FUTURA

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The start of life
The journey from oocyte to embryo was discussed at the 117th ITC

New seminar venue in Europe
First English-language communication training near Mainz

Gracht: a fond farewell
The 28th alumni seminar at Gracht Castle was the last at this venue

The cover illustration shows a simplified model of the moon's phases, which govern rhythms of marine life. Researchers use the marine bristle worm *Platynereis dumerilii* to study the impact of the moon and sun on the reproductive cycle of these worms as well as the effects of pollution on marine ecosystems.
Read more about *Platynereis dumerilii* on page 8.

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NEW PHD PROJECTS, FIRST ROUND 2018

PHD RESULTS

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GRACHT 2018: A FOND FAREWELL

PUBLISHING INFORMATION

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A PHD – WHAT IS IT WORTH TO YOU?

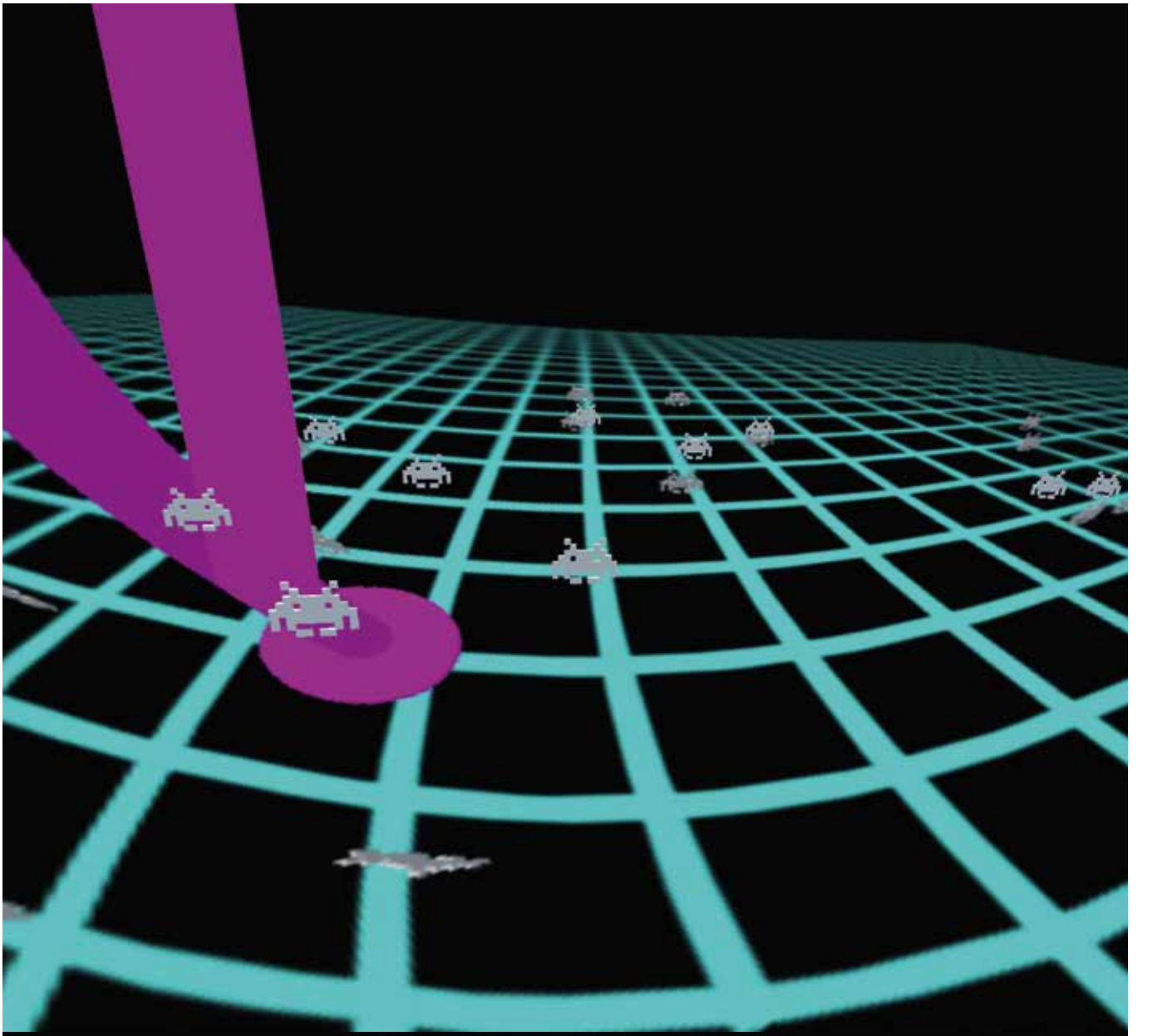
»Is it not a privilege to be given such an education?«

The status of PhD candidates can differ greatly depending on a country's tradition, its education and research system. In Sweden, they usually consider themselves employees, while in Great Britain they are seen as students. In other countries such as Germany, the debate is still ongoing, and the Max Planck Society and its PhD candidates have reached a compromise: candidates are given so-called support contracts which include social benefits while allowing them to focus predominantly on their thesis project.

Employees or students? Contracts or stipends? The answers have far-reaching consequences for PhD candidates, institutions, and science as a whole. To name but one: the obligations of someone bound by an employment contract are quite different from the freedom a stipend holder has (and which should be respected by supervisors and institutes alike). As usual, such freedom comes at a price: more responsibility, more risk.

In the public discussion about the status of PhD candidates, the meaning and worth of a PhD seem to have been relegated to the background. A PhD ranks among the best opportunities one can be given. It is a high-calibre training to become an independent researcher and it proves useful far beyond the academic world: You learn to identify important problems, develop clear hypotheses, establish and apply the right methods to test them, and interpret the resulting data while keeping in mind the limitations of the approaches used. Analytical and critical thinking skills are honed. Embracing and critically evaluating one complex issue after another and solving the attendant problems becomes daily routine. And yes, it also tries and trains perseverance and tolerance of frustration. A PhD allows candidates to aim high, to follow their love of a subject with creativity and ample freedom. It is a time to explore limits and develop potential and talent with the help of supervisors and the additional courses today's PhD programmes offer. And all this hopefully also leads to what lures and beckons explorers: the moment of discovery. However, it is unlikely to be attainable without dedication to the goal and hard work, often to the exclusion of much else, at least for a time. Nobody promises that earning a top-level qualification such as a PhD is an easy path to take. And yes, research is an unpredictable journey that does not always lead to the destination one has aimed for.

The prestigious PhD title opens doors to responsible and well-paid positions in academia and elsewhere. Is it not a privilege to be given such an education? Is it not an opportunity to be seized with both hands? Should one not make the most of this "investment" phase? Past European generations and today's refugees from war-ridden countries have experienced what it means to lose everything. What you can take with you wherever you go are your skills and your education. They remain the best investment and security net for your future that you can possibly think of.

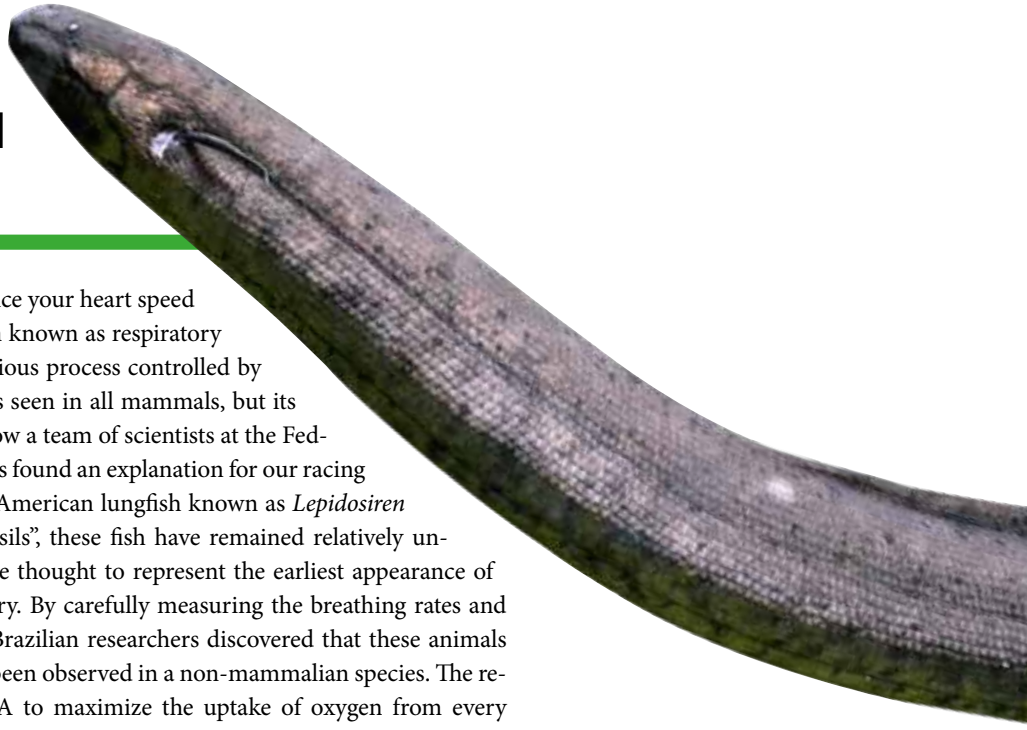


A HOLODECK FOR FISH

By Andrew Straw, University of Freiburg, Germany, and Kristin Tessmar-Raible, University of Vienna, Austria

Animals and their nervous systems have evolved in a multifaceted, natural environment not well reflected in most lab settings. Virtual realities can help by providing more complex stimuli. BIF alumna Kristin Tessmar-Raible and Andrew Straw developed a setup in which a single fish becomes part of a swarm of artificial agents (in this case space invaders) that can be controlled by the researchers to study the fish's behaviour. Also new is that the fish can choose to leave an environment. The magenta post acts as a "teleporter", transporting it to a different environment.

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



HUMAN HEARTBEATS IN ANCIENT LUNGFISH

If you take a deep breath, you may notice your heart speed up very slightly. This is a phenomenon known as respiratory sinus arrhythmia (RSA) – an unconscious process controlled by the autonomic nervous system. RSA is seen in all mammals, but its purpose has always been a mystery. Now a team of scientists at the Federal University of Sao Carlos, Brazil, has found an explanation for our racing hearts by studying a species of South American lungfish known as *Lepidosiren paradoxa*. Often viewed as "living fossils", these fish have remained relatively unchanged over millions of years and are thought to represent the earliest appearance of lung-like organs in evolutionary history. By carefully measuring the breathing rates and heartbeats of lungfish in the lab, the Brazilian researchers discovered that these animals show RSA too – the first time this has been observed in a non-mammalian species. The researchers found that lungfish use RSA to maximize the uptake of oxygen from every breath. But humans and other mammals do not have to do this, because our lungs are more efficient, so it looks as though RSA is an evolutionary relic that we retain today even though we no longer need it.

REFERENCE

Monteiro DA, Taylor EW, Sartori MR, Cruz AL, Rantin FT, Leite CAC (2018) Cardiorespiratory interactions previously identified as mammalian are present in the primitive lungfish. *Science Advances* DOI: 10.1126/sciadv.aaq0800

BODY CLOCK REGULATES DNA LOOPING

Many organisms have a strong circadian rhythm – an internal body clock that governs sleep, wakefulness, and a range of other biological processes. Over the years, scientists have found many genes that drive circadian rhythms, as well as plenty of others that are switched on or off in a cyclical fashion. Now researchers at the Ecole Polytechnique Fédérale de Lausanne in Switzerland have delved deeper into this biological "clockwork", focusing on how the organization of DNA within the nucleus changes according to the body clock. Using a technique called 4-C, which reveals whether specific regions of DNA are touching or not, the researchers found that genes and some of their control switches (enhancers) came together and fell apart in precise rhythms over a 24-hour period in cells from healthy mice. This reveals an underlying circadian pattern of DNA twisting and looping that helps to control gene activity. The rhythmic looping was disrupted in animals lacking an enhancer that controlled a key circadian gene called *Cryptochrome 1*, and their body clocks sped up. As well as showing that altering a non-coding region of the genome disrupts circadian rhythms, the findings reveal another layer of moving parts within this complex biological clockwork.

REFERENCE

Mermet J, Yeung J, Hurni C, Mauvoisin D, Gustafson K (2018) Clock-dependent chromatin topology modulates circadian transcription and behavior. *Genes Dev* DOI: 10.1101/gad.312397.118

Photos: Vassil/Wikimedia Commons (top); Stock (bottom)

SHINING A LIGHT INSIDE THE BRAIN

One of the greatest challenges in medical imaging is seeing inside the "black box" of the skull to look at the brain. Light doesn't penetrate very far into biological material, so most brain scanning techniques rely on X-rays (CT), magnetic fields (MRI), or radioactive markers (PET). But a team of scientists at the Institute for Basic Science in Daejeon, South Korea, have discovered how to get a deeper view using light alone. The technique relies on a process called time-gating: when a light beam is fired into a biological sample and the light waves scatter in all directions, only the ones coming back to the detector at a certain time are captured and analysed. This way, researchers can gain a more accurate picture of what is inside down to double the depth of current light-based techniques. So far the method has been used to see through the relatively thin skull of a young mouse, but the researchers want to use it to look at nerve cells, bone marrow, and brain tissue in living animals and eventually humans. Furthermore, precision beams could be used to non-invasively activate light-sensitive drugs inside brain tumours, leaving healthy cells unharmed. Light could also be used to charge electronic devices implanted in the brain, or to stimulate genetically engineered biological sensors.

REFERENCE

Hong J, Jeong S, Kang S, Lee Y-R, Choi W (2018) Focusing of light energy inside a scattering medium by controlling the time-gated multiple light scattering. *Nature Photonics* DOI: 10.1038/s41566-018-0120-9

Male zebra finches with a defective *FoxP2* gene have difficulty learning how to sing correctly.

2,000

km

That's how far the invasive comb jellyfish *Mnemiopsis leidyi* can travel in just three months thanks to a push from strong ocean currents. For an animal of about one centimetre, this is a fast and long journey. Researchers have found that if the jellies arrive at a new location in the ballast water of ships, they ride the currents to spread rapidly in these new regions, which may cause major problems for native sea life.

Source: Jaspers, C. et. al. (2018) Ocean current connectivity propelling the secondary spread of a marine invasive comb jelly across western Eurasia. *Global Ecology and Biogeography*, DOI: 10.1111/geb.12742

SONGBIRD GENES HOLD CLUES TO HUMAN SPEECH DISORDERS

The sound of birdsong is unmistakeable, yet it has more in common with human speech than you might imagine. Humans are unique among mammals in mastering a huge number of detailed speech patterns as we grow up. Many songbirds also have to learn very complex songs when they are young, but how they do this is a mystery. To find out more, a team of scientists based at the University of California Los Angeles and Stanford University in the USA focused on just one species of songbird: the zebra finch. When a young male zebra finch practises its song, the activity of a gene called *FoxP2* decreases in a special part of the brain called Area X, leading to changes in the activity of thousands of other genes. *FoxP2* is also found in humans, where it is known to play an important role in speech. The researchers used genetic engineering to give finches a version of *FoxP2* that could not be turned down and found that the birds then had problems learning to sing. Adding in different-sized versions of *FoxP2* led to strange-sounding, abnormal songs. The next step is to find out if the same molecular pathways are at work in humans when children learn to speak, which could shed light on the origins of speech impairments.

