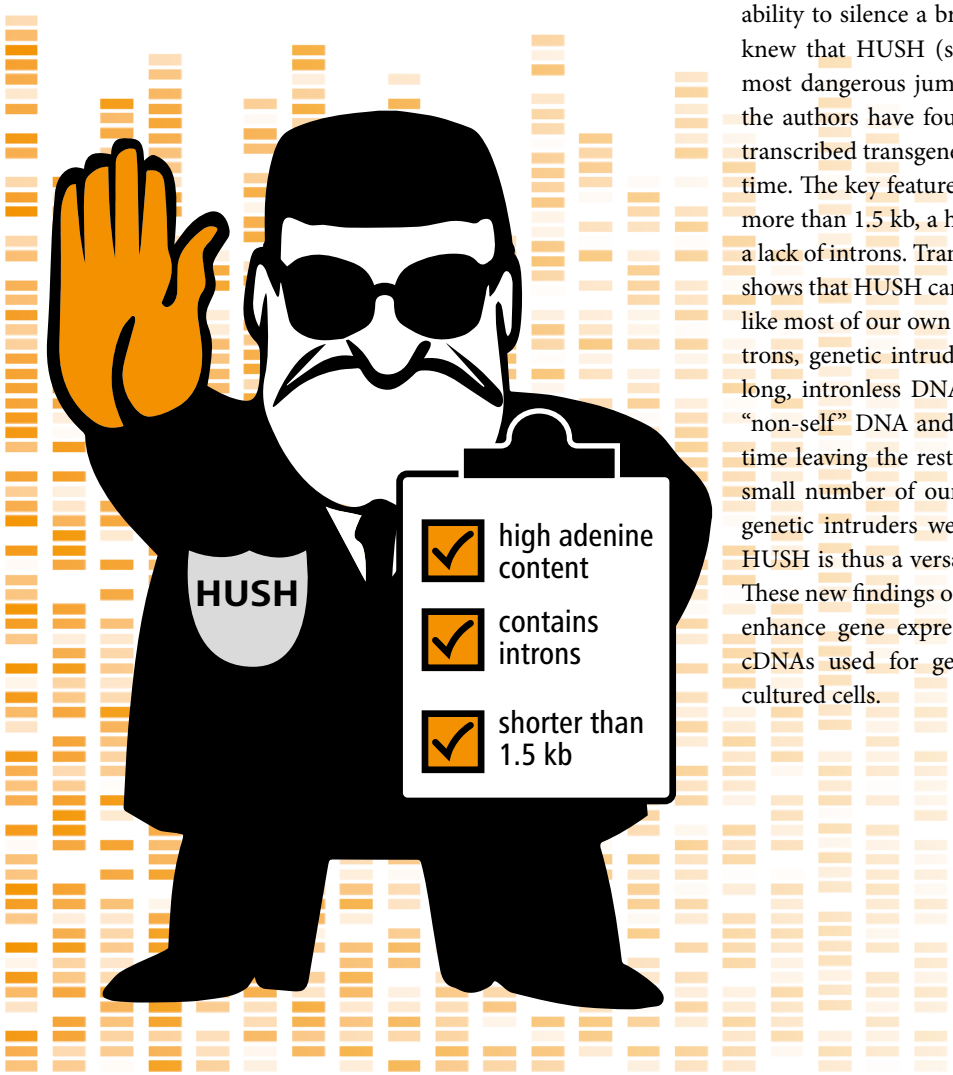
They are 3D printed. During the summit we walked into a tent and it took 30 seconds to take the pictures. We got the files, so now I can print myself in all sizes.

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.

