FUTURA

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Vaccinations
One of medicine's greatest success stories – and how it will continue

Projects and Results
New PhD projects and completed theses by BIF fellows

A BIF Fellow's Guide
Discover Lisbon, seafaring city and capital of Portugal

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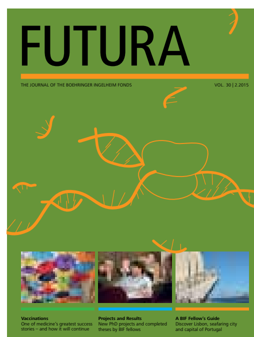

The cover illustration shows a simplified model of RNA interference (RNAi), which degrades RNA before translation, effectively silencing the corresponding gene. Former BIF fellow Katarzyna Kowalik determined the conditions under which RNAi leads to epigenetic silencing, substantially widening the potential of RNAi for research. Read more on page 65.

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THE YEAR 2015 AT BIF

»In 2015, the share of non-Germans among the almost 700 applicants of 66 nationalities topped 70%.«

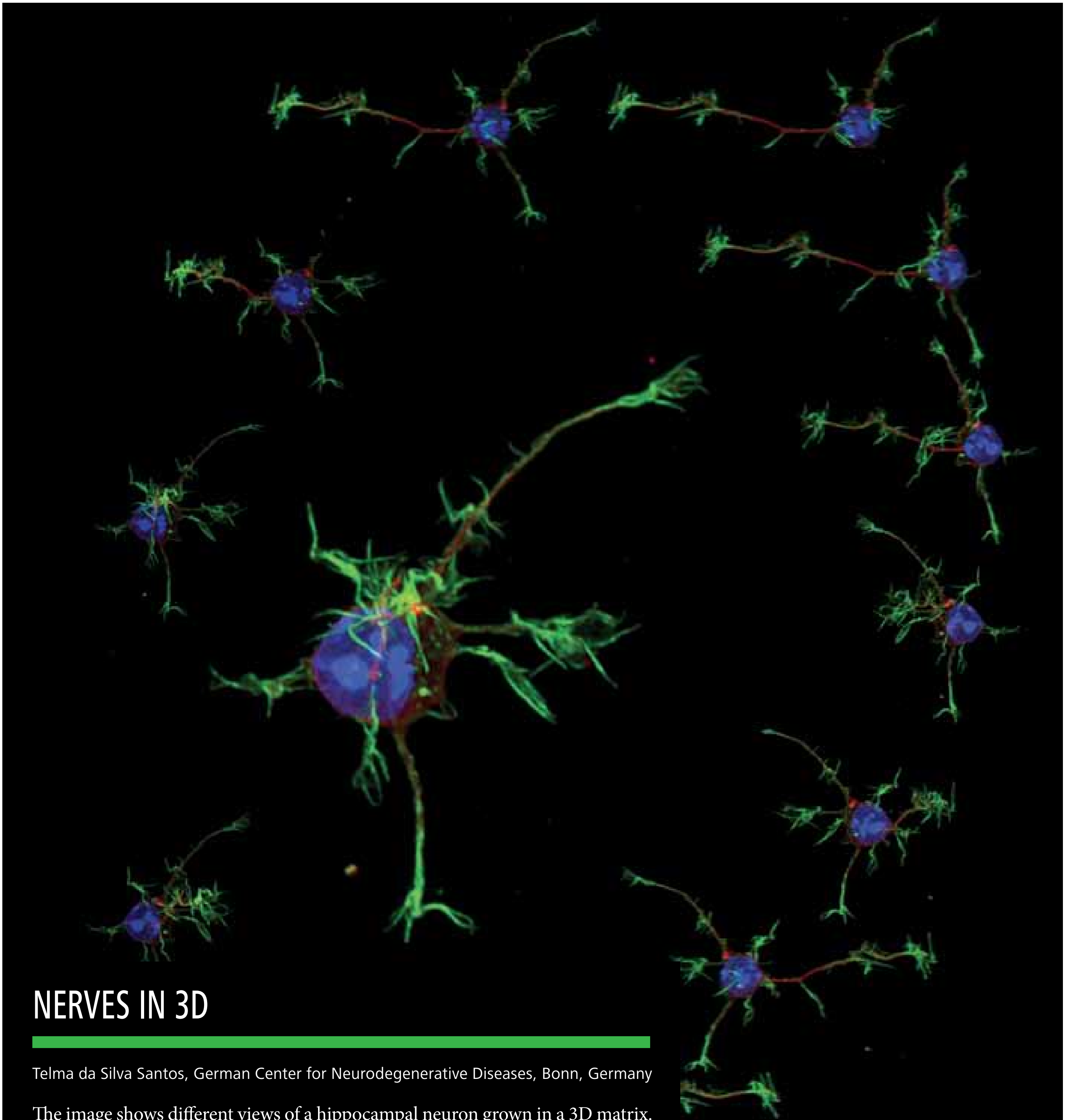
The funding programmes of the Boehringer Ingelheim Fonds (BIF) for young researchers are becoming more and more international. Take our PhD fellowships with which we support some 110 students worldwide at any one time: In 2015, the share of non-Germans among the almost 700 applicants of 66 nationalities topped 70%; among the 51 new fellowship holders it was only slightly lower. Currently, some 20% of our 110 PhD fellows work overseas, 26% in Germany, and 54% in other European countries. Germany, the United Kingdom, the United States, and Switzerland are most successful in attracting talent; they have led BIF's statistics for many years. The 2015 numbers for our Travel Grant programme for postdocs and PhDs who are not BIF fellows also reflect the high degree of internationalization: some 300 applications from 42 nations with 75% of the awardees being non-German and 21% from overseas.