REFERENCE

Hilliard AT, Heston JB, Burkett ZD, Day NF, Kimball TH, Aamodt CM (2018) FoxP2 isoforms delineate spatiotemporal transcriptional networks for vocal learning in the zebra finch. *ELife* DOI: 10.7554/eLife.30649

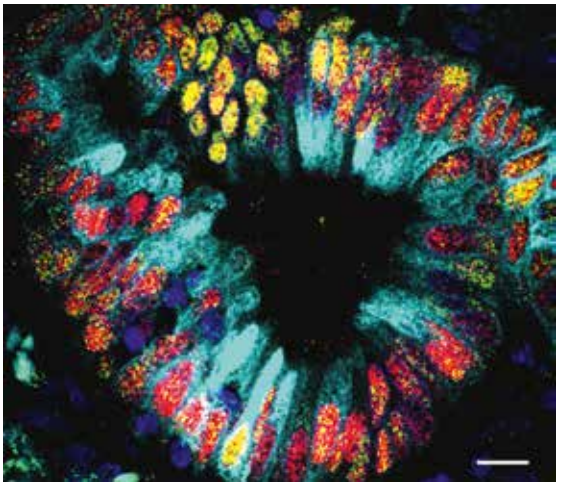
Photos: Hallshadow/iStock (top); Tata Lab at Duke University (bottom right)

SHAPE-SHIFTING CANCER CELLS

Cancer researchers at Duke University School of Medicine in the USA were surprised to discover a miniature stomach, small intestine, and duodenum lurking inside their lung tumour samples. It was already known that the genetic chaos inside cancer cells makes them highly plastic, enabling them to change their characteristics as a tumour grows and becomes resistant to therapy – a major challenge for successful treatment. Even so, this was a remarkable transformation. Investigating further, the scientists discovered that a gene called *NKX2-1* was missing or switched off in the shape-shifting lung cancer cells. The gene normally acts as a master switch during development, pushing embryonic cells down a pathway towards becoming, for example, lung rather than gut. Once they have lost *NKX2-1*, these lung cancer cells quickly regress back to an earlier state and embark on an alternative journey, transforming into gut tissues. Activating additional cancer-promoting genes gives the cells even more flexibility, creating tumours resembling mid- and hind-gut tissues. The team plans to use this system to study how plasticity enables tumours to resist treatment and to find new ways to block it.

REFERENCE

Konkimalla A, Bara A, Tata PR, Chow RD, Saladi SV, Aleksandra Tata A (2018) Developmental history provides a roadmap for the emergence of tumor plasticity. *Dev Cell* DOI: 10.1016/j.devcel.2018.02.024



Some lung cancer cells with errors in the transcription factor *NKX2-1* begin to resemble the cells of the stomach and gut.

The larvae and embryos of the marine bristle worm *Platynereis dumerilii* are transparent, making it easy for researchers to observe their internal structures.

PROFILE OF **PLATYNEREIS DUMERILII**

By Dr Mitch Leslie

The marine bristle worm is a useful phylogenetic model in evo-devo biology research. Due to its slow rate of evolution, it provides insights into the common origins of invertebrates and vertebrates.

When marine bristle worms (*Platynereis dumerilii*) are in the mood to mate, thousands and thousands of them perform their version of synchronized swimming. While the female pirouettes, the male zooms around her, releasing clouds of sperm to fertilize her eggs.

The worm's striking reproductive cycle originally prompted researchers to start raising *P. dumerilii* in the lab in the 1950s to study how they synchronize themselves. Today, they also employ these animals to study how the sun and moon govern the marine rhythms of life as well as issues ranging from regeneration to the evolutionary origin of different muscle types to the effects of pollution on marine ecosystems.

P. dumerilii is also a revealing model because of its position in the phylogenetic tree of animals and its slow rate of evolution. With models such as fruit flies, nematodes, zebrafish, and mice, researchers have covered two of the major animal groups, or superphyla. But *P. dumerilii* gives them a member of a third superphylum that also includes earthworms, snails, and leeches.

The worm's have other virtues as well. They thrive in the lab, feeding on spinach or algae and the occasional sprinkle of fish food. Their embryos and larvae are transparent, making it easy to observe internal structures. In addition, a variety of tools are available to study the worms. Researchers can silence genes, insert new ones, and eliminate individual cells to determine their function.

P. dumerilii has made a splash in the field of evo-devo, or evolutionary developmental biology, in which researchers try to understand how different developmental patterns have evolved and shaped animals' bodies. For instance, the light-sensitive cells in the eyes of invertebrates differ from those in vertebrates, which could indicate that the eyes of these two groups evolved independently. But scientists discovered that *P. dumerilii* carries invertebrate light-sensitive cells and cells that are similar to the vertebrate versions, suggesting that the two types of eyes have a common origin.

WHO AM I? A FEW FACTS

- I grow up to 6 cm long.
- I live for up to 18 months.
- I feed on algae and live in seawater.
- I am studied mainly in developmental biology, evolutionary biology, chronobiology, and neurobiology.

Photos: Jürgen Berger, MPI for Developmental Biology, Tübingen (left); Friedrich Hempelmann (1911) (right)

Hard work: a tortoise emerging from its egg.

Photo: shutterstock

THE START OF LIFE

By Dr Alex Reis

How does an organism ensure the right mix of creativity and fidelity when reproducing via eggs and sperm – this was one of the questions discussed at the 117th International Titisee Conference in April 2018.

Karl Ernst von Baer (1792–1876) carefully removed a yellowish structure he had spotted in the ovary of a dog and studied it in 1826. Inside was a precious cell – the oocyte. This was the first time mammalian oocytes were observed under the microscope. Von Baer called it "ovulum" or "little egg" and, at a time when many contradicting theories abounded, he made an important leap to establish that all animals develop from an egg. He also described the initial stages of development in the dog embryo, carefully placing earlier stages near the ovary and more advanced stages of development closer to the uterus. In view of the fact that he had a very rudimentary microscope and the concept of a cell had not been developed yet, his results were nothing short of amazing. How an organism develops from an oocyte to an embryo is again such a fast-moving field that it was the topic of the 117th ITC in April of this year. The conference title: "From Oocyte to Embryo – Illuminating the Origins of Life". During the four-day conference, which was organized and financed by the Boehringer Ingelheim Fonds and chaired by Melina Schuh, director at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, and Takashi Hiiragi, group leader at the European Molecular Biology Laboratory (EMBL) in Heidelberg, about 50 international top-level scientists discussed their latest findings in the scenic setting of Lake Titisee, Germany.

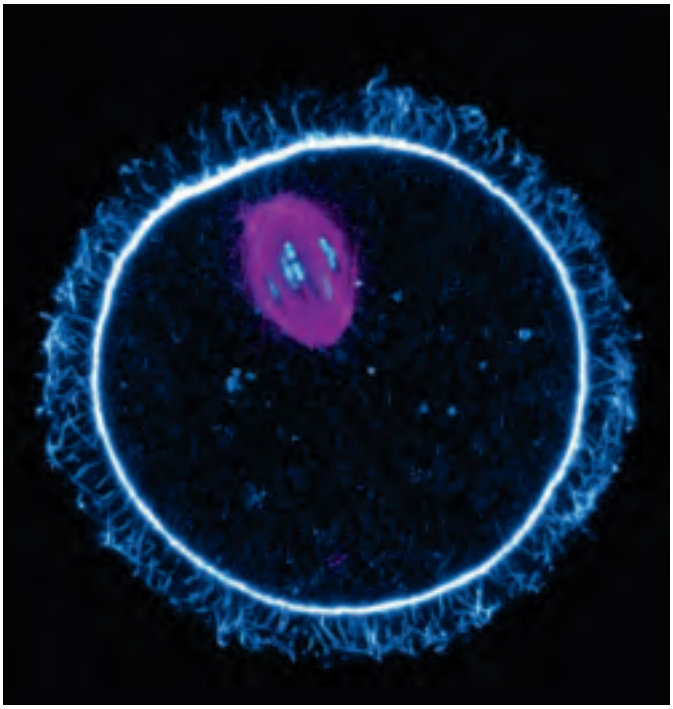
As von Baer spotted in his dog, the story of a mammalian female's eggs starts with primordial germ cells which in humans turn into 1–2 million oocytes at birth, carefully tucked away inside small follicles in the ovary, ready for a long wait – remaining dormant until puberty. They are arrested in an early stage of meiosis – a special form of cell division to form germ cells. It involves →

two divisions and results in daughter cells with only a single set of chromosomes. This ensures that after sperm and oocyte merge, the zygote has the standard double set of chromosomes and thus two copies of each gene – one from the father and one from the mother. At the onset of puberty, follicles start a complex maturation process leading to an increase in the size of the oocytes. Shortly before ovulation, one – or several – oocytes complete the first of the two meiotic divisions by segregating homologous chromosomes. Unlike the corresponding stage during sperm formation, however, this is a highly asymmetrical division into egg cell and the so-called polar body. The egg gets to keep almost all of the cytoplasm, while the polar body receives next to nothing apart from half of the chromosomes and soon degenerates.

After ovulation, the oocyte starts the second meiotic division, but once again halts halfway through. At this stage, its fate hangs on timely fertilization and the egg balances in limbo between certain death and the possibility of producing offspring. If a sperm does penetrate the oocyte, the second meiotic division is completed by separating the sister chromatids. One set of chromatids is destined for the second polar body, and therefore also doomed to a fleeting existence.

While the sperm makes an obvious and important genetic contribution to the zygote, the major player here is obviously the oocyte, contributing not only half of the genetic make-up, but also nearly all the organelles needed for development.

As a consequence, the calibre of oocytes is crucial on several levels. Not only does their limited number impose restrictions on the reproductive lifespan of every woman, but their malfunction often results in infertility or major congenital disorders, such as Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), and Turner syndrome (monosomy X).



A mouse oocyte during meiosis I: Actin cortex (cyan), spindle (microtubules, magenta), and chromosomes (cyan).

In every cell division – and the oocyte is no exception – the cellular machinery responsible for ensuring accurate chromosome segregation is the so-called spindle. In mitotic cells and male gametes (at least in the animal kingdom), the assembly of this highly dynamic structure is guided by small barrel-shaped structures called centrosomes.

Oocytes, however, stand in a class of their own as the only cells to go through cell division without the help of these structures. The most important cell division to create life – where the spindle must function with the highest fidelity – is thus achieved without guiding centrosomes.

"Because the egg is so important, you would expect it to be particularly efficient," says German cell biologist Melina Schuh. "But in fact, a very large fraction of human eggs contains an abnormal number of chromosomes – a condition known as aneuploidy."

It is estimated that one in four pregnancies is not carried to term, and often the embryo is lost even before the woman realizes she is pregnant. To make things worse for us as a species, there is some evidence that human eggs are particularly poor at monitoring whether chromosomes are correctly attached to the spindle prior to segregation, independently of age. For humans, in evolutionary terms, this may not be such a big problem, as they have only very few pregnancies over their life time. If the embryo is able to go through the initial stages, there is a good chance it can reach the end of the pregnancy. In animals which rely on large litters, such as mice, many losses would mean significantly smaller litters on a regular basis, and therefore reduce their ability to reproduce significantly. This might

There is evidence that human eggs are particularly poor at monitoring whether chromosomes are correctly attached to the spindle prior to segregation, independently of age.

Photo: Melina Schuh, Elsevier

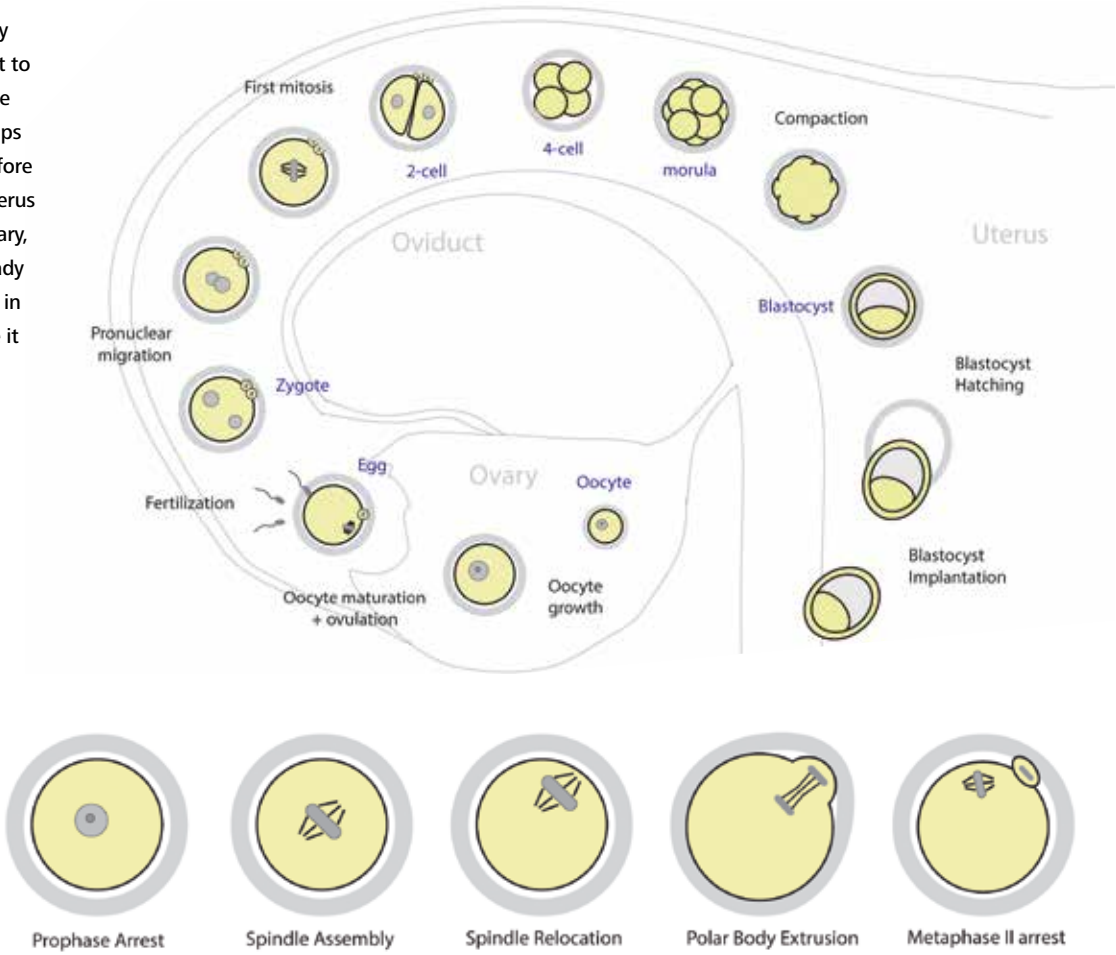
be one reason why mouse and human oocytes differ quite a bit: for example, mice have so-called acentriolar microtubule-organizing centres, which help the spindle to assemble efficiently in the absence of centrosomes. Human oocytes do not have detectable acentriolar microtubule-organizing centres, and their spindles need much longer to form and are more likely to be unstable.

Schuh has been investigating the spindle for years, first at the MRC Laboratory of Molecular Biology in Cambridge, UK, and now as director at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany. In 2015, introducing a revolutionary imaging technique, her team was the first to be able to image how live meiosis progresses in 100 human oocytes. Not only did they stumble on an incredibly long process – a whopping ~16 hours, compared to five hours in mouse oocytes and only around half an hour in mitotic cells – they also often found abnormalities in the spindle. It was not uncommon for them to find an unstable spindle structure with multiple poles that was interacting with the chromosomes in an erroneous manner.

"The spindle continuously gets larger and gets extensively reorganized, and the prolonged multipolar spindle stages may favour abnormal interactions between the spindle and chromosomes," says Schuh this unusually long process. The researcher notes that this may be similar to what happens in cancer cells, which often also have multipolar spindles with excessive centrosomes.

Schuh's team also discovered another unexpected component of the spindle in mammalian oocytes. For a long time, the researchers were puzzled by the presence of large amounts of actin in the spindle. "This was surprising because spindles are thought to consist mostly of microtubules and microtubule-dependent motor proteins, and actin is typically not thought to be important in the spindle," says Schuh. What made this observation even more interesting was that different species, including humans, cows, and sheep, all had the same large amounts of actin.

Reducing the levels of actin by knocking out the factor required for making the actin filaments resulted in mouse eggs more likely to have problems. These eggs were less efficient in aligning their chromosomes in the centre of the spindle, but instead carried chromosomes at their spindle poles. However, the actin does not interact with the chromosomes directly, but instead promotes the formation of specialized microtubules within the spindles which →



Eggs are fertilized by sperm in the oviduct to form the zygote. The embryo then develops into a blastocyst before it implants in the uterus (top). Within the ovary, the oocyte has already matured but arrests in metaphase II before it ovulates (bottom).