INTRONLESS AND TRANSCRIBED? HUSH WILL SILENCE YOU

Intruders in your home usually try to steal something, but intruders in your genome – mobile genetic elements such as retroviruses and transposons – want to move in and stay. They do so by reverse-transcribing their RNA into so-called complementary DNA (cDNA) and then inserting it into the genome. Once inside, they might not stay put, but move around randomly by repeating the process and thus damaging important genes. This behaviour has earned them the name "jumping genes". Luckily, we have a police force that protects our genome by silencing these elements via repressive chromatin. Marta Seczynska of the laboratory of Paul Lehner at Cambridge University, UK, has now shown that one part of the police force, the HUSH complex, has the remarkable ability to silence a broad range of different genetic intruders. We knew that HUSH (short for human silencing hub) silences the most dangerous jumping genes, young L1 retrotransposons, but the authors have found that HUSH also silences many different transcribed transgenes, even when encountering them for the first time. The key features of the targeted transgenes were a length of more than 1.5 kb, a high adenine content in the sense strand, and a lack of introns. Transgenes with introns were not silenced, which shows that HUSH can specifically recognize intronless DNAs. Unlike most of our own genes, which are built up from exons and introns, genetic intruders usually lack introns. The recognition of long, intronless DNA enables HUSH to distinguish "self" from "non-self" DNA and silence genetic intruders, while at the same time leaving the rest of our genome alone. Well, mostly alone: a small number of our genes that lack introns and thus resemble genetic intruders were also recognized and silenced by HUSH. HUSH is thus a versatile defender against genetic housebreakers. These new findings on how HUSH works also explain why introns enhance gene expression and why it is so difficult to express cDNAs used for gene therapy or ectopic gene expression in cultured cells.



REFERENCE

Seczynska M, Bloo, S, Cuesta SM, Lehner P (2022) Genome surveillance by HUSH-mediated silencing of intronless mobile elements. *Nature* **601**: 440–445. https://doi.org/10.1038/s41586-021-04228-1

Marta Seczynska, fellow 2018–2020

NOBEL PRIZE FLASHBACK: FIRST NEANDERTHAL DNA ANALYZED BY BIF FELLOW

Svante Pääbo received the 2022 Nobel Prize in Physiology or Medicine for his discoveries relating to the genomes of extinct hominins and human evolution. A major breakthrough came with the extraction and analysis of the first mitochondrial DNA sequences from a Neanderthal, published in 1997. The first author was BIF fellow Matthias Krings – hence, our first "flashback" spotlight.

Neanderthals coexisted with anatomically modern humans in Eurasia for tens of thousands of years. But how much of our genetic heritage – if any – comes from Neanderthals? In the 1990s, this question was hotly debated with only a slim hope of getting genomic evidence from Neanderthal fossils. After thousands of years, bones contain only trace amounts of DNA, which have been broken into short fragments, chemically modified, and almost certainly contaminated with modern human DNA. But Matthias Krings, then a PhD student in Svante Pääbo's laboratory and now a senior partner at the consulting company Catenion, took on the challenge of extracting and sequencing the very first Neanderthal DNA ever. To make this possible, he received a sample of the original Neanderthal type specimen found near Düsseldorf, Germany, in 1856.

He focused on the so-called hypervariable region I of the mitochondrial DNA (mtDNA) control region. This stretch was the go-to sequence for genetically comparing populations at this time and also the basis for the theory of an "African Eve" – i.e., the most recent common ancestor of all modern humans originating in Africa. To start with, Matthias picked sequences, so-called primers, that bound to his target region in modern humans and chimpanzees. These, he theorized, should also bind to the corresponding Neanderthal sequence. He pushed the PCR method to its limits and ultimately

In 1997, fossil human genetic material was extracted for the first time from this piece of the upper arm bone of the "original Neanderthal" from the Neander Valley.

was able to compile the entire sequence of his target region of almost 400 base pairs from as little as 50 putative Neanderthal mtDNA fragments in his PCR assay. To validate that these sequences were the real deal, he had to go beyond usual practices: the work took place in special clean rooms. The steps leading to the final results, from extraction to amplification of DNA, were repeated in different ways and rigorously monitored for contamination with contemporary DNA. The results were even validated by a different laboratory. The authors set new standards in paleogenomics.

By comparing this sequence with modern humans and chimpanzees, Matthias showed that the Neanderthal mtDNA is genetically distinct and falls outside the variation of modern humans. He clearly stated the limits of having just one sample and using strictly maternally inherited mtDNA. Still, many headlines read: Neanderthals are not our ancestors.

We now know – thanks in large part to Svante Pääbo's later work – that Neanderthals and Denisovans interbred with modern humans and thus left us with some of their nuclear DNA. Still, Matthias's paper represents a tremendous milestone in understanding our origins.

Skullcap of *Homo neanderthalensis* from the Neander Valley photographed from the left side.