What about gender balance? BIF's three funding programmes give very different answers testifying to the complexity of the issue. Among our PhD fellows, the ratio of men to women has been more or less 50:50 for many years now. In our Travel Grant programme, women clearly dominate, comprising 65–70% of applicants and awardees alike. Our research fellowships for medical students show the reverse ratio – with 65% of applicants and fellows being male.

BIF's summer and alumni seminars, communication trainings, and informal meetings in Europe and the United States are as important as the stipends. Each year, a few fellows are also permitted to participate in our two International Titisee Conferences. This "package" offers ample opportunities to improve skills, discuss research in depth, build trust and networks, and profit from more experienced fellows and scientists. In addition, BIF's staff helps to solve minor and major problems encountered on the sometimes thorny path to a PhD.

Such comprehensive support seems more important than ever to navigate the unpredictable paths in academia. Even the recently published Imboden Report, an evaluation of Germany's Excellency Initiative, quite bluntly calls academic career options – not only in Germany – a "Vabanque-Spiel" (hazardous venture). A venture that not necessarily the best ones may embark on anymore. Even from our BIF fellows, who are highly motivated and interested in research, we hear that a growing number thinks that risk and reward are off kilter. Being among the most talented, they have a wide range of options, and currently many options appear more promising than academia. So far, a rather high percentage of BIF's nearly 1,300 alumni have remained in academia, which has led to 189 professors and some 90 independent group leaders.

While hard to measure in numbers, the contribution of BIF's knowledge network and comprehensive support, as well as the motivation and enthusiasm other fellows pass on, has been immense – and is required more than ever, if we want to keep the most talented in academic research.



NERVES IN 3D

Telma da Silva Santos, German Center for Neurodegenerative Diseases, Bonn, Germany

The image shows different views of a hippocampal neuron grown in a 3D matrix. So far, *in vitro* studies limit cells to two dimensions, forcing them to adapt to rigid and flat surfaces. Especially for neurons with their far-reaching dendrites and delicate structures, this changes their shape drastically. It may even alter their biology. Growing cells in a 3D matrix allows us to study them in a more natural state, gaining more valid answers. Actin is stained green, microtubules red, and the nucleus is blue.

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.

This photo went viral in 2015. Why people's perceptions differed were puzzling questions – until now.

THAT DRESS: THE ILLUSION EXPLAINED

In February 2015, social media quickly spread a picture of an amazing colour-changing dress. For some people it looked blue and black. For others it looked white and gold. You either saw it one way or the other, with no in-between. It created a media buzz and hit the major news channels. So how could the same picture look so different to different people?