Graphic: Dean Clift, MRC Laboratory of Molecular Biology Melina Schuh, MPI BPC

in turn attach to the chromosomes. These microtubules form the engine that pulls chromosomes apart. When there is no actin, the engine fails, making it harder to align and separate chromosomes.

The surprisingly slow process of spindle assembly in mammalian oocytes may also have advantages, though, as it gives the oocytes enough time to align their chromosomes. When Marie-Emile Terret, senior researcher at the Parisian Collège de France, forced a mitotic spindle onto mouse oocytes by up-regulating spindle protein kinesin-14, the results were catastrophic. This shift toward a fast-acting mitotic-like spindle was sufficient to severely impair chromosome alignment. It seems that a mitotic spindle and the very slow meiotic division are a bad combination, contributing to massive errors during chromosome segregation. The researchers argue this could be relevant to other systems as well, such as human oocytes, where meiosis is even slower. "It is possible that mouse oocytes, and maybe also human oocytes, eliminated centrosomes to prevent a mitotic-like mode of spindle assembly during meiosis, thereby safeguarding against further increases in aneuploidy levels, already high during this specific division in these species," says Terret.

Why it is such a long process in the first place? There are many theories, but using 3D live imaging, Tomoya Kitajima, professor at the Japanese RIKEN Center for Biosystems Dynamics Research, offered a possible explanation. The researcher showed that the long delay is caused, at least partly, by multiple attempts of trial-and-error before chromosomes find stable attachments to the spindle. The problem is that, in the absence of centrosomes, the spindle starts off as a tangled balled-up mess of microtubules, and only slowly starts to take shape. During this period, impatient chromosomes still attempt to link up, but almost 90% are faulty attachments that need correction. It is not unreasonable to speculate that a fast centrosome-based spindle would be too forgiving of these errors and therefore result in an even higher aneuploidy rate.

This ability of chromosomes to try multiple connections happens thanks to a mechanism in mouse oocytes unravelled by Katja Wassmann, research director at the French Centre National de la Recherche Scientifique, CNRS, in mouse oocytes. Her team, based at the Institute de Biologie in Paris, developed an ingenious way in which an Eg5 kinesin inhibitor affects spindle tension, but not chromosome attachment. "When tension is reduced, a mechanism, named error correction pathway, is activated. It will detach microtubules that are not under tension, and this will lead to SAC activation," says Wassmann. In a nutshell, the cell "assumes" that all chromosomes attached but not under tension are in the wrong position. If that is the case the SAC – short for spindle assembly checkpoint – is activated, which stops cell division until all connections are corrected.

Despite this mechanism, many have speculated that the SAC is still less effective in meiosis than in mitosis. One of the reasons suggested is the large size of the oocyte. Kitajima had wanted to test this for a long time, but it was not until he observed co-worker Hirohisa Kyogoku's expertise in micro-manipulation that he was prepared to do so. Kyogoku created oocytes with either half or double the amount of cytoplasm by removing some cytoplasmic material or joining an intact and an enucleated oocyte together. In the double-sized oocytes the duo found several defects – in particular, an enlarged faulty spindle with severely delayed chromosome segregation.

It seems bigger is not always better and certainly a combination of bigger cell and a proportionally bigger spindle is asking for trouble. The researchers speculated that XL size dilutes critical components to control segregation, lowering the stringency of the checkpoint, but also that large spindles lose the ability to keep chromosomes in place. The smaller oocytes, by contrast, showed less segregation errors and a better functioning, more concentrated spindle. However, there has to be a trade-off as the oocyte needs to be big enough to support post-fertilization embryo development.

It seems bigger is not always better and certainly a combination of bigger cell and a proportionally bigger spindle is asking for trouble.

One last challenge for the spindle is one of position. As described above, meiotic cell divisions need to be asymmetrical to ensure that the oocyte retains most of the maternal stores accumulated during growth, which means the spindle needs to go on a little journey from the centre to the edge of the cell. The big question is, without centrosomes, how does the meiotic spindle become oriented and positioned?

Marie Emile has shown this short migration is mediated by a now familiar player for the spindle: actin. The process starts with a thick layer around the edges of the cell connected to a loose mesh in the cytoplasm, which in turn is connected to a denser cage surrounding the spindle. The second ingredient in this mechanism is myosin, which locates at the spindle poles generating localized contractions and pulling the spindle towards the edge on both sides. As the spindle does not form exactly at the cen- →

EMBRYO RESEARCH LEGISLATION

Recently, scientists have started to investigate human embryos in addition to mouse embryos. This research allows them to get a better picture of the first stages of human development, which is fundamental in understanding the causes of early developmental defects that can result in miscarriages and birth defects. The use of embryonic stem cells for therapeutic purposes holds out the promise of new treatments for a variety of conditions, such as spinal cord injury, cerebral palsy, and Parkinson's.

Technician using a light microscope to inject human sperm into a human egg cell during *in vitro* fertilization (IVF). This technique is known as intracytoplasmic sperm injection (ICSI).

In Europe, legislation regulating this research is as diverse as European traditions and languages. Even for the countries that allow this type of research, regulations vary widely. With a lot of caveats, Belgium, Sweden, and the UK are currently at the forefront of human embryo research, allowing the creation of human embryos specifically for research under strict rules. Research is only allowed if it has been approved by a qualified ethics committee and if it is deemed to lead to major scientific advances or medical treatments, but embryos must be destroyed 14 days after fertilization. Under similar rules – albeit with variations – other countries are allowing the use of surplus embryos following IVF treatments. This is the case in Bulgaria, the Czech Republic, Denmark, Estonia, Finland, France, Greece, Portugal, Spain, and Switzerland. Other countries, such as Austria, Germany, Italy, Lithuania, Poland, and Slovakia, follow a more restrictive approach where research on embryos is banned. In some countries, the legislation allows for the use of imported embryonic stem cell lines, but in most cases destroying human embryos may even carry a prison sentence. On the other hand, Cyprus, Ireland, Luxembourg, Malta, and Romania have yet to define their legislation on the subject. Due to this disparity amongst EU members, in 2002, the EU developed its own legal framework for research funded from the EU budget. The current version, from 2007, has been renewed for the duration of Horizon 2020. Above all, national legislation must be respected at all times, and EU projects must follow the laws of the country where the research is being done. In addition, projects applying for funding must be peer-reviewed within strict ethical parameters, and EU funds must never be used for the creation of new stem cell lines.

In the US, the situation is just as confusing. Former president Barack Obama lifted some of the legal constraints in this area, but it is up to individual states to decide whether to allow (and fund) this type of research. Connecticut, Massachusetts, California, and Illinois are among the states that allow research using human embryos. While President Trump has not made his official position clear, some of his cabinet members are strong opponents of human embryo research.

On the opposite end of the scale, China has one of the most permissive approaches to research using human embryos. Nevertheless, there are guidelines to follow that strictly forbid any research aimed at human reproductive cloning. The embryos must come from the "surpluses" of IVF treatments, germ cells donated voluntarily, or blastocysts obtained by somatic cell nuclear transfer.

Photo: Science Photo Library/PHANIE/BURGER

tre, there is always one side closer to the edge and typically that is the side that wins.

Finally, when the time is right, fertilization creates a single cell known as the zygote. Curiously, chromosomes need to travel from the periphery back to the centre of the zygote, where the two sets of chromosomes from both parents merge together. As before, chromosomes rely on the actin bus to take them on this journey. Once again, Terret and her team showed that actin-dependent mechanics are crucial for zygotic spindle positioning, ensuring the centring of the two pronuclei from oocyte and spermatocyte.

The two pronuclei coming together after fertilization mark the end of meiosis and the beginning of mitosis. When and how exactly this happens differs between species. Hiiragi and his EMBL colleague Jan Ellenberg, BIF alumnus and EMBL group leader, recently were surprised to find that in mice the nuclei of sperm and oocyte do not merge immediately after fertilization, but that the parental chromosome sets stayed separated at least for the first division after fertilization. To this end, the zygote built two spindles – something that so far was only known for insects and their like. This double spindle might be double trouble, as it increases the likelihood of faulty chromosome segregation. As the resulting errors are similar to errors seen in human embryos during fertilization processes, it might be the same in humans.

Curiously, the first several cell divisions continue to occur without centrosomes, as even the one coming from the sperm is destroyed and not rebuilt until the 64-cell stage. As time progresses and one cell splits to become two, and two to become four, and four to become eight, the embryo faces yet another challenge: compaction and cell differentiation from the 8-cell stage onwards.

ITC chair Takashi Hiiragi and his former EMBL colleague Jean-Leon Maitre, now group leader at the Institut Curie in Paris, have long been fascinated by the mechanical forces shaping the early embryo. Similar to the process in which the spindle is pulled to the edge and then pushed back to the centre, the duo has identified that the principal mechanism squeezing cells together during compaction at the 8-cell stage is the combination of actin and myosin. Similarly, asymmetrical divisions at 16-cells will eventually form the blastocyst with distinct inside and outside lineages.

"Cells can recognize their position: 'outside' if there is a contact-free surface and 'inside' if a cell is surrounded entirely by contact, but crucially, this is not the only factor", says Hiiragi. The duo showed that, by interfering with the generation of contractile forces using maternal myosin knockout embryos, cells not only fail to acquire the correct position, but also fail to sense where they are. In a disorientated manner, cells go to the surface of the embryo but adopt an inner cell mass fate. Without tension, cells completely lose the ability to know where they are and what they need to do.

"By positioning the cells, actomyosin defines which cells become placenta and which cells become embryo. It's a very important process, not just shaping [the embryo], but also the fate of the cells", says Maitre. Cells can react to different tension patterns and contact asymmetry and respond with different signals – such as the Yap/Hippo pathway inside but not outside cells – eventually leading to differentiation into the inner cell mass, giving rise to the foetus, and the trophectoderm, the cell layer later forming part of the placenta.

For a while on this journey, the early embryo floats freely down the fallopian tube heading for the uterus, but its period of freedom soon approaches its end. Finally, as the inner cell mass and the trophoectoderm establish themselves, it is time to lose the protection of the egg coating, the zona pellucida, and to make direct contact with the lining of the uterus. Building the placenta can start in earnest, marking the end of pre-implantation embryo development.

Embryology has come a long way since von Baer. This classical field has gone from simple observations to exact measurements and the understanding of mechanisms, thanks to the development of new methods for studying oocytes and embryos. Researchers are now able to measure minute changes in gene expression or follow cellular mechanisms with 3D live imaging. Research efforts are focused on understanding more about the reasons behind the decline in oocyte quality with higher maternal age, with the aim of developing a potential treatment. Given the increasing trend to have children later in life, this topic will become more relevant to our society.

Only a few days after the end of the ITC conference, students in Tartu, Estonia, gave von Baer's statue its yearly champagne bath. The statue was unveiled ten years after his death in 1876, and has pride of place in local Toome Hill Park. It may sound like a bit of a strange tradition, but every Walpurgis Night (30 April) students recognize the contributions of this great scientist by washing his head with the bubbly drink.

Karl Ernst von Baer was the first scientist to observe a mammalian oocyte under a microscope – in 1826.

Photo: shutterstock

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 *.

POLYCOMB REPRESSIVE COMPLEX 2 IS TARGETED TO UNMETHYLATED CG MOTIFS

cf. BIF FUTURA, VOL. 28 | 2.2013

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Institute: Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland
Supervisor: Prof. Dirk Schübeler

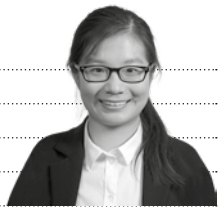
Every cell in the human body has the same genetic information, yet many different cells and tissues perform very distinct functions. Certain genes are active only in specific tissues, but how that specificity is established is not clear. The goal of my PhD project was to address this question using embryonic stem cells, which differentiate into different cell types at a very early stage of development. I focused on mechanisms of gene silencing by chromatin-modifying complexes, namely Polycomb group proteins. Despite their importance during development, it is largely unknown how these complexes are recruited to target genes and how they regulate transcriptional repression. My group's working hypothesis was that Polycomb recruitment is encoded in the underlying DNA sequence and is dependent on transcription factors. I therefore investigated the contribution of transcription factor motifs, CG motifs, and DNA methylation to Polycomb recruitment, as well as the role of Polycomb recruitment in transcriptional silencing. I developed an assay to test many DNA sequences with various sequence properties for their ability to drive recruitment of Polycomb repressive complex 2 (PRC2) in mouse embryonic stem cells. The assay involved integrating hundreds of sequences into a defined genomic location in parallel. I found that high density of unmethylated CG motifs within a synthetic backbone sequence is sufficient to recruit PRC2. Furthermore, to link PRC2 recruitment with transcriptional repression, I used CRISPR-Cas9 technology to delete a core PRC2 component and then used RNA sequencing to monitor the transcriptional response. Upon depletion of global methylation levels on Lys27 in histone H3 (H3K27), I observed no significant changes in gene expression. However, PRC2 targets were deregulated globally during differentiation into neuronal progenitors. These results show that recruitment of PRC2 and subsequent H3K27 methylation is important for cell-fate transition, but is not required for gene repression at the steady state of mouse embryonic stem cells. Taken together, my work suggests that PRC2 mediates the onset of cellular differentiation and tissue formation, but does not maintain tissue-specific programs.

PUBLICATIONS

The results of this project have not yet been published.

PROSTHECOBACTER BtubAB PROTEINS FORM BACTERIAL MINI MICROTUBULES

cf. BIF FUTURA, VOL. 30 | 2.2015



XIAN DENG
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Institute: MRC Laboratory of Molecular Biology (LMB), University of Cambridge, Cambridge, UK
Supervisor: Dr Jan Löwe

Microtubules – the dynamic yet stiff, hollow tubes built from αβ-tubulin protein heterodimers – are thought to be present only in eukaryotic cells. In 2002, a pair of tubulin-like genes, bacterial tubulin A (*btuba*) and B (*btubb*), were identified in *Prosthecobacter* bacteria. Both genes were shown to have higher sequence homology to eukaryotic tubulins than to FtsZ, the prokaryotic homologue of tubulin, or other bacterial homologues. Crystal structures also revealed that the proteins have a closer similarity to αβ-tubulins than to their prokaryotic equivalents. Although the complex of the two bacterial tubulins, BtubAB, is known to form filaments in the presence of guanosine triphosphate, little is known about the filament architecture. During my PhD, I determined a 3.6 Å structure of the *in vitro* BtubAB filament using cryo-electron microscopy and cryo-electron tomography, which revealed a hollow tube consisting of four protofilaments. A closer look showed that BtubAB filaments have many conserved microtubule features, including an overall polarity, similar longitudinal contacts, M-loops in lateral interfaces, and the presence of the seam, a structural hallmark of microtubules. In collaboration with me, postdoc Gero Fink investigated the dynamic properties of BtubAB filaments using total internal reflection fluorescence microscopy. He showed that BtubAB filaments display treadmilling as well as dynamic instability, another hallmark of eukaryotic microtubules. In addition, our studies show that the third protein in the *btub* gene cluster, BtubC (previously known as bacterial kinesin light chain), binds along protofilaments every 8 nm, significantly reduces the rate of BtubAB filament catastrophe (stochastic shrinkage), and increases the rate of rescue (repolymerization). Based on our data, we concluded that *Prosthecobacter* BtubAB forms bacterial "mini microtubules". Although the biological function of BtubAB has yet to be determined, our work reveals that some bacteria contain regulated and dynamic cytomotive microtubule systems that were once thought to be useful only in much larger and sophisticated eukaryotic cells.