REFERENCE

Krings M *et al* (1997) Neandertal DNA sequences and the origins of modern humans. *Cell.* PMID: 9230299

Matthias Krings, fellow 1994–1997

Photos: LVR-LandesMuseum Bonn/ J.Vogel (bottom left); 1997 Cell Press (top right)

PERSPECTIVES

FROM DRUGS TO PROSTHETICS,
FROM LAB TO MANAGEMENT

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

INTERVIEW WITH UWE SCHLOKAT: POSTDOC FELLOWSHIP, 1987–1989

Uwe Schlokat (b. 1957) did his PhD with Peter Gruss in Heidelberg before conducting postdoctoral research in gene regulation at Genentech. In 1989, he joined the Austrian company Immuno (later Baxter, now Takeda), where he developed plasma-derived products while juggling lab work and project management. In the aftermath of 9/11, he led the development, within one year, of a state-of-the-art smallpox vaccine (also suitable for monkeypox) for the US government. At 55 he accepted the position of CEO at a small Malaysian biotech company, growing it from a research to a clinical stage company. He held several board positions in Singapore, Malaysia, Portugal, and Austria before returning to Austria in 2018 to serve as vice-president of global project management for OttoBock, a world leader in hi-tech prosthetics and orthoses. In early 2022, he left OttoBock to focus on more philanthropic topics but continues to lecture as adjunct professor of biotechnology at the University of Applied Sciences Krems in Austria.

With all your experience, are you a mentor to younger people?

Yes, I love to work with young people, with all their dreams, ideas, and motivation. They're so full of energy! It started long ago when a student asked me whether I could be her mentor. I now have quite a few mentees and gladly give advice to BIF fellows. I advise all young researchers to seek a mentor. Pick someone you like and trust, someone that values you in return. My advice especially for young BIF fellows: don't be intimidated when you see a group of older alumni sitting together at a BIF meeting. You may profit from us more than we need you, but we like answering your questions and giving our perspective. BIF provides unique opportunities by making all these contacts available and letting you tap into this type of network.

How did you build your personal network and in what ways do you use it?

In my younger years, I didn't give it much thought. Today, when I know old acquaintances are more proficient than me, I give them a call. If there is mutual trust, they will be open, often even telling me things in confidence. Such connections need to be honest and do not happen overnight, so start early. I only allow people into my network that I've worked with in one way or another and I value, as well as students from my teaching roles.

How do you shape your teams?

In the beginning I picked people who were absolute experts in their fields, and I failed miserably. I was much more successful when I switched strategies and began hiring good, motivated people with whom the chemistry was right and who fit into my team. I believe that an excellent team is what ultimately makes the difference. It's important that you trust one another. Feedback needs to be given *and* taken in a constructive manner. I strongly believe that there are no incapable people. If someone isn't performing well in their role, it's likely that the person is simply in the wrong position.

What's your advice for job applicants?

Always be authentic. Look at what is described in the ad. Yes, mention the awesome work you're proud of and your excellent publication(s), but focus clearly on what is demanded and the experience you have to offer in regard to the job. In interviews, what often counts more than the factual answer is your response, your approach to a question. Often men have done something once and present themselves as experts, while women have done something two hundred times, but still say they have "some experience in". I'd like to encourage them to be more confident.

What skill would you have liked to learn earlier?

I'm a "learned extrovert". I had to learn how to better present and confidently portray all I've done and achieved. You have to market the great things you do, but careful – this differs across cultures. How to get key messages across in a simple and convincing way is a valuable skill that you need in all areas of life. If you pitch to stakeholders, you need to show your enthusiasm and present a good story. Storytelling is crucial.

PROFILES

PROFESSOR IVAN DIKIC
Institute: University of Frankfurt, Germany, Postdoctoral Award: 1997

PROFESSOR STEPHANIE DIMMELER
Institute: University of Frankfurt, Germany
Fellowship: 1991–1992

Two BIF alumni were among the 253 researchers to receive ERC Advanced Grants worth up to 2.5 million euros over five years. This was the third such award for both. A total of 1,735 proposals were submitted for the 2021 call, 625 million euros was disbursed, and the success rate was just below 15%.

In his project "ER-REMODEL – Endoplasmic Reticulum Remodelling via ER-Phagy Pathways", **Ivan Dikic** aims to explore the novel idea that ubiquitin drives receptor clusters, which in turn ignite the process of membrane remodelling.

In her project "Neuroheart – The Cardiac Neurovascular Interface in Ageing", **Stephanie Dimmeler** will study how nerves and blood vessels communicate in ageing hearts and why renewal lessens with age.