Neuroscientists from University Clinic Bergmannsheil in Bochum, Germany, suggest the phenomenon is caused by different brain activation patterns. Using functional MRI scans that pick up brain activity in real time, the researchers compared brain activity between subjects who saw the dress as blue/black and those who saw it as white/gold.

First, volunteers looked at plain squares of the same colours as the dress. They all identified the colours correctly and showed now differences in brain activity. However, when the subjects were shown the photo of the dress, the scientists found that people who saw it as white/gold had a highter activation of frontal and parietal brain areas. Frontal regions are particularly involved in higher cognitive processes such as selective attention and decision making, while parietal areas process visual information. This is the first ever optical illusion that people seem unable to mentally control and 'see both ways', giving a valuable new tool for neurobiologists studying visual processing.

REFERENCE

Schlaffkea L, Golischa A, Haaga LM, Lenza M, Hebaa S, Lisseka S *et al* (2015) The brain's dress code: How The Dress allows to decode the neuronal pathway of an optical illusion. *Cortex* **73:** 271–275

EXTRA SPERM KEEP EMBRYOS ALIVE

One sperm creates life, but many sperm maintain it.

Biology throws up all sorts of puzzles and strange behaviours. But the observation that numerous sperm usually penetrate a bird's eggs – even though only one is needed for fertilization – has had scientists scratching their heads for a long time. Researchers from the University of Sheffield, UK, have revealed that in zebra finches and domestic chickens these extra sperm are essential for early embryo development. Although only one sperm fertilizes the egg, without extra sperm, the newly developing embryo quickly dies. This latest finding proves there is a biological function for polyspermy. The researchers found more evidence of its importance, when they discovered that female birds can actually regulate the number of sperm that get into the egg. When sperm is in short supply, the female allows a higher proportion to reach and penetrate the egg to allow roughly the same total number of sperm. Exactly how the non-fertilizing sperm promote and protect the embryo is now a matter of intense debate that will keep biologists scratching their heads a little while longer.

REFERENCE

Hemmings N, Birkhead TR (2015) Polyspermy in birds: Sperm numbers and embryo survival. *Proc Royal Soc B* **282:**20151682

Photo: Caitlin McNeill/Tumblr (top); iStock

TICK TOCK, THE MUTATION CLOCK

We tend to think of mutations as "freaks of nature", errors in the genetic code created when DNA is incorrectly copied during cell division. Scientists recently discovered that cells can acquire mutations with clockwork regularity, as if the cell is actually programmed to make mistakes at set intervals. These mutations accumulate and probably contribute to ageing and cause many human cancers. The investigators screened 10,250 cancer genomes from 36 different types of cancer. They identified 33 "mutational signatures", of which two had clock-like characteristics. Indeed, the mutation rate of these two signatures was so steady, it was possible to calculate the age of the patient simply by counting the number of signature mutations they had acquired. This is the first time that mutational clocks have been identified and "timed". Clinicians could compare the genomes of a primary tumour and any metastasis, and now calculate how long it has taken the cancer to spread. They could use similar techniques to predict how soon a cancer may change or develop chemoresistance, helping them to plot the best course of treatment.

REFERENCE

Alexandrov LB, Jones PH, Wedge DC, Sale JE, Campbell PJ, Nik-Zainal S *et al* (2015) Clock-like mutational processes in human somatic cells. *Nat Genet* **47:** 1402–1407

The horned frog (*Ceratophrys cranwelli*) hunts by burying itself in loose soil, staying motionless and waiting for prey to get close.

DUCK TAPE? WHAT ABOUT FROG TONGUE?

What happens when you put a frog behind glass with a cricket on the other side? Splat! Out shoots the tongue towards its prey; it hits the glass with a satisfying whack. The frog may be frustrated, but not Dr Thomas Kleinteich from Kiel University, Germany. By connecting the glass to a light source, he was able to see all the spots where the tongue touched the glass. Filming this contact with a high-speed camera, he and his colleague could observe exactly how the tongue hit and then disconnected.

Micro-tomography of the tongue shows it is a single muscle, made up of lots of little threads called fibrils. When the muscle pulls the tongue back into the frog's mouth, the tendrils fan out under the surface of the tongue. The force is spread evenly over the entire surface of the tongue. Just like the adhesive on sticky tape, each fibril breaks its contact with the surface one at a time.