PUBLICATIONS

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MECHANISTIC STUDIES OF PROTEIN AGGREGATION

cf. BIF FUTURA, VOL. 29 | 2.2014

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Protein misfolding and aggregation are linked to more than fifty human pathologies, which are becoming increasingly prevalent. For example, the aggregation of α-synuclein has been linked to Parkinson's disease and other neurogenerative disorders. During my PhD, I developed an approach to study the mechanism of α-synuclein aggregation, as well as a methodology to quantify the ability of protein aggregates to disrupt membranes. Together with my colleagues, I used spectroscopy and imaging to characterize the influence of disease-associated mutations in α-synuclein on its aggregation mechanism. We found that the mutations influence individual events in the aggregation process and alter the structural characteristics of the aggregates. We showed that this approach enables the identification of the mode of action of a small molecule that inhibits protein aggregation. One of the damaging effects associated with protein aggregation is the disruption of membranes. We therefore developed a total internal reflection microscopy-based assay that measures membrane integrity using surface-tethered vesicles filled with environment-sensitive dye molecules. We showed that the assay can be used to identify aggregates that permeabilize the lipid bilayer. This technique allows us to characterize the ability of molecular chaperones and antibodies to counteract the membrane permeation. These methodologies therefore not only enable studies that advance our understanding of the mechanisms and damaging effects associated with protein aggregation, but also potentially provide platforms for the development of therapeutics.

PUBLICATIONS

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DEEP TISSUE METASTASIS IMAGING WITH SHORT-WAVELENGTH INFRARED QUANTUM DOTS

cf. BIF FUTURA, VOL. 30 | 2.2015

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Supervisor: Prof. Moungi Bawendi

Fluorescence imaging is a transformative biomedical imaging technology, as it is a low-cost, high-sensitivity method for real-time molecular imaging *in vivo*. Imaging in the shortwave infrared region (SWIR; 1–2 µm) promises higher contrast, sensitivity, and penetration depths than conventional visible and near-infrared fluorescence imaging. However, the lack of versatile SWIR emitters has prevented the general adoption of SWIR imaging in both academic and clinical settings. In my PhD project, I developed SWIR-emissive quantum dots (QDs) that enable the non-invasive, real-time imaging of haemorrhagic stroke, the measurement of brain vasculature hemodynamics in tumour microenvironments, and the quantification of metabolic activity in murine models. Building on these probes, I created a platform technology for the molecular targeting of biological entities with SWIR fluorescent tags that has the potential to enable the detection of small metastatic lesions in deep tissue. As the use of QD-based probes will remain limited to pre-clinical settings due to the presence of heavy metals, we investigated alternative approaches for clinical use. I found that the commercially available contrast agent indocyanine green, which has been approved by the US Food and Drug Administration, exhibits optical properties suitable for *in vivo* SWIR fluorescence imaging. My findings suggest that SWIR fluorescence imaging can be implemented alongside existing clinical imaging modalities, offering the possibility of translating this high-contrast technique to clinical applications.

PUBLICATIONS

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MOLECULAR FORCE ANALYSIS OF THE VERTEBRATE KINETOCHORE PROTEIN CENP-T

cf. BIF FUTURA, VOL. 28 | 2.2013

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Supervisor: Dr Carsten Grashoff

Accurate chromosome segregation during mitosis depends on tightly regulated mechanical forces co-ordinating with complex cell-cycle signalling cascades. Evidence of a direct role for these forces in the correction of erroneous kinetochore-microtubule interactions has accumulated in recent years, but little was known about their molecular nature and magnitude. In my PhD project, I quantified kinetochore forces using fluorescence resonance energy transfer (FRET)-based tension sensor modules. These modules are comparable to conventional spring scales, but are built from two fluorescent proteins connected by a flexible, calibrated linker peptide that elongates under force. A functional biosensor is built by genetically integrating the sensor module into a protein of interest. I analysed centromere protein T (CENP-T), which forms a direct link between the microtubule-binding Ndc80 complex and the centromeric region of the DNA. Quantitative FRET analysis using the well-established F40 sensor module demonstrated that CENP-T experiences force during metaphase. Subsequent analysis with the stiffer HP35 module revealed that these forces do not exceed the sensor's lower threshold of 6 pN. To analyse these forces in more detail, I developed a third sensor module, named FL, with almost switch-like unfolding kinetics between 3 and 5 pN, and thus greater sensitivity than F40, which gradually elongates between 1 and 6 pN. Experiments with FL revealed that mechanically engaged CENP-T molecules can bear forces of at least 3 pN, which is significantly higher than the average force previously reported for a related kinetochore protein. In addition to its kinetochore-specific application, the new sensor is a valuable tool for the mechanobiology community, as its switch-like unfolding kinetics enable the fraction of mechanically engaged molecules to be quantified for the first time.

PUBLICATIONS

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FUNCTIONAL ANNOTATION OF KINASES THROUGH PERTURBATION AND TRANSCRIPTIONAL PROFILING

cf. BIF FUTURA, VOL. 29 | 1.2014

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Supervisor: Dr Sebastian Nijman

Kinases are an important class of enzymes that play key roles in a wide range of cellular processes, including cell division, differentiation, and migration. Kinases can rapidly alter or adjust cellular behaviour through the orchestrated modification of hundreds of target proteins. Although many kinases have been studied in detail, a large proportion of kinase genes have not yet been assigned a particular function. Genetic screening approaches have proven to be powerful tools to study gene function and have provided insights into fundamental biological processes. However, systematic and unbiased studies have largely been limited to model organisms. Complex disease-relevant genotypes and phenotypes cannot be studied in their entirety in lower organisms, which creates a need for systematic approaches in human cells. During my PhD, I established a large-scale reverse genetic screening platform and provided a proof-of-concept study for a scalable and generic approach to quantify phenotypes in human cells. First, I focused on the set-up of a quantitative phenotypic read-out using a novel application of RNA sequencing. I demonstrated that this shallow sequencing method is scalable and suitable as a read-out for reverse genetic screening. Second, I focused on the implementation of this method in haploid human cells to functionally annotate tyrosine kinases in signalling pathways. By comparing transcriptional signatures of stimulated wild-type cells to isogenic kinase knockout cell lines, I revealed known and unexpected interactions between different tyrosine kinases and pathways. By developing and validating this technical approach in a biological context, my study not only contributes to our understanding of kinase genes but also provides a novel tool for the systematic study of other gene families – and eventually all human genes.

PUBLICATIONS

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THE ROLE OF RIPK1 IN TNF-INDUCED CELL DEATH IN INTESTINAL EPITHELIAL CELLS

cf. BIF FUTURA, VOL. 30 | 1.2015

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Supervisor: Prof. Michael Karin

Inflammatory bowel diseases (IBDs) are chronic processes that affect the gastrointestinal tract. In many IBD patients, epithelial damage and inflammation depend on the proinflammatory cytokine tumour necrosis factor (TNF). Normally, intestinal epithelial cells (IECs) are insensitive to TNF-induced death due to activation of NF-κB (nuclear factor kappa light chain enhancer of activated B cells) through transcription of anti-apoptotic genes. Therefore, how TNF became a potent inducer of IEC death and tissue injury remains a mystery. Epithelial NF-κB activation in human IBD samples correlates with caspase-3 cleavage, a marker of apoptosis. This is unexpected, as in most cell types, including IECs, transient TNF signalling inhibits apoptosis due to IKKβ (inhibitor of NF-κB kinase subunit β)-dependent NF-κB activation. IKKβ(EE)IEC mice, in which a constitutively active IKKβ variant is expressed specifically in IECs, display a basal inflammatory phenotype and severe TNF-dependent destruction of the epithelial layer. This chronic inflammation enhances the expression of TNF α-induced protein 3 (TNFAIP3, also known as A20), a downstream NF-κB target gene that functions as a negative feedback regulator and has been associated with IBD. In my PhD project, I showed that IECs from IBD patients have higher A20 expression than controls, which also coincided with the presence of cleaved caspase-3. I also found that mice overexpressing A20 in IECs were more susceptible to TNF-induced cell death, challenging the view that A20 is predominantly an anti-inflammatory protein. In both cases, the outcome was dependent on receptor interacting protein kinase 1 (RIPK1), which serves as a key regulator of life and death in TNF-exposed cells. Upon TNF challenge, an increase in A20 levels favours the formation of the Ripoptosome, a protein complex in which the kinase activity of RIPK1 plays a critical role in activating caspase-3 downstream and inducing apoptosis. I showed that genetic and pharmacological inhibition of RIPK1 prevented Ripoptosome activation and apoptosis in IECs downstream of TNF. My work suggests that chronic NF-κB activation increases A20 levels, unravelling previously unknown functions of A20, and proposes RIPK1 as a new druggable target for treating IBDs.

PUBLICATIONS

The results of this project have not yet been published.

DESIGN PRINCIPLES OF CpG ISLAND PROMOTER ACTIVITY

cf. BIF FUTURA, VOL. 29 | 2.2014

DOMINIK HARTL
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Supervisor: Prof. Dirk Schübeler

Gene expression patterns are regulated by promoters. In humans and mice, two-thirds of promoters have a higher density of the dinucleotide CpG than the rest of the genome, and are known as CpG islands (CGIs). If and how CpGs contribute to promoter activity and regulation remains unclear. The goal of my PhD was to gain insights into this matter by studying the role of CpGs in mouse embryonic stem cells. I used chromatin immunoprecipitation sequencing to monitor binding of four transcription factors that are thought to play a role in CGI activity. I found that CpG density, together with the presence of the transcription factor binding site, correlates with binding, indicating that CpG density might contribute to transcription. To test this hypothesis, I monitored the activity of more than 250 mutated promoters, including artificial promoter sequences, on genomic DNA. I established a reporter assay that allowed me to quantify their transcriptional activity at a defined chromosomal locus in parallel. I found that the number of CpGs is proportional to transcriptional activity in the presence of transcription factor binding sites. However, I also discovered that high CpG density is not sufficient for transcription. My lab previously showed that high CpG density counteracts the effects of DNA methylation, a chromatin mark linked to inactivity. To assess how this interplay affects the role of CGIs in transcription, I tested the activity of promoter mutants in mouse embryonic stem cells lacking DNA methylation. Mutants with low CpG density were more transcriptionally active in cells that lacked DNA methylation than in wild-type cells, which suggests that high CpG density may be required to generate a chromatin environment that is permissive for transcription. My work underlines the importance of the sequence context for transcription, and provides an experimental framework for rigorously testing the putative regulatory roles of dinucleotides in regulatory elements.

PUBLICATIONS

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CHROMATIN DYNAMICS IN DNA DAMAGE AND REPAIR

cf. BIF FUTURA, VOL. 29 | 1.2014

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Institute: Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland
Supervisor: Prof. Susan M. Gasser

Genomic DNA in eukaryotes is highly organized and packed into chromatin. The most basic unit of chromatin is the nucleosome, in which DNA is wrapped around a core of histone proteins. Nucleosome occupancy along DNA influences chromatin properties and genome integrity. Chromatin remodellers slide, space, or eject nucleosomes from DNA. In response to DNA damage, these actions enable access to lesions and ensure proper restoration of chromatin structure after repair. DNA with double-strand breaks (DSBs) located in heterochromatic regions of high nucleosome occupancy relocates to the edge of such domains, which requires DNA mobility. In budding yeast and human cells, increased chromatin mobility in response to DNA damage has been observed both at DSBs and at undamaged sites where no lesion could be detected. Higher local mobility of DSBs correlated with efficient repair by homologous recombination (HR). As the mechanisms that underlie DNA damage-driven chromatin mobility were elusive, the goal of my PhD was to determine whether changes in nucleosome occupancy are involved. I challenged yeast cells with DNA-damaging agents and followed the amount of histones by immunoblotting, quantitative mass spectrometry, genome-wide nucleosome mapping, and fluorescence microscopy. I found that histone levels dropped 20–40% in response to DNA damage. The loss of histones was mediated by the proteasome and required the DNA damage checkpoint and chromatin remodeller function. Chromatin decompaction and increased fibre flexibility accompanied histone degradation, but also occurred when histone levels were reduced by other means. Such artificially induced reductions also increased the rates of HR. My findings uncovered a previously unknown pathway that triggers the proteasomal degradation of histones in response to DNA damage. This provides a mechanism for enhanced chromatin mobility and more efficient HR. My results also suggest that modulation of nucleosome occupancy could be used to increase HR rates in DNA-editing technologies such as CRISPR-Cas9.

PUBLICATIONS

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CYTOPLASMIC FUNCTIONS OF THE tRNA LIGASE COMPLEX IN HEALTH AND DISEASE

cf. BIF FUTURA, VOL. 28 | 2.2013

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Discipline: Molecular Cell Biologist, Diploma
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Supervisor: Dr Javier Martinez

Transfer RNA (tRNA) ligases splice together discontinuous tRNA genes to produce functional tRNAs for protein synthesis. These enzymes have also been implicated in biological processes such as unconventional mRNA splicing, RNA repair, and the replication of RNA viruses. The only known substrate of the mammalian tRNA ligase complex, which has a catalytic subunit called RTCB, was the tRNA exon halves that it splices together to create whole tRNAs. The aim of my PhD project was to identify and characterize new functions of the mammalian tRNA ligase complex. Using inducible, small hairpin RNA-mediated depletion of RTCB and its co-factor archease in HeLa cells, I identified the mammalian tRNA ligase complex as the RNA ligase responsible for splicing the X-box binding protein 1 (*XBP1*) mRNA as part of the unfolded protein response. This unconventional splicing reaction causes a frame shift and enables expression of the transcription factor XBP1s. In the absence of RTCB and archease, *XBP1* mRNA splicing fails and XBP1s is not expressed. Using RNA sequencing, I revealed that HeLa cells in which RTCB and archease were depleted show alterations in the activation of major cellular signalling pathways such as extracellular signal-regulating kinase / mitogen-activated protein kinase signalling or transforming growth factor β signalling. Cells lacking tRNA ligase accumulated in the G_0/G_1 phase of the cell cycle, leading to reduced proliferation kinetics in comparison to control cells. These functions of RTCB seem to be independent of its role in *XBP1* mRNA splicing, as overexpression of XBP1s fails to restore normal signalling levels or proliferation kinetics. Although depletion of RTCB and archease in four cancer cell lines revealed mainly cell line-specific transcriptome changes, all the cell lines that I generated were less competitive than control cells, suggesting that their proliferation kinetics were affected. Taken together, my results have the potential to offer new avenues for the treatment of diseases in which XBP1s has been implicated, such as multiple myeloma, triple-negative breast cancer, and chronic lymphocytic leukemia.

PUBLICATIONS

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STRUCTURAL BASIS OF MITOCHONDRIAL TRANSCRIPTION INITIATION AND PROCESSIVE ELONGATION

cf. BIF FUTURA, VOL. 29 | 2.2014

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

Cellular respiration depends on transcription of the mitochondrial genome by the mitochondrial RNA polymerase (mtRNAP). However, the mechanistic basis of this process and its regulation are poorly understood. During my PhD research, I used X-ray crystallography to determine the structures of proteins involved in mitochondrial transcription and of their functional complexes with mtRNAP and nucleic acid. To initiate transcription, mtRNAP associates with mitochondrial transcription factor A (TFAM) and mitochondrial transcription factor B2 (TFB2M) at the promoter to form the initiation complex (IC). The structures of TFAM and mtRNAP were known, but no structural information for TFB2M or the IC was available. Thus, I solved the structures first of human TFB2M and then of the entire IC bound to promoter DNA. These structures reveal how TFAM interacts with mtRNAP to recruit it to the promoter, and how TFB2M enables mtRNAP to melt the DNA strands and stabilizes this open complex. Following initiation, TFAM and TFB2M are lost and the polymerase recruits the transcription elongation factor (TEFM) to ensure processive elongation of the transcript. Without TEFM, transcription prematurely terminates at a G-quadruplex sequence, and the RNA is used as a primer for DNA replication. Hence, mtRNAP acts as a primase, and its activity is regulated by TEFM. To elucidate how TEFM achieves this, I determined the structures of TEFM and of the anti-termination complex consisting of TEFM bound to transcribing mtRNAP. These structures demonstrate that TEFM stabilizes the elongation complex by forming a clamp around the DNA and binding the single-stranded portion of the transcription bubble. TEFM also interacts with mtRNAP to form an RNA exit path, presumably preventing formation of the G-quadruplex. Taken together, my results substantially deepen our understanding of human mitochondrial transcription and provide the framework for investigating how this important process is regulated.