FLORIAN WILFLING
Institute: MPI or Biophysics, Frankfurt, Germany
Fellowship: 2009–2012

Florian Wilfling has been selected for an ERC Starting Grant and is one of around 400 grantees from more than 4,000 applicants. He will receive 1.5 million euros for his project "Intrinsic Autophagy Receptors: Identity and Cellular Mechanisms", in which he will investigate how specific cargoes are collected in the cell and how this process is synchronized with autophagosome formation.

PROFESSOR BENJAMIN JUDKEWITZ
Institute: Charité – Universitätsmedizin Berlin, Germany
Fellowship: 2006–2008

PROFESSOR TIM NIKOLAI SIEGEL
Institute: University of Munich (LMU), Germany
Fellowship: 2005–2008

PROFESSOR MARION SILIES
Institute: University of Mainz, Germany
Fellowship: 2006–2008

Three BIF fellows have received ERC Consolidator Grants. These are awarded to mid-career scientists and come with up to two million euros in funding for a five-year period. The 2021 call attracted 2,652 proposals, 313 of which were funded with a total of 632 million euros, making for a success rate of about 12%.

Benjamin Judkewitz received a grant for his project "GlassBrain: Brain-Wide Processing and Whole-Body Biophysics of Directional Sound". The researchers in his team aim to be the first to reveal the entire processing chain at the single-cell level from acoustic stimulus and mechanical transmission in the body to brain-wide neuronal activity.

In his project "SwitchDecoding: Decoding the Path to Cellular Variation within Pathogen Populations", **Tim Nikolai Siegel** will study the parasite responsible for sleeping sickness, *Tryanosoma brucei*, to understand the mechanisms that produce genetic variability in pathogen populations and those that enable pathogens to evade the immune defences of their host.

The main objective of **Marion Silies**' project "Adaptive Functions of Visual Systems" is to understand how the brain deals with rapidly changing light conditions and how it encodes self-motion. To do so, her team will develop genetic models not only of fruit flies, but also of other insects such as hover flies.

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

PROFESSOR LUDGER JOHANNES
Institute: Institut Curie, Paris, France
Fellowship: 1993–1995

PROFESSOR MICHAEL SIEWEKE
Institute: Technical University Dresden, Germany
Fellowship: 1991–1992

Three BIF fellows have received ERC Proof-of-Concept (PoC) Grants, which come with 150,000 euros over a period of 1.5 years and enable grantees to explore the commercial or societal potential of ideas arising from projects previously funded by an ERC.

Simon Elsässer has been awarded his second PoC Grant for the project "hmqPro: Highly Multiplexed, Quantitative Protein Biomarker Profiling". Grants also went to **Ludger Johannes** for his project "Lectibodies to Eliminate Tumours" and to **Michael Sieweke** for "ONCOMAC: Allogeneic Macrophages for Cancer Therapy". This was the first time these latter two researchers won such grants.

A BIF FELLOW'S GUIDE TO ...

PARIS

Travelling is fun – especially if you get insider tips from locals! In each edition of FUTURA, one fellow shows you around their city. In this edition your guide is Felix Streicher, who reports from Paris, France, best known as the City of Love.

FACTS & FIGURES

Country: France
Population: about 2.2 million
Area: 105.4 km²
Students: about 650,000
Famous for its romantic and cultural atmosphere, high-quality cuisine, fashion, and strikes.

WHERE TO STAY

The People Hostel: situated in Belleville, one of the liveliest neighborhoods in Paris, with an amazing rooftop terrace!
Hotel Madrigal: this charming boutique hotel is very close to the Institut Pasteur, so don't be surprised if you hear a lot of science talk among the Pasteurians who meet in its bar.

NIGHTLIFE

Rue Mouffetard: a street full of cafes, bars, and restaurants located in a vibrant student neighborhood.
Ground Control: a former postal sorting centre transformed into a cultural space where almost anything can happen.
La Mezcaleria: a speakeasy-style bar hidden behind the kitchen of the 1K Hotel.

RESTAURANTS

La Felicità 1**:** a selection of street food vendors in a beautifully decorated old warehouse.
Académie de la Bière: offers an extensive selection of international beers and exquisite moules frites.
Andia: a spectacularly beautiful restaurant in a repurposed train station.

ACTIVITIES

Winter: Paris has an endless number of amazing museums. My favorites are the Palais de Tokyo 2 and Centre Pompidou.
Spring: walk along the Promenade Plantée, the world's first elevated park walkway, or enjoy the cherry blossoms at the impressive Père Lachaise cemetery.
Summer: fetch a beer and hang out at the Canal Saint Martin 3.
Autumn: don't miss the Nuit Blanche on the first Saturday of October, when art galleries, museums, and other cultural institutions remain open for the night.