Kleinteich says that the unsticking of the tongue is "like trying to vertically pull a strip of Sellotape off a surface, instead of starting from one end – you need significantly more strength to do so." But it also enables the animal to lift insects, or even larger prey, in one go, no glue required!

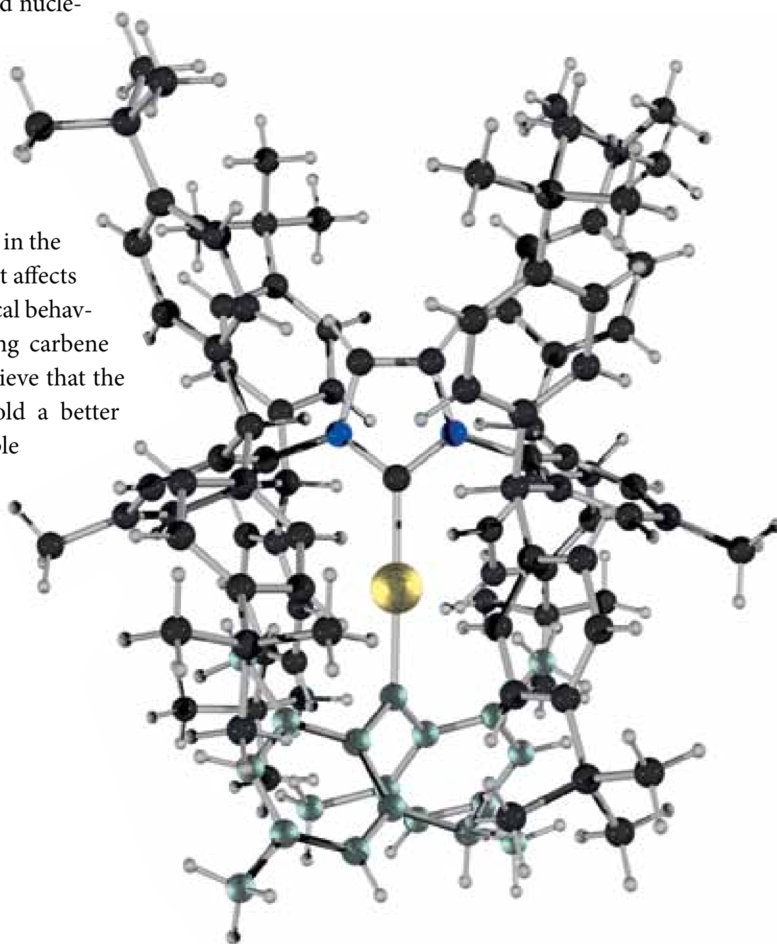
REFERENCE

Kleinteich T, Gorb S (2015) Frog tongue acts as muscle powered adhesive tape. *R Soc Open Sci* **2:** *150333*

RELATIVITY: A GOLDEN OLDIE

In November 2015 Einstein's General Theory of Relativity celebrated its centennial birthday. This incredible theory – the idea that gravity curves space and time – has been a bedrock of physics ever since. But relativity not only explains weird astronomy like gravitational lensing and black holes. Einstein's precursor theory – special relativity and the formula $E = mc^2$ – also offers insights into some of the fundamental properties of matter.

Using some clever chemistry, a team from Heidelberg successfully isolated for the first time stable complexes containing a gold atom double-bonded to carbon (gold carbene). They also managed to prepare a copper carbene and a silver carbene, but these two compounds were much more unstable than the gold carbene. They found that gold carbene had a much shorter and stronger metal-carbon bond than the other metal carbenes. Only relativity could explain their discovery. Team leader Professor Bernd Straub argues that electrons orbit around the gold nucleus at velocities close to the speed of light; additional motion energy cannot increase their speed futher, so instead they increase in mass. As this effect occurs in the outermost electron shell, it affects gold's bonding and chemical behaviours, like the extra strong carbene bond. The researchers believe that the same effect also gives gold a better chance of activating a triple bond between two carbon atoms.



Gold carbene model: The Au=C double bond in the gold carbene compound is the bond between the large golden atom in the middle and the slightly greenish atom below. The position of the atoms was derived from an X-ray crystal structure analysis.