PUBLICATIONS

Hillen HS, Parshin AV, Agaronyan K, Morozov YI, Graber JJ, Chernev A *et al* (2017) Mechanism of transcription anti-termination in human mitochondria. *Cell* **171:** 1082–1093.e13

Hillen HS, Morozov YI, Sarfallah A, Temiakov D, Cramer P (2017) Structural basis of mitochondrial transcription initiation. *Cell* **171:** 1072–1081.e10

PRIMING NUCLEIC ACID SYNTHESIS WITH A EUKARYOTIC-LIKE ARCHAEAL PRIMASE

cf. BIF FUTURA, VOL. 28 | 2.2013

SANDRO HOLZER
Discipline: Biochemist, MSc
Institute: Department of Biochemistry, University of Cambridge, Cambridge, UK
Supervisor: Prof. Luca Pellegrini

DNA replication depends on primase, the specialized polymerase responsible for synthesizing the RNA primers that are subsequently elongated by replicative DNA polymerases. In eukaryotic and archaeal replication, primase is a heterodimer of two subunits: the small subunit, PriS, and the large subunit, PriL. PriS contains the active site for polymerization, while PriL has an iron–sulphur cluster domain that is required only during the initiation step, when a chemical bond is formed between two free nucleotides at the very beginning of primer synthesis. The mechanistic relevance of the iron–sulphur cluster in primer synthesis is contentious: the view of the iron–sulphur cluster as a purely structural feature of PriL opposes the idea that it might be directly involved in the catalysis of primer initiation. Recently, a third primase subunit, PriX, was identified in the hyperthermophilic archaeon *Sulfolobus solfataricus*. PriX is essential for primer synthesis and is structurally related to the iron–sulphur cluster domain of eukaryotic PriL. By solving a high-resolution crystal structure of the heterotrimeric primase (PriSLX) with bound nucleotide, I showed that PriX contains a nucleotide-binding site. Interpretation of the crystal structure combined with mutational studies allowed me to demonstrate homology between the nucleotide-binding residues in PriX and residues in eukaryotic PriL that are essential for the initiation of primer synthesis. I fused PriX with a truncated version of PriL that lacked the iron–sulphur cluster domain and showed that the chimera retained wild-type *S. solfataricus* levels of primer synthesis. Thus, my results demonstrate that in *S. solfataricus*, PriX has evolved to replace PriL as the subunit endowing primase with the unique ability to initiate nucleic acid synthesis. As all archaeal and eukaryotic primases are structurally related, the first description of the PriX nucleotide binding site together with the finding that the iron–sulphur cluster is not catalytically relevant represents an important addition to the general mechanistic understanding of primer synthesis.

PUBLICATIONS

Holzer S, Yan J, Kilkenny ML, Bell SD, Pellegrini L (2017) Primer synthesis by a eukaryotic-like archaeal primase is independent of its Fe-S cluster. *Nat Commun* **8:** 1718

Yan J, Holzer S, Pellegrini L, Bell SD (2017) An archaeal primase functions as a nanoscale caliper to define primer length. Proc Natl Acad Sci U S A 115: 6697-6702

USING DNA SEQUENCING TO MAP CONNECTIONS IN THE BRAIN

cf. BIF FUTURA, VOL. 28 | 2.2013

JUSTUS KEBSCHULL
Discipline: Neuroscientist, MSc
Institute: Cold Spring Harbor Laboratory/Watson School of Biological Sciences, Cold Spring Harbor, NY, USA
Supervisor: Prof. Anthony Zador

The brain is the most complex organ of the body, formed by billions of neurons and trillions of synapses, all precisely connected by more than 150,000 kilometres of wiring. Understanding how the brain processes information relies on understanding these connections. However, we still lack a fine-resolution map of neural connectivity in mammals, because current microscopy-based brain-mapping techniques require the brain to be traced one neuron at a time. During my PhD, I reimagined the problem of brain mapping in terms of high-throughput DNA sequencing. I developed MAPseq, a method that allows 100,000 cells to be traced in parallel, making brain mapping faster and more economical than ever before. MAPseq uniquely labels thousands of neurons with different RNA sequence tags, or barcodes, using a simple injection of a diverse pool of barcoded Sindbis virus particles. Inside every brain cell, the barcode is amplified and trafficked into axons, where it can be detected by bulk sequencing of dissected brain areas, ultimately reporting the target of each axonal process. I applied MAPseq to the locus coeruleus, a brain area involved in arousal, and to the visual cortex. By mapping hundreds of individual neurons in each area, my colleagues and I revealed surprising connectional specificity, including projectional cell types and underlying organizing principles, in both areas. Expanding on these results, we multiplexed MAPseq to map the mouse neocortex, creating a large-scale map comparable to the Allen Mouse Brain Connectivity Atlas, but at single-cell resolution and from just a single animal, instead of hundreds, in a two-week experiment. In complementary work, we demonstrated that, in principle, a similar sequencing approach can be used to solve the problem of mapping synaptic connectivity, using a method called SYNseq.

PUBLICATIONS

Han Y*, Kebschull JM*, Campbell RAA*, Cowan D, Imhof F, Zador AM, Mrsic-Flogel TM (2018) The logic of single-cell projections from visual cortex. *Nature* **556:** 51–56

Peikon ID*, Kebschull JM*, Vagin VV*, Ravens DI, Sun Y, Brouzes E *et al* (2017) Using high-throughput barcode sequencing to efficiently map connectomes. *Nucleic Acids Res* **45:** e115

Kebschull JM, Garcia da Silva P, Reid AP, Peikon ID, Albeanu DF, Zador AM (2016) High-throughput mapping of single neuron projections by sequencing of barcoded RNA. *Neuron* **91:** 975–987

GENETIC DISSECTION OF FLAVIVIRIDAE HOST FACTORS THROUGH GENOME-SCALE CRISPR SCREENS

cf. BIF FUTURA, VOL. 30 | 1.2015

ANDREAS PUSCHNIK
Discipline: Microbiologist, BSc
Institute: School of Medicine, Stanford University, Stanford, CA, USA
Supervisor: Prof. Jan Carette

Viruses bind to cell surface receptors to enter host cells, where they co-opt intracellular organelles to replicate their genome and form new infectious particles. The *Flaviviridae* family, which includes dengue virus (DENV) and hepatitis C virus (HCV), causes severe disease in humans. Despite differences in their pathogenesis and mode of transmission, DENV and HCV have similar replication strategies. However, a detailed understanding of the host factors involved in viral infection is lacking. The goal of my PhD project was to find these host factors using a CRISPR genetic screening approach. I mutagenized human hepatocytes and infected them with the virus. Cells containing a mutation in a gene required for infection thrived, as they were resistant to the virus. In the DENV screen, I identified multi-protein complexes associated with the endoplasmic reticulum (ER) that are involved in signal sequence recognition, *N*-linked glycosylation, and the ER-associated degradation pathway. I showed that DENV replication was almost completely abrogated in cells lacking the oligosaccharyltransferase (OST) complex. Viral entry and replicon studies pinpointed viral RNA replication, but not entry or translation of the virus, as the critical step requiring the OST complex. Treatment using an OST inhibitor strongly reduced infection with DENV and other mosquito-borne flaviviruses, such as Zika and yellow fever virus. By contrast, the most significant genes identified in the HCV screen included viral entry receptors, RNA-binding proteins, and metabolic enzymes. I found a link between HCV replication and intracellular levels of flavin adenine dinucleotide, which could be targeted to block viral replication. Overall, my work has demonstrated a remarkable divergence in the host factors required by DENV and HCV and illuminated potential host targets for antiviral therapy.

PUBLICATIONS

Puschnik AS, Marceau CD, Ooi YS, Majzoub K, Rinis N, Contessa JN, Carette JE (2017) A small molecule oligosaccharyltransferase inhibitor with pan-flaviviral activity. *Cell Rep* **21:** 3032–3039

Puschnik AS, Majzoub K, Ooi YS, Carette JE (2017) A CRISPR toolbox to study virus-host interactions. *Nat Rev Microbiol* **15:** 351–364

Marceau CD*, Puschnik AS*, Majzoub K, Ooi YS, Brewer SM, Fuchs G *et al* (2016) Genetic dissection of *Flaviviridae* host factors through genome-scale CRISPR screens. *Nature* **535:** 159–163

THE DEVELOPMENT OF CELLULAR TUBES DEPENDS ON THE PROCESSING OF ENVIRONMENTAL CUES

cf. BIF FUTURA, VOL. 30 | 2.2015

JULIA SAUERWALD
Discipline: Biochemist, Diploma
Institute: Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland
Supervisor: Prof. Stefan Luschnig

Branching morphogenesis governs molecular and cellular processes within multiple cell types during development to build tubular organs, such as the mammalian vasculature, lung, and kidney. While substantial information has been gathered about the regulation of branching morphogenesis, the mechanisms used by developing vessels to invade the surrounding tissue are not well understood. Using the *Drosophila* tracheal (respiratory) system as a model system, I analysed the mechanistic interplay between developing vessels and their cellular environment during the invasion of branch sprouts into surrounding tissues. First, I found that tracheal branching, which involves dynamic regulation of gene expression, depends on efficient mRNA processing in cells surrounding the tracheal branch primordia. I showed that *faint sausage* (*fas*), a subunit of the pre-mRNA processing factor 19 (Prp19) complex, is essential for efficient pre-mRNA splicing. During branching morphogenesis, *fas* is predominantly required in the cellular environment to regulate chemoattractive guidance of branch sprouts. This regulation likely includes the dynamic production, processing, and distribution of the chemoattractive fibroblast growth factor (FGF) Branchless (Bnl). Second, I established tracheal invasion into *Drosophila* flight muscles as a model to investigate how sprouting branches enter into multinucleated muscle fibres. Using tissue-specific RNA interference, I showed that matrix metalloproteinase 1 (Mmp1) is required in tracheal cells for proper invasion into muscle cells. Apart from its known function in extracellular matrix remodelling, Mmp1 modulates the levels of FGF Bnl in a tissue culture assay. Hence, Mmp1 produced by tracheal cells may contribute *in vivo* to establishing a self-generated chemotactic gradient through depletion of FGF Bnl around invading branches. Matrix metalloprotease-mediated degradation could represent a new strategy to modulate the distribution of a chemoattractant during growth factor-induced chemotaxis. My studies provided a conceptual framework for how branch invasion is modulated by integration and processing of environmental cues.

PUBLICATIONS

Sauerwald J, Soneson C, Robinson MD, Luschnig S (2017) Faithful mRNA splicing depends on the Prp19 complex subunit *faint sausage* and is required for tracheal branching morphogenesis in *Drosophila*. *Development* **144:** 657–663

DEVELOPMENTALLY REGULATED GTP BINDING PROTEIN 1 (DRG1) CONTROLS MICROTUBULE DYNAMICS

cf. BIF FUTURA, VOL. 29 | 1.2014

ANNA KATHARINA SCHELLHAUS
Discipline: Biochemist, Diploma
Institute: Friedrich Miescher Laboratory of the Max Planck Society (FML), Tübingen, Germany
Supervisor: Prof. Wolfram Antonin

The cell undergoes enormous structural rearrangements in order to divide. Chromatin condenses and the nuclear envelope breaks down at the beginning of mitosis. Microtubules form the mitotic spindle, which is necessary for chromosome segregation. At the end of mitosis, these events are reversed: chromatin decondenses and the nuclear envelope reforms. The activity of microtubules, the highly dynamic cytoskeletal filaments forming the mitotic spindle, is regulated by many microtubule-associated proteins. During my PhD project, mass spectrometry revealed that developmentally regulated GTP binding protein 1 (DRG1) interacts with several spindle assembly factors, so I characterized it in the context of microtubules. DRG1 is a highly conserved GTPase, but its function is largely unknown. By incubating taxol-stabilized microtubules with recombinant DRG1, followed by centrifugation, I showed that DRG1 binds directly to microtubules. Single-molecule total internal reflection fluorescence microscopy showed that after binding, DRG1 can either remain immobile or diffuse on the microtubule lattice. Using *in vitro* approaches with recombinant DRG1 and fluorescently labelled tubulin or microtubules, I found that DRG1 promotes microtubule polymerization and prevents depolymerization. Small interfering RNA-mediated knockdown of DRG1 in HeLa cells delayed progression from prophase to anaphase. I also showed that many different domains of DRG1 bind microtubules, but full-length DRG1 is needed to promote microtubule polymerization, bundling, and stabilization. By contrast, GTP activity is not necessary for these functions. In summary, I identified DRG1 as a microtubule-binding protein with manifold microtubule-associated functions that are crucial for timely spindle assembly during mitosis, which is deregulated in many diseases such as cancer.

PUBLICATIONS

Schellhaus AK, Moreno-Andres D, Chugh M, Yokoyama H, Moschopoulou A, De S *et al* (2017) Developmentally regulated GTP binding protein 1 (DRG1) controls microtubule dynamics. *Sci Rep* **7:** 9996

Schellhaus AK*, De Magistris P*, Antonin W (2016) Nuclear reformation at the end of mitosis. *J Mol Biol* **428:** 1962–1985

Magalska A, Schellhaus AK, Moreno-Andres D, Zanini F, Schooley A, Sachdev R *et al* (2014) RuvB-like ATPases function in chromatin decondensation at the end of mitosis. *Dev Cell* **31:** 305–318

RECONSTITUTING THE EMERGENCE OF VISUAL CORTICAL FEATURE SELECTIVITY

cf. BIF FUTURA, VOL. 29 | 1.2014

MANUEL SCHOTTDORF
Discipline: Physicist, MSc
Institute: Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany
Supervisor: Prof. Fred Wolf

Information processing in the nervous system requires the co-ordinated activity of neurons interacting in complex circuits. Despite efforts to understand the design principles underlying most neuronal circuits, they remain one of the greatest challenges facing science. In my PhD project, I developed synthetic hybrid circuits comprising simulated and living neurons, in which key features of the wiring diagram – or connectome – could be artificially modified. The first component of the hybrid circuit was a computational model of the mammalian early visual system. Using digital holography and optogenetics, I interfaced the output of this *in silico* simulation with a surrogate visual cortex – a recurrent network of living neurons *in vitro*. A key component of the mammalian visual system is the primary visual cortex, which comprises nerve cells that detect features, such as specific aspects of visual scenes. Feedforward and recurrent circuits contribute to feature selectivity, but their interplay is enigmatic because they are hard to control selectively and specifically *in vivo*. By manipulating the structure of the simulated connectome, I showed that recurrent circuits in the surrogate cortex were sufficient to spontaneously generate feature selectivity. Many neurons responded selectively to the orientation of simulated moving gratings, and reverse correlation with a checkerboard stimulus revealed excitatory and inhibitory subregions that were a good indicator of the preferred orientation. Linear, orientation-tuned neurons – called simple cells – were historically the first indicator from a single-cell perspective that the cortex might do something more than represent afferent neuronal inputs. I found that simple cells can emerge naturally in recurrent circuits. My work demonstrates that this synthetic biology approach complements classical neurobiology, broadens our understanding of neuronal circuits, and extends the realm of questions that can be addressed experimentally.