BEST SIGHTS

Sacre Coeur 4**:** my absolute favorite view of Paris. Stay late and listen to some amazing street musicians while taking in the city of lights.
Le Marais: one of Paris's central districts; a visit is essential to understand what the city is all about.
Les Catacombes de Paris: the catacombs are old underground limestone quarries that hold the remains of about six million Parisians – truly fascinating!

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

Felix Streicher
Institut Pasteur,
Paris, France
Supervisor: Nolwenn Jouvenet, PhD
29 years

Photos: Felix Streicher

PROFILES

PROFESSOR BARBARA TREUTLEIN
Institute: ETH Zurich, Switzerland
Fellowship: 2009–2010

PROFESSOR EDWARD LEMKE
Institute: Institute of Molecular Biology (IMB), Mainz, Germany
Fellowship: 2003–2005

Two BIF fellows have been elected as EMBO members. In 2022, 67 outstanding life scientists working in 22 different countries were honoured in this way, joining a community of more than 1,900 leading life scientists.

Barbara Treutlein uses single-cell genomic, imaging, and computational tools to dissect human organoid formation and to understand how processes fail in human disease. She is also attempting to engineer better organoid models.

Edward Lemke develops innovative techniques using synthetic and chemical biology in order to study the biological dynamics of intrinsically disordered proteins at high temporal and spatial resolution.

UPCOMING EVENTS

1–5 MARCH 2023
126th International Titisee Conference, Titisee, Germany
The 126th ITC, titled "NeuroAI – Connecting Advances in Machine Learning and Neuroscience", will be chaired by Caswell Barry and Matthew Botvinick (both London, UK) and bring together leading neuroscientists and machine learning researchers to advance knowledge in these interconnected fields.

17–18 MARCH 2023
Meetings of BIF's Board of Trustees
The trustees decide on the allocation of fellowships, review the proposals for the International Titisee Conferences, and handle all matters of fundamental importance for the foundation.

BIF'S SISTER FOUNDATIONS

HEINRICH WIELAND PRIZE 2022 FOR XIAOWEI ZHUANG AND NOBEL PRIZE FOR 2012 LAUREATE CAROLYN BERTOZZI

On 6 October, Xiaowei Zhuang of Harvard University, Cambridge, USA, received the 2022 Heinrich Wieland Prize worth 100,000 euros. The Boehringer Ingelheim Foundation honoured her for developing groundbreaking imaging methods and using these to make seminal discoveries in cell biology and neurobiology. Her super-resolution STORM method enables us to observe how structures approximately 100 atoms (10 nanometres) wide do their work in human cells, while her MERFISH technique for genome-scale imaging produces clear snapshots of the activity of 10,000 genes within one cell at the same time. Over the past 16 years, Zhuang and scientists worldwide have used these inventions to gain valuable insight into complex biological processes.

Professor Xiaowei Zhuang has, for example, discovered an unexpected part of the cell skeleton in nerve cells. This element is found in the brains of organisms as diverse as worms and humans and is essential for signalling. Both STORM and MERFISH are invaluable to the scientific community and are widely used in academic and commercial laboratories worldwide. MERFISH is one of the key technologies behind the Human Cell Atlas, a global initiative to map all the cells in the human body, characterize these cells on a molecular basis for a better understanding of human health, and diagnose, monitor, and treat disease.

The award was presented during a festive ceremony following a scientific symposium at Nymphenburg Palace in Munich, Germany.

The 2022 Nobel Prize for Chemistry was announced the day before the symposium. One of the three recipients is the 2012 Heinrich Wieland Prize laureate Carolyn Bertozzi. Professor Bertozzi was honoured with the Nobel Prize for the same work that earned her the Heinrich Wieland Prize 10 years ago: the development of click and bioorthogonal chemistry. This groundbreaking field has enabled us to study vital processes in living beings and has paved the way for her pioneering insights into the biological functions of cellular sugars in health and disease. There are now five awardees of the Heinrich Wieland Prize who have subsequently won the Nobel Prize.

Photo: Boehringer Ingelheim Stiftung (HWP); private (Treutlein); IMB Mainz (Lemke)

Boehringer Ingelheim Fonds
Stiftung für medizinische
Grundlagenforschung

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

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