REFERENCE

Hussong MW, Hoffmeister WT, Rominger F, Straub BR (2015) Copper and silver carbene complexes without heteroatom-stabilization: Structure, spectroscopy, and relativistic effects. *Angew Chem Int Ed Engl* **54:** 10331–10335

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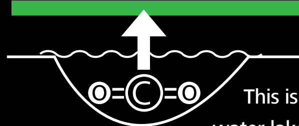
MILLION



This is the number of freshwater lakes and water courses worldwide. They account for nearly a quarter of all carbon dioxide (CO_2) released into the atmosphere yearly. Recent analysis of data from over 5,000 lakes in Sweden suggests global warming could increase these emissions, especially from lakes in the northern hemisphere.

Source: Weyhenmeyer GA, Kosten S, Wallin MB, Tranvik LJ, Jeppesen E, Roland F (2015) Significant fraction of CO_2 emissions from boreal lakes derived from hydrologic inorganic carbon inputs. *Nat Geosci* **8**: 933-936

Photo: Kleinteich (top); Matthias Hussong and Bernd F. Straub, Heidelberg University (right)

Vaccines do not prevent exposure to pathogens, but by stimulating the production of antibodies, they help shield the body from dangerous infections.

TEACHING THE BODY TO **PROTECT** ITSELF

By Kirsten Achenbach

Vaccines are one of medicine's greatest success stories, and prevent about 7,000 deaths worldwide each day – according to the WHO – and eliminate much suffering, sickness, and even permanent disability. "We must make this the decade of vaccines", said Bill Gates at the World Economic Forum's annual meeting in 2010.

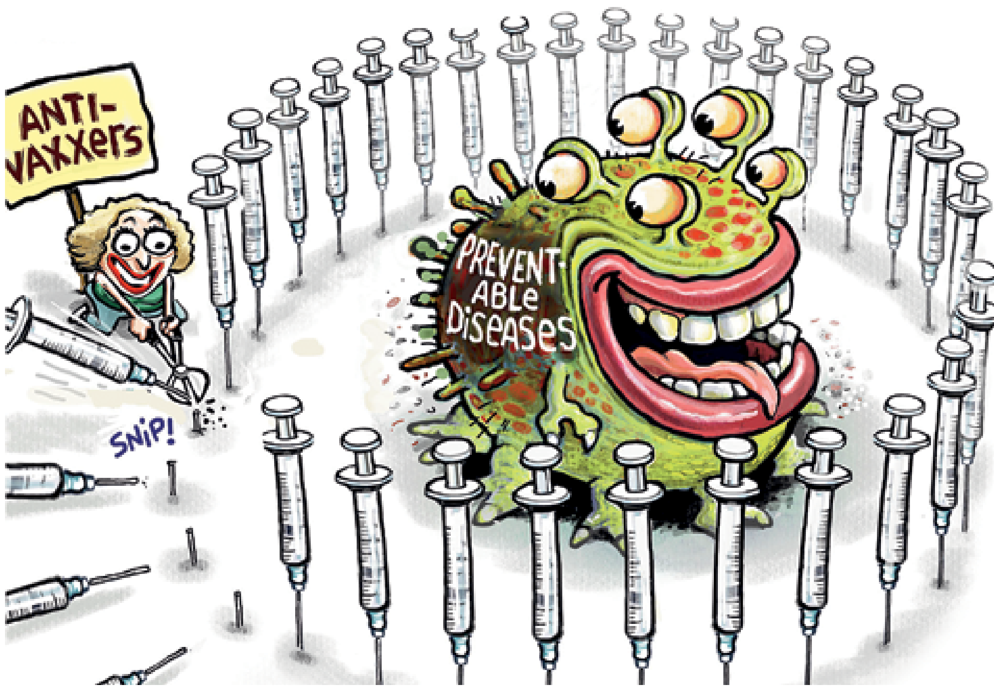
He backed his call to action with a pledge of ten billion dollars in funding from his charity, the Bill and Melinda Gates Foundation, to research, develop, and deliver vaccines to the world's poorest nations. It's not hard to see why Gates is so intense about vaccines. Thanks to polio vaccination, for example, more than 13 million people are now walking who otherwise would have been partly paralysed, and another