PUBLICATIONS

Samhaber R*, Schottdorf M*, El Hady A*, Bröking K, Daus A, Thielemann C *et al* (2016) Growing neuronal islands on multi-electrode arrays using an accurate positioning-μCP device. *J Neurosci Methods* **257:** 194–203

Schottdorf M*, Keil W*, Coppola D, White L, Wolf F (2015) Random wiring, ganglion cell mosaics, and the functional architecture of the visual cortex. *PLoS Comp Bio* **11:** e1004602

MOLECULAR MECHANISMS OF RNP REMODELLING BY THE SPLICEOSOMAL BRR2 RNA HELICASE

cf. BIF FUTURA, VOL. 29 | 1.2014

MATTHIAS THEUSER
Discipline: Biochemist, Diploma
Institute: Freie Universität Berlin, Berlin, Germany
Supervisor: Prof. Markus C. Wahl

The splicing of pre-messenger RNA (pre-mRNA) to create messenger RNA is facilitated by the spliceosome. This large molecular machine, which consists of small nuclear ribonucleoproteins (RNPs) and many other factors, assembles anew on each pre-mRNA substrate. During each splicing cycle, the spliceosome is repeatedly remodelled by spliceosomal RNA helicases. The most dramatic remodelling events take place during spliceosome catalytic activation and require the Brr2 RNA helicase, which unwinds a protein-decorated U4/U6 small nuclear RNA (snRNA) duplex. Structural snapshots captured by cryo-electron microscopy have provided detailed insights into various spliceosomal intermediates, including the stages before and after Brr2 activity. However, the detailed molecular mechanisms underlying Brr2-mediated spliceosome activation remained unresolved. To investigate these mechanisms, I reconstituted reduced, recombinant U4/U6 particles from yeast *in vitro*. Using enzymatic assays, I probed the influence of wild-type and mutant spliceosomal proteins and RNAs on Brr2-mediated U4/U6 snRNA duplex unwinding and RNP remodelling. All the U4/U6-bound proteins that I tested inhibited unwinding, whereas the C-terminal portion of Prp8, a large spliceosomal protein, efficiently restored this activity. RNP disruption assays revealed that Brr2 releases protein-decorated U4 snRNA and protein-free U6 snRNA, the latter of which can subsequently be incorporated into the spliceosome's active site. Moreover, my data indicate that Brr2 translocates only a limited distance on the U4 snRNA strand, and that U4/U6 disruption is completed by U6 conformational switching into an alternative stem loop structure. In summary, I observed a hitherto unreported activity of an RNA helicase, which involves translocation of the enzyme on the same RNA molecule that is part of the displaced RNP. My results thus aid in understanding not only spliceosome catalytic activation but also RNA helicase mechanisms.

PUBLICATIONS

Theuser M, Höbartner C, Wahl MC, Santos KF (2016) Substrate-assisted mechanism of RNP disruption by the spliceosomal Brr2 RNA helicase. P*roc Natl Acad Sci USA* **113:** 7798–7803

Liu S, Mozaffari-Jovin S, Wollenhaupt J, Santos KF, Theuser M, Dunin-Horkawicz S *et al* (2015) A composite double- / single-stranded RNA binding region in protein Prp3 supports tri-snRNP stability and splicing. *eLife* **4:** e07320

THIOESTER PROTEINS ACT AS ADHESINS OF GRAM-POSITIVE PATHOGENIC BACTERIA

cf. BIF FUTURA, VOL. 30 | 1.2015

MIRIAM WECKENER
Discipline: Biochemist, MSc
Institute: Biomedical Sciences Research Complex, University of St Andrews, St Andrews, UK
Supervisor: Dr Ulrich Schwarz-Linek

Before they can colonize their host and cause an infection, pathogenic bacteria need to closely interact with and bind to host cells in a process called adhesion. The bacterial surface proteins responsible for this interaction are called adhesins. My PhD project focused on two adhesins from the gut pathogen *Clostridioides difficile* that have been predicted to facilitate interaction with the host through a covalent bond in a thioester domain (TED). TEDs contain an intramolecular, self-generated thioester bond between cysteine and glutamine side chains. I recombinantly expressed the two TEDs, CdTIE-A-TED and CdTEP-TED, in *Escherichia coli*, purified and crystallized them, and determined their structures. CdTIE-A-TED has the minimal features of a TED: an α-helical bundle and a β-barrel that contains the thioester bond. By contrast, CdTEP-TED is an elongated protein that contains the characteristic features of a TED but is extended by β-sheets at the N- and C-termini. Using nuclear magnetic resonance spectroscopy, I studied the effect of small molecules, which can lyse the thioester bond, on the CdTIE-A TED structure. I showed that the TED mostly remains in its native conformation; only amino-acid residues near the thioester bond are affected. I obtained the same result when small-molecule inhibitors reacted specifically and covalently with the thioester bond-forming residues. The residues are bridged by the inhibitor, so the thioester bond is no longer reactive. Using a cell assay, I showed that the thioester bond needs to be intact for the TED to act as an adhesin. In mammalian cell culture experiments, the two TEDs adhered to lung epithelial cells in which the acute inflammatory response had been induced. Through my work, I discovered that TEDs in *C. difficile* play an active role in facilitating covalent adhesion to host tissues, a mechanism not previously characterized for these bacteria. The adhesion to inflamed cells might indicate that TEDs play a role in later stages of infection, after the host has sensed the pathogen and the immune system has been activated. The specificity of the reaction between a small molecule inhibitor and the thioester bond raises the possibility of developing novel drugs targeted specifically at TEDs in Gram-positive bacteria.

PUBLICATIONS

The results of this project have not yet been published.

PROTEIN LIPIDATION IN THE CONTEXT OF ADULT NEUROGENESIS

cf. BIF FUTURA, VOL. 28 | 2.2013

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

Neural stem and progenitor cells (NSPCs), which are found in the mammalian brain, are characterized by their ability to self-renew and to give rise to all neural cell types. Alterations in neurogenesis, the process that produces neurons from NSPCs, can lead to neuropsychiatric diseases such as epilepsy or cognitive ageing. Although newly generated lipids are essential for NSPCs to proliferate, the relevant mechanisms were unknown. The aim of my PhD project was to characterize how changes in lipid metabolism affect NSPC activity. I hypothesized that the transition between quiescence and proliferation is associated with major changes in protein lipidation, a post-translational modification that facilitates fast adaptations in protein localization and function. I used mass spectrometry to identify more than 300 lipidated proteins in mouse NSPCs that were not previously known to carry a fatty acid modification. I also discovered that a change in NSPC proliferation rate alters the lipidation of some of these proteins. To validate this large-scale analysis, I selected some of the candidate proteins and confirmed that they were modified with fatty acids. To identify the number and location of the modified amino-acid residues, I used acyl-poly-ethylene glycol exchange assays. With this new information, I could then use CRISPR-Cas9-mediated gene editing to mutate the lipidated residues in NSPCs and use acyl-biotin-exchange assays to confirm the loss of lipidation. Live-cell microscopy enabled me to characterize changes in protein trafficking towards the plasma membrane in these mutated NSPCs. Luciferase assays helped me to characterize how the loss of lipidation changes the activity of signalling pathways that are required to maintain NSPC quiescence. By showing that protein lipidation is a key regulator of NSPC proliferation and quiescence, my work may lead to a better understanding of the molecular mechanisms underlying neurogenesis in the healthy and the diseased brain.

PUBLICATIONS

Knobloch M, Pilz G-A, Ghesquière B, Kovacs WJ, Wegleiter T, Moore DL *et al* (2017) A fatty acid oxidation-dependent metabolic shift regulates adult neural stem cell activity. *Cell Rep* **20:** 2144–2155

Rolando C, Erni A, Grison A, Beattie R, Engler A, Gokhale PJ *et al* (2016) Multipotency of adult hippocampal NSCs *in vivo* is restricted by Drosha/NFIB. *Cell Stem Cell* **19:** 653–662

MD FELLOWS 2018

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 2018.

QUYNH BACH

Identifying the molecular causes of rare human thymic stromal cell defects

JULIA BUNGENBERG

Molecular factors of trans-synaptic α-synuclein spread

LENNARD HALLE

Local function of GM-CSF in acute myocardial infarction

TORBEN HUNCK

Splicing factor mutations in MDS/AML

CHRISTIAN KLEIMEYER

The role of adipose tissue NOD-like receptors and inflammasomes in health and metabolic syndrome disorders

SAMUEL LOEBELL

Molecular determinants of the response of cancer cells to CDK4/6 inhibition

JULIA MATTHIAS

The role of the extra-large G protein α (XLas) in kidney failure-induced FGF23 elevation

JONAS PRENISSL

The transcription factor GATA3 and its influence on human regulatory T-cell function and plasticity in multiple sclerosis

MAX TRÄGER

Characterization of tumour–stroma interactions in KRAS-mutated organoid models of human pancreatic cancer

IDENTIFYING THE MOLECULAR CAUSES OF RARE HUMAN THYMIC STROMAL CELL DEFECTS

QUYNH BACH

Duration: 05/17–08/17

Project at: University of Oxford, Weatherall Institute of Molecular Medicine, Oxford, UK

Supervisor: Professor Georg A. Holländer, MD

Home University: Charité – Universitätsmedizin Berlin

MOLECULAR FACTORS OF TRANS-SYNAPTIC α-SYNUCLEIN SPREAD

JULIA BUNGENBERG

Duration: 05/17–05/18

Project at: Tufts University School of Medicine, Department of Neuroscience, Boston, MA, USA

Supervisor: Professor Thomas Biederer, PhD

Home University: University Hospital Bonn

LOCAL FUNCTION OF GM-CSF IN ACUTE MYOCARDIAL INFARCTION

LENNARD HALLE

Duration: 09/17–08/18

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

Supervisor: Professor Filip K. Swirski, PhD

Home University: University Heart Center Freiburg

SPLICING FACTOR MUTATIONS IN MDS/AML

TORBEN HUNCK

Duration: 10/17–09/18

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

Supervisor: Professor Stephanie Halene, MD

Home University: Medical Center – University of Freiburg

THE ROLE OF ADIPOSE TISSUE NOD-LIKE RECEPTORS AND INFLAMMASOMES IN HEALTH AND METABOLIC SYNDROME DISORDERS

CHRISTIAN KLEIMEYER

Duration: 10/17–09/18

Project at: Weizmann Institute of Science, Department of Immunology, Rehovot, Israel

Supervisor: Eran Elinav, MD, PhD

Home University: Medical Center – University of Freiburg

MOLECULAR DETERMINANTS OF THE RESPONSE OF CANCER CELLS TO CDK4/6 INHIBITION

SAMUEL LOEBELL

Duration: 11/17–10/18

Project at: Stanford University Medical Center, Departments of Pediatrics and Genetics, Stanford, CA, USA

Supervisor: Professor Julian Sage, PhD

Home University: German Cancer Research Center (DKFZ)

THE ROLE OF THE EXTRA-LARGE G PROTEIN α (XLAS) IN KIDNEY FAILURE-INDUCED FGF23 ELEVATION

JULIA MATTHIAS

Duration: 09/17–09/18

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

Supervisor: Professor Murat Bastepe, MD, PhD

Home University: Charité – Universitätsmedizin Berlin

THE TRANSCRIPTION FACTOR GATA3 AND ITS INFLUENCE ON HUMAN REGULATORY T-CELL FUNCTION AND PLASTICITY IN MULTIPLE SCLEROSIS

JONAS PRENISSL

Duration: 10/17–09/18

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

Supervisor: Professor David A. Hafler, MD

Home University: University Hospital Mannheim

CHARACTERIZATION OF TUMOUR–STROMA INTERACTIONS IN KRAS-MUTATED ORGANOID MODELS OF HUMAN PANCREATIC CANCER

MAX TRÄGER

Duration: 08/17–10/17

Project at: Massachusetts General Hospital, Edwin L. Steele Laboratories for Tumor Biology, Charlestown, MA, USA

Supervisor: Professor Dan G. Duda, DMD, PhD

Home University: Münster University Hospital

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.

GRACHT 2018: A FOND FAREWELL

By Kirsten Achenbach

On the first weekend of July, for the 28th time, we welcomed participants to the BIF alumni seminar at Gracht Castle. Sadly, as those present already know, it was our last time there, as the castle has been sold and, effective October 2018, will be used as a stress medicine and prevention centre. In the future, we will have to find a new and (given its popularity) larger home for our alumni seminar.

Photo: Kirsten Achenbach





The motto of the last Gracht meeting was "You and me, similar but different." Together with around 120 particpants it explored human diversity from different angles, including human evolution, our gut microbiomes, personalized medicine, epigenetics, and human perception. In keeping with the theme, the talks were given by an equally diverse mix: reknown paleoantrophologist Friedemann Schrenk from the Senckenberg Museum in Frankfurt, bioinformatician Peer Bork from the EMBL in Heidelberg, our alumni pathologist Ruth Knückel Clarke from the university clinic in Aachen, Alexander Meissner, director at the MPI for Molecular Genetics, and artist and synaesthete Christine Söffing. On Saturday afternoon, spurred by last year's positive response, we again offered a round of speed-dating. In an orchestrated series of four-minute mini "dates", the participants met new people or learned new things about already familiar ones. To judge by the animated discussions afterwards, this format again proved to be a success. As has become tradition, our Sunday talk by Miles Hewstone from the Oxford Centre for the Study of Intergroup Conflict, Oxford, UK, gave a very different take on the subject. Under the heading "Why can't we live together", he presented field studies showing how prejudices can be lessened and acceptance of differences can be furthered.

As if to make up for it being the last time, Gracht Castle offered the best weather, filling the terrace to capacity at meal times and during the coffee breaks, creating the usual BIF atmosphere for catching up, networking, and discussing science, career options and – how could it be otherwise – food and wine.

BIF's first alumni seminar in 1990 took place with only 30 participants. This year, Sandra sent out invitations to about 900 BIF alumni living and working in Europe. We had to turn people down again this year because the lecture room, the Rittersaal, was bursting at the seams. During the very first seminar, BIF alumni reported on research conditions abroad in countries as diverse as New Guinea and the USA. They also discussed how BIF could shape its relationships with its alumni. Apparently, relationships and the venue have proved excellent; after all, about 90 per cent of our alumni stay in touch with us and BIF returned to Gracht 27 times. Topics covered over the course of the years range from how to fund research to its evaluation and ethics, from bacterial life in the deep sea to human biology in outer space, from autoimmunity to epidemics, from career options to science policy. Not only was knowledge imparted, but the participants formed friendships (or more) and launched scientific and commercial collaborations. A big heartfelt "thank you" goes to the staff of Gracht Castle, especially Hartmut and Sylvia Althoff, who have always made us feel welcome, solved every problem with a smile, and met even the strangest request – e.g. finding 100 "instruments" such as spoons, bottles, trays, trashcans, empty canisters, and keyboards for our sound workshop last year. Without them and their team, we would not have been able to create that special Gracht feel. They and their castle are now a cherished part of BIF's history.

1 **The Rittersaal** has a great, charming atmosphere, but it has grown too small for us.
2 **Piotr Szwedziak and Christiane Boeddinghaus** enjoying the relaxed atmosphere.
3 **Animated discussions** about everything from science to fiction, from work to private interests, are a hallmark of our alumni seminars, here Ivana Gasic.
4 **Underneath the heraldic sign** of the Metternich family at the entrance to Gracht Castle is the 1538 proclamation of ownership by Hieronymus Wolff, who also bore the name Metternich.
5 **Scientific collaborations** and friendships begin and are nurtured at BIF's seminars, here Christina Helbig, Gerrit Praefcke, Boris Klockow, and Christian Ungermann.

Photos: Ayse Tascí-Steinebach (1,3); Christian Ungermann (2, 5); Schloss Gracht (4)

A CASTLE WHERE HISTORY WAS MADE

As a tribute to the long history we share with Gracht Castle, we have put together a short overview of its history. Gracht Castle itself was first mentioned in 1433, although it probably had existed before then. The first picture is from 1642. It shows the castle divided by a moat that separates the main part from the outbuildings and gives the castle its name – Gracht being a German word for canal. Over four centuries, from 1638 to 1957, the castle was the seat of the aristocratic Wolff Metternich family from Hesse – one of its ancestors had married the heiress to the castle. The fortunes of the next few generations of Metternichs prospered and many of its members held high church and state offices. They were bishops and knights, ministers and barons, shaping and influencing Germany's history. The first of the barons, Reichsfreiherr Johann Adolf (1592–1669), for example, held many influential posts in the court of Maximilian I, Elector of Bavaria, which brought him prestige and wealth. Using Versailles as his role model, he started to rebuild Gracht as a prestigious, albeit much smaller, castle with impressive gardens. Nowadays, only a small part of the gardens remain. But it was not only the people in the grand house who shaped history. Carl Schurz (1829–1906) was born and grew up in the tenants' quarter of the castle. He took part in the German revolution of 1848/49, and because of that had to leave Germany. He fled first to Switzerland, then to France, Scotland, and London. In 1852, he and his wife immigrated to the USA, where she founded the first kindergarten in the country. Carl achieved enough for several lives: he helped Abraham Lincoln to win the election of 1860, was the US ambassador to Spain, served as a general in the Union, shaped the civil service reform, and was the first German-born American senator and then Secretary of the Interior from 1876 to 1881. In 1957, the Metternichs sold Gracht Castle to the town of Liblar. After changing hands several times, in 1973 it was bought by the Universitätsseminar der Wirtschaft (USW), an education centre for managers. The USW was later integrated into the European School of Management and Technology (ESMT), a private university for managers, which has recently sold the castle.

The first illustration of Gracht from 1642

Born in Gracht: Carl Schurz (1829–1906), German revolutionary and US Secretary of the Interior

Photos: Schloss Gracht (top); Detail Matthäus Merian d. Ä (middle); gemeinfrei (bottom)

PERSPECTIVES

FROM TECHNICAL ASSISTANT TO PROFESSOR

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 PETRA BOUKAMP, GERMAN CANCER RESEARCH CENTER (DKFZ), HEIDELBERG, AND LEIBNIZ INSTITUTE FOR ENVIRONMENTAL MEDICINE, DÜSSELDORF, GERMANY

Photo: Deutsche Krebsgesellschaft

Petra Boukamp, born in Bremen in 1949, joined the German Cancer Research Center (DKFZ) in Heidelberg in 1972 as a technical assistant. She then started studying biology in Heidelberg and received her PhD in Kaiserslautern in 1987. After several research stays at the University of Irvine, California, she continued at the DKFZ, where she established an independent research group. In 2001, she became head of its Division of Genetics of Skin Carcinogenesis and in 2004 also a professor at the University of Heidelberg. After reaching official retirement age in 2015, she took up a post as team leader at the Leibniz Institute for Environmental Medicine in Düsseldorf, while finishing her work at the DKFZ. She received, among other prizes, the 2017 German Cancer Award for Experimental Research from the German Cancer Society for her 3D models of human skin, which are invaluable for skin cancer research and other fields.

What would you see as your greatest achievements?

It's difficult to reduce this to only a few things. First, I am happy and proud that all our early data are still valid. Then, partly due to our results showing the devastating effect of UVA on skin, the WHO acknowledged it as a carcinogen and Germany introduced much stricter laws for tanning booths. And of course our human skin models. I managed to establish the first human immortalized skin keratinocyte line, the HaCaT cells, used in more than 600 laboratories worldwide. We were among the first to establish 3D organotypic culture models and manage to drive this artificial skin "as close as possible to real life". As it survives for more than six months, we can use it to study, for example, how stem cells are maintained and regenerated. Furthermore, our unique human skin tumour invasion model allows investigations of how skin cancers start out and how drugs work, while reducing animal experiments.

You never took the direct path – looking back, how do you see this?

My father's generation still thought that daughters didn't need to study because they would marry and become housewives anyway. I was, however, allowed to train as a biological technical assistant (BTA) and later studied biology while continuing to work, thanks to a very supportive boss. Doing it this way proved a great advantage in the lab. The other PhD students had to learn lab skills and routines the hard way very quickly. It also taught me the technical needs – and how long it takes to actually do experiments, something that "bosses" easily forget. For a long time, I conducted my own experiments just to give it a try and enjoy, or suffer, the outcome, a feeling typically lost when merely supervising.

What do think science and especially young researchers need?

Science needs more time and less pressure to answer the big questions. Pressure to publish only in high-ranking journals and create excitement may drive science into a biased or even the wrong direction. We may easily overlook what is not obvious, discarding it like an artefact if it doesn't fit the big picture. To me, a successful scientist needs never-ending enthusiasm. Stay in academia only if science is a real passion. It doesn't make you happy every day. Experiments often go wrong, but if they work and prove your hypothesis, all the pain is forgotten and gives way to exaltation.

Do you have passions other than science?

Gardening comes close, on my balcony at home or at our home on Mallorca. I also collect umbrellas. The latest one is from Gracht Castle – as a memory of many wonderful alumni seminars.

Knowing what you do – how do you protect your skin?

To me, the sun is best enjoyed in the shade. Sunscreen is important, but can give a false sense of security.

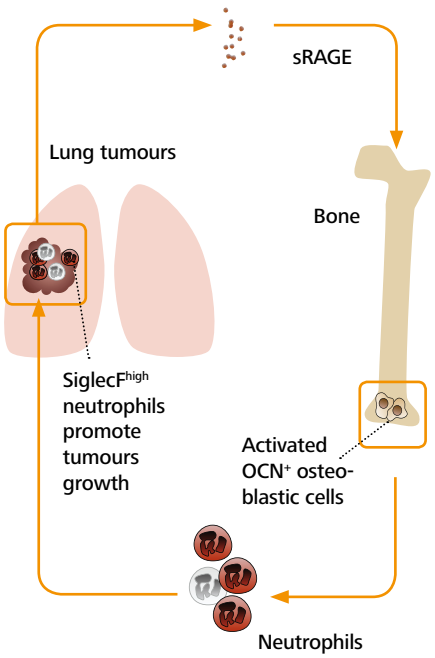
PAPERS IN THE SPOTLIGHT

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.

HOW LUNG CANCER GETS THE BONE MARROW'S HELP

Solid tumours often contain a large number of immune cells, which derive from circulating precursors originating in the bone marrow. Camilla Engblom from Mikael Pittet's group at the Massachusetts General Hospital in Boston, USA, has now found in mice that even without metastases in the bone, lung tumours can remotely ramp up the activity of certain bone marrow cells, increasing bone density. In addition, these osteocalcin-expressing osteoblast (OCN$^+$) set off a cascade of events that reach back to the tumour, helping it to grow. They induce the production and release of a subset of immune cells called neutrophils, which in turn have a range of tumour-promoting activities. These neutrophils overexpress the cell surface receptor lectin SiglecF and genes fostering cancerous processes. They are also 70 times more numerous in lung cancers than in normal lung tissue. Deleting OCN$^+$ cells in mice – and consequently SiglecF^{high} neutrophils – slowed lung tumour growth visibly. Also, cancer patients who showed a SiglecF^{high}-gene expression profile had poorer disease prognosis.

In addition, Engblom and her colleagues identified a potential messenger in the blood of tumour-bearing mice contributing to the remote control of the osteoblasts: sRAGE, the soluble receptor for advanced glycation end products. Finding and blocking such tumour messengers may offer worthwhile targets for fighting many different types of cancer at the systemic level as it stops the body from aiding and abetting the enemy involuntarily.



Cancer's long arm: via a blood-borne messenger, lung tumours can activate cells in the bone marrow which then induce tumour-invading and growth-promoting neutrophils.

REFERENCE
Engblom C, Pfirschke C, Zilionis R, Da Silva Martins J, Bos SA, Courties G, *et al* (2017) Osteoblasts remotely supply lung tumors with cancer-promoting SiglecF(high) neutrophils. *Science*. **358**, eaal5081

Camilla Engblom, fellow 2014–2016

BARCODING SINGLE NEURONS REVEALS PATTERNS

Finding out which nerve cells talk with each other, a map of the brain so to speak, could go a long way towards understanding the brain. But so far it has taken years to map just dozens of cells, although efforts are underway to change that. Thanks to Justus Kebschull and the MAPseq technique he developed in the lab of Anthony Zador at the Cold Spring Harbor Laboratory, New York, USA, we now have a fast, cheap, and reliable way to pinpoint, for potentially hundreds of thousands of neurons, where they send their far-reaching axons to. This enables us to detect patterns in their wiring and thus lay the foundation for matching anatomy and function. MAPseq uses an RNA virus to label each neuron with a randomly-generated, unique nucleotide string or "barcode". These barcodes travel along the branches of the neuron's axon all the way to wherever it projects to. Looking at six areas in the primary visual cortex of mice, Justus and his co-authors detected the barcodes by high-throughput sequencing, revealing which of the areas each cell had reached. Their results refute the popular idea that neurons usually project to only one area in the cortex: of 591 neurons followed, 44 percent connected to more than one area. The large data set also revealed six wiring patterns that showed up more often than chance connections would have predicted. Justus suggests that one neuron sharing its information with different brain areas at the same time helps to generate visual representations and multimodal associations. And the best part: getting the data only took three weeks! By comparison, tracing the axons of just 71 fluorescently

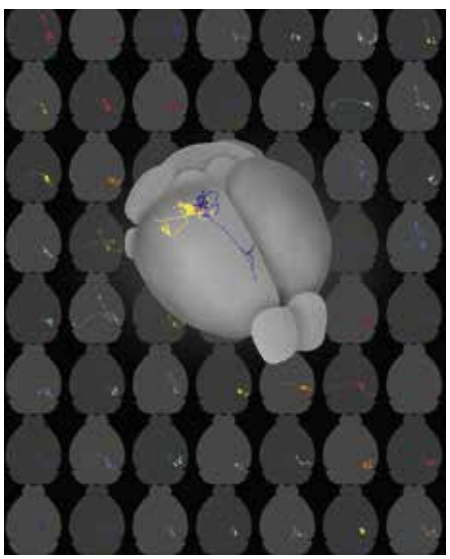
Photos: Engholm (bottom left)

marked neurons with light microscopy took them three years. For this, they included the whole brain showing that more than 70% of the cells connected to more than one and some to up to seven areas. Comparing just the six areas included in both mappings, the results matched well, validating the new technique.

REFERENCE

Han Y*, Kebschull JK*, Campbell RAA*, Cowan D, Imhof F, Zador AM, Mrsic-Flogel TD (2018) The logic of single-cell projections from visual cortex. *Nature* **556**: 51–56

Justus Kebschull, fellow 2013–2015

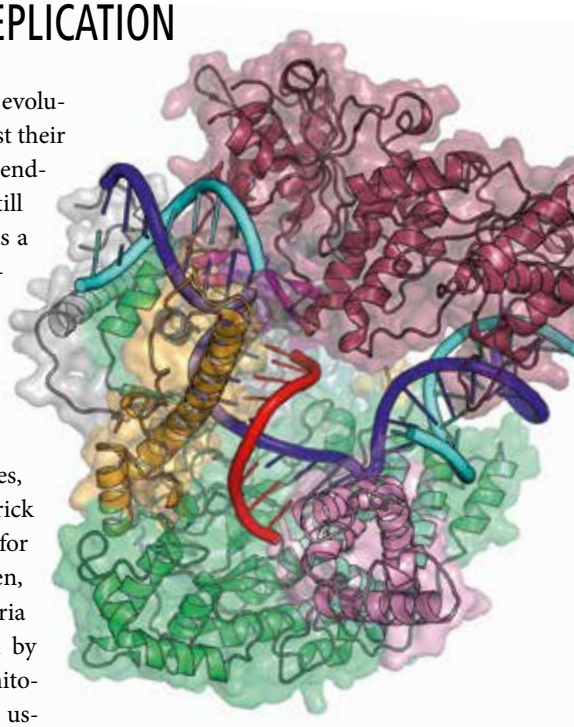


Using fluorescence-based single-cell tracing, the team needed three years to map 71 neurons in a mouse brain, a selection of which is shown here. The new MAPseq enabled them to map 591 neurons in three weeks.

Photos: Sainsbury Wellcome Centre for Neural Circuits and Behaviour (bottom); Hillen (right)

RNA KINKS SWITCH MITOCHONDRIA FROM TRANSCRIPTION TO REPLICATION

Mitochondria are a relict from our evolutionary past – captured cells that lost their freedom, but not all of their independence. Most notably, mitochondria still retain some of their DNA as well as a separate replication and transcription machinery. These organelles basically deliver all the energy for eukaryotic cells, and thus need to regulate both their number and activity very carefully to cater to the needs of their cell. In two studies, Hauke Hillen from the group of Patrick Cramer at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, showed how mitochondria initiate and keep up transcription by analysing the 3D structure of the mitochondrial transcription machinery using X-ray crystallography. First, he studied the RNA polymerase bound to its two initiation factors TFAM and TFB2M. He found that TFAM helps to tether the polymerase to the place where transcription starts. TFB2M then twists the polymerase so that one of its hairpin loops is positioned between the DNA strands. TFB2M also keeps the two DNA strands apart.



The initiation factor TFAM docks to the DNA (blue) and recruits the mitochondrial RNA polymerase (mtRNAP); the initiation factor TFB2M positions it and separates the DNA strands.

In the second study, the group probed how mitochondria ensure that their RNA is faithfully transcribed. The researchers analysed the mitochondrial elongation complex (EC), consisting of polymerase, DNA, RNA, and the elongation factor TEFM. They found that when the factor binds to the polymerase both form a clamp that can slide along the DNA. This creates a channel which keeps the nascent RNA chain away from the DNA and prevents the RNA chain from forming kinks – e.g. near the start signal for DNA replication. If present, these kinks are possibly so large that they keep the EC from travelling further and transcribing the DNA strand. The authors speculate that these kinks could result in the EC dissolving, ending transcription. The already synthesized, short RNA strand would then act as a primer to start replication, explaining how the RNA polymerase also acts as the mitochondrial primase.

Additionally, the team compared these structures and their function to their counterparts in eukaryotic nuclei, bacteria, and bacteriophages, finding them to be different, but following similar conceptual and mechanistic principles. Hauke's studies thus decipher the fundamental workings of human mitochondrial transcription and gene regulation and show a possible switching mechanism between transcription and DNA replication.

REFERENCE

Hillen HS, Morozov YI, Sarfallah A, Temiakov D, Cramer P. (2017) Structural basis of mitochondrial transcription initiation. *Cell* **171**:1072–1081

Hillen HS, Parshin AV, Agaronyan K, Morozov YI, Graber JJ, Chernev A, *et al* (2017) Mechanism of transcription anti-termination in human mitochondria. *Cell* **171**:1–12

Hauke Hillen, fellow 2014–2016

NEW VENUE, NEW COMMUNICATION SEMINAR

By Kirsten Achenbach

In November 2017, BIF held its first English-language communication training in Europe in Köngernheim, Germany, as a counterpart to the seminars in Cold Spring Harbor for the fellows working in North America.

The participants in the first seminar in Köngernheim consisted of 25 of the growing number of non-German-speaking fellows working in Europe. The Untermühle, a 15th-century mill turned hotel, offers state-of-the art seminar facilities. It is not only conveniently close to the BIF office in Mainz, but more importantly, it also has everything we need to help create the special BIF seminar atmosphere: a location conducive to learning, competent and friendly staff, and of course good food. The training programme included writing, presenting, and graphic design skills and, as usual, was packed. It started at 8 am and continued until after dinner. But as the pictures show, we also provided some much-needed physical activity in a nearby climbing hall. In May 2018, we held the second "Mainz Seminar", with a further 25 fellows coming to Köngernheim. However, instead of grey November skies, we enjoyed beautiful summer weather, discussing and networking in the courtyard under the leaves of the mill's old stately oak. The next seminar is planned for 2019 – in summer.

1 **It is not only about work,** but also about workout – the excursion provided a much-needed opportunity for exercise.

2 **The courtyard of the Untermühle** is the perfect place to relax after a long seminar day and to discuss more science.

3 **The seminar room** before the arrival of the BIF fellows.

4 **The participants** in the first seminar at the Untermühle in November 2017.

5 **Shhh –** everybody is concentrating while they write about their interview partner's project.

Photos: Jordan's Untermühle (2, 3); Kirsten Achenbach (1, 4, 5)

PROFILES

PROFESSOR MICHAEL BOUTROS
Institute: German Cancer Research Center, Heidelberg, Germany
Fellowship: 1997–1999

PROFESSOR JAN SIEMENS
Institute: University of Heidelberg, Germany
Fellowship: 2001–2003

PROFESSOR ANDREAS MAYER
Institute: Université de Lausanne, Switzerland
Fellowship: 1993–1995

PROFESSOR EDWARD LEMKE
Institute: European Molecular Biology Laboratory, Heidelberg, Germany
Fellowship: 2003–2005

JANOS SZABADICS
Institute: Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest
Fellowship: 2002–2004

DR ZOLTAN NUSSER
Institute: Hungarian Academy of Sciences, Budapest
Postdoc-Fellowship: 2000–2006

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

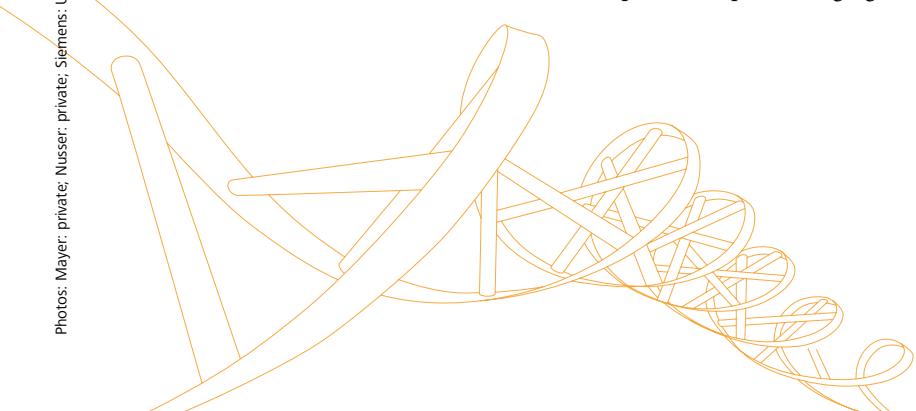
In the last two rounds for **ERC Proof of Concept Grants** worth up to 150,000 euros, three of our alumni were successful: Michael Boutros, Edward Lemke, and Christine Selhuber-Unkel. For Christine, it is the second PoC grant within one year. The PoC grants top up the researchers' existing ERC grants to help investigate business opportunities, establish intellectual property rights, or conduct technical validation for their frontier research findings. The projects are called, respectively, "REMATCH – Image-Based Analysis for Drug Discovery and Repurposing", "Radio-Click – A Versatile Ultrafast Click Platform for Antibody-Based Radio Diagnostics", and "Strain-Stiffening Polymer Structures for Orthotics".

Two BIF fellows have received **ERC Consolidator Grants** worth around two million euros: **Jan Siemens** will build on his outstanding research on temperature detection and thermoregulation. He discovered the first sensor that detects body temperature in the brain, TRPM2. When it detects temperatures in excess of 39°C in mice – and presumably humans – it starts a signalling cascade which leads to cooling. This work was already supported by a previous ERC Starting Grant. It was also recognized by two science prizes last year, both worth 10,000 euros: the Sir Hans Krebs Prize of the Society of Friends of the University of Hannover and the PHOENIX Pharmaceutics Science Award in the "Pharmacology and Clinical Pharmacology" category. **Janos Szabadics** will study the fundamental signalling principles of axons that relay excitation to and within the hippocampus. These previously inaccessible small axons, whose size matches the majority of cortical axons, will be investigated by direct patch clamp and imaging methods.

In April, two fellows were awarded **ERC Advanced Grants** of up to 3.5 million euros for a ground-breaking, high-risk project. **Andreas Mayer** will study a novel pathway for intracellular phosphate reception and signalling and explore the role played by acidocalcisomes in it. He hopes to identify key functions of these poorly understood organelles. **Zoltan Nusser** will receive 2.5 million euros over the course of five years for his project "Proteomic Fingerprinting of Functionally Characterized Single Synapses". His aim is to test whether the large functional diversity of chemical synapses of otherwise homogeneous nerve cells is indeed caused by quantitative molecular differences.

SIGRID MILLES
Institute: Institut de Biologie Structurale, Grenoble, France
Fellowship: 2010–2012

Sigrid Milles has received an **ERC Sarting Grant** to develop an integrative approach using nuclear magnetic resonance and single-molecule fluorescence to study intrinsically disordered protein regions (IDR), which often contain short interaction motifs with vital functions for the cell. She will study IDRs with different types of motifs and interaction partners to understand their function in endocytosis and other biological processes.



Photos: Mayer: private; Nusser: private; Siemens: Uniklinik Heidelberg; Szabadics: private; Boutros: DKFZ.de; Lemke: IMB, c_T. Hartmann; Selhuber-Unkel: private; Milles: private

PROFILES

PROFESSOR PETRA DERSCH
Institute: Helmholtz Centre for Infection Research (HZI). Braunschweig, Germany
Fellowship: 1992–1994

Petra Dersch has been elected to the Senate of the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) in the area of life sciences. The 37-member Senate is the central self-administration body of the DFG and is responsible for decisions on funding and the organization of review, assessment, and decision-making processes. Petra Dersch heads the Department of Molecular Infection Biology at the HZI and is professor of microbiology at TU Braunschweig, where she studies intestinal bacteria, especially *Yersinia* species and how they adhere to the gut wall, penetrate it, and ultimately spread within the host. Earlier this year, she was also elected to the European Academy of Microbiology.

CHRISTOPH ENGEL
Institute: University of Regensburg, Germany
Fellowship: 2011–2013

Christoph Engel has been awarded the Bayer Early Excellence in Science Award for Biology by the Bayer Science & Education Foundation. He has received the 10,000 euro award for his outstanding work in resolving the 3D structure of RNA polymerase I and the first complete model of the Pol I transcription cycle, which was developed from the structural data.

PROFESSOR CHRISTIAN HAASS
Institute: University of Munich (LMU), Germany
Postdoc-fellowship: 1990–1991

Together with three other researchers, Christian Haaß has been awarded the one-million-euro Brain Prize 2018 by the Lundbeck Foundation in Denmark for helping to map out the genetics and biological processes that underpin the devastating effects of Alzheimer's. Their ground-breaking research has far-reaching implications for new therapeutic interventions and for understanding other neurodegenerative diseases of the brain.

HAUKE HILLEN
Institute: Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
Fellowship: 2014–2016

The German Society for Biochemistry and Molecular Biology awarded Hauke Hillen this year's Bayer Pharmaceuticals PhD Prize. The biochemist has received the award for his work on the regulation of a molecular machine that activates the genes in the power plants of living cells.

PROFESSOR TOBIAS JANOWITZ
Institute: Cold Spring Harbor Laboratory, NY, USA
Fellowship: 2001–2003

Tobias Janowitz has accepted a professorship at the Cold Spring Harbor Laboratory which will be linked to a clinical professorship. He started in August and will continue his work on the host response to cancer and immunotherapy.

TIMO KUSCHMA
Institute: McKinsey & Company, Berlin, Germany
MD Fellowship: 2014–2015

ISABEL SCHELLINGER
Institute: Angiolutions and University Nuremberg-Erlangen, Germany
MD Fellowship: 2013–2014

Two of our MD alumni, Timo Kuschma and Isabel Schellinger, have been named to the "30 under 30 Europe" list for healthcare and science by the magazine *Forbes*. The list "comprises the most impressive young entrepreneurs that are reshaping Europe, and the world, for the better", according to Randall Lane, editor of Forbes and CCO at Forbes Media. Honourees are judged on leadership and disruption; entrepreneurial mind-set and results; and the likelihood of changing their field over the next half-century. **Timo** is recognized for research that led to an early-stage therapy for pancreatic cancer as well as for work advising health care companies on data-driven analytics. **Isabel** is recognized for discovering a new way that small abdominal aortic aneurysms grow and cofounding the company Angiolutions to develop the first minimally invasive device to prevent the aneurysms from growing to potentially life-threatening size.

Photos: Dersch: private; Engel: Bayer AG; Haaß: Lundbeck Foundation; Hillen: MPI für BC; Janowitz: private; Kuschma: private; Schellinger: private

ANNELI PETERS
Institute: Max Planck Institute of Neurobiology, Martinsried, and University of Munich (LMU), Germany
Fellowship: 2007–2010

Anneli Peters has received the Sobek Junior Research Award from the Roman, Marga, and Mareille Sobek Foundation for her outstanding research on the contribution of autoreactive T and B cells to multiple sclerosis (MS) pathogenesis. The award comes with 15,000 euros and is jointly presented with Amsel, a society for people with MS in the German state of Baden-Württemberg, and DMSG, the German MS society.

MANUEL SCHOTTDORF
Institute: Princeton Neuroscience Institute, NJ, USA
Fellowship: 2014–2015

Manuel Schottdorf has been awarded the Otto Hahn Medal, with which the Max Planck Society honours its best PhD students. He developed hybrid circuits of living and simulated nerve cells, enabling him to study the relationship between network structure and function. This synthetic approach to brain research will open up new questions in experimental study.

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

Michael Sieweke has been awarded one of the prestigious Humboldt Professorships and earlier this year started his tenure at the DFG Research Center for Regenerative Therapies in Dresden, a Cluster of Excellence at TU Dresden. The Humboldt Professorships come with up to five million euros for the first five years and are meant to attract top international researchers to Germany. Michael's research is located at the interface of immunology and stem cell research and the recent findings of his group have laid the groundwork for new cellular therapy approaches in regenerative medicine and brought them closer to clinical application.

PROFESSOR BARBARA TREUTLEIN
Institute: Technical University of Munich and MPI for Evolutionary Anthropology, Leipzig, Germany
Fellowship: 2009–2010

Barbara Treutlein has accepted a call to serve as professor at the Department of Biosystems at ETH Zurich, Switzerland. Her research focuses on human developmental biology and the formation of various complex organs such as the liver and brain. She will start on 1 January 2019.

PROFESSOR DANIEL WACKER
Institute: Icahn School of Medicine at Mount Sinai, New York, USA
Fellowship: 2010–2012

Daniel Wacker has been appointed assistant professor at the Department of Pharmacological Sciences at the Icahn School of Medicine at Mount Sinai. In his lab, he studies serotonin-mediated signalling, which controls mood, sleep, cognition, and consciousness, and develops new therapeutics using structure-based drug design. By combining X-ray crystallography, cryo-EM, and pharmacological assays, he analyses how neurotransmitters and drugs stabilize different receptor conformations that cause physiological effects.

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

As announced in March, Detlef Weigel will receive the Barbara McClintock Prize 2019 for Plant Genetics and Genome Studies from the Maize Genetics Executive Committee. This award recognizes him as one of the most outstanding plant geneticists of the present era while at the same time memorializing the unequalled contributions of Dr McClintock to this field.

Photos: Peters: Amsel e.V./M. Stollbeck; Schottdorf: private; Sieweke: Humboldt-Stiftung/Elbmotion; Treutlein: Ronny Barr/MPI-EVA; Wacker: private; Weigel: c_MPI DB

A BIF FELLOW'S GUIDE TO ...

LYON

Travelling is fun – especially if you get insider tips from locals! In this edition, BIF fellow Bahadir Cagri Cevrim shows you around Lyon, France, the home of nouvelle cuisine and late chef Paul Bocuse.

FACTS & FIGURES

Country: France
Population: About 500,000
Area: About 48 km²
Students: About 64,000
Famous for gastronomy, the Lumières brothers, the Festival of Lights, trompe l'œil, and traboules.
Websites: www.lyon.fr, www.onlylyon.com

WHERE TO STAY

Le Flâneur Guesthouse: an international magnet for backpackers with frequent concerts and cultural events in the bar.
Studio Peniche: the place to go if you want to sleep in a real boat cabin in the city centre.
Fourvière Hotel: designed by the architect of the Basilica of Fourvière in the 19th century and elegantely restored.

NIGHTLIFE

Boats on Rhône River 1**:** for everything from a relaxed glass of wine to a party.
Look Bar: its decoration and signature cocktail – a secret recipe – have not changed since the 1920s.
La Sucre: the sugar factory now offers amazing art, exhibitions, and the craziest parties in town.

RESTAURANTS

La Garet: a traditional Lyonnais "bouchon" with a great atmosphere, but not for vegetarians.
Café Comptoir Abel: offers the best of the local cuisine.
Brasserie Georges: the go-to place for Alsatian cuisine, a fusion of French and German.
L'Auberge du Pont de Collonges: the three-star restaurant of the late Paul Bocuse

ACTIVITIES

Visit the Fête de Lumières 4, Europe's oldest light festival with amazing light shows and lots of hot wine!
Walk, cycle, or chill along the rivers. Amble through Parc de la Tête d'or or, for swimming, go to Lake Miribel.
Listen to world-famous singers during Le Nuits de Fourvière in the 2,000-year-old Roman theatre.

BEST SIGHTS

Basilica of Notre Dame de Fourvière 2**:** historic landmark offering impressive architecture, beautiful view of the city, and amazing rose gardens.
Les Muses de l'Opera: roof bar on the architectural masterpiece of the Opera of Lyon, an excellent place to watch the sun set over the city with a drink.
Jardin de Couriosité 3**:** The park's great views may even allow you to see the Alps on a clear day!

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

Bahadir Cagri Cevrim is 29 years old and comes from Turkey. He is studying at the University of Lyon and is supervised by Professor Michalis Averof.

Photos: Elena Volterrani

AN ADDITIONAL 106 MILLION EUROS FOR THE INSTITUTE OF MOLECULAR BIOLOGY (IMB)

In May, the Boehringer Ingelheim Foundation (BIS), BIF's sister foundation, and the state of Rhineland-Palatinate announced that they will fund the Institute of Molecular Biology (IMB) at the University of Mainz with a further 106 million euros from 2020 to 2027. The foundation will contribute about 54 million euros and the state 52 million euros. The IMB was initiated by a 100-million-euro donation pledged by the BIS in 2009, which provides its core funding until 2020. The institute's research focuses on epigenetics, developmental biology, and genome stability. "Today, the institute is well on its way to establishing itself as a top centre for international life science research and becoming a scientific showpiece of Rhineland-Palatinate," said Minister-President Malu Dreyer at the press conference. "We are committed to excellence in scientific research in Mainz and Germany and understand our involvement as a service to society. The continued aim of our initiative with the IMB and the University of Mainz is to support excellent life science research and to attract internationally outstanding scientists to Mainz," declared Christoph Boehringer, chairman of the Executive Board of BIS. "We can only succeed in this by offering them academic freedom and the best possible conditions for their research. Thus, together with this new initiative, we are providing the University of Mainz with more than 200 million euros in funding until 2027." Professor Christof Niehrs, scientific and founding director of the IMB, said: "This unprecedented financial support provides our scientists with the freedom to pursue their self-defined goals and the optimal conditions to successfully conclude ambitious projects. This will further enhance the Mainz campus's international appeal."

BIF's SISTER FOUNDATIONS

The Institute of Molecular Biology will receive a further 106 million euro from the Boehringer Ingelheim Foundation and the state of Rhineland-Palatinate.

Photo: Ule Ruhland

UPCOMING EVENTS

21–25 SEPTEMBER 2018

BIF fellows' meeting in Woods Hole

Seminar for alumni and PhD and MD fellows working in North America. Participants will present their scientific results. The programme is complemented by keynote presentations and talks on topics such as career opportunities, and includes tours of the scenic surroundings on Cape Cod.

10–14 OCTOBER 2018

118th International Titisee Conference

Wendy A. Bickmore, Edinburgh, and Sarah A. Teichmann, Cambridge, both UK, will chair the 118th ITC titled "Cell Heterogeneity & Tissue Architecture". Scientists who investigate topics such as tissue architecture and gene regulation, who generate *ex vivo* organoid models of tissues, or who analyse and model these complex datasets will discuss how different cell types form, communicate, and work together to form and maintain tissues.

Participation is by invitation only.

17–18 NOVEMBER 2018

Meeting of BIF's Board of Trustees

The trustees decide on the allocation of fellowships, review the proposals for the International Titisee Conferences, and settle all the foundation's matters of fundamental importance.

14 DECEMBER 2018

BIF's Christmas party in December

BIF's Christmas party for fellows and alumni starts at 6 pm on our premises in the centre of Mainz. Meet other fellows and alumni and celebrate with us. Food and drink will be provided.

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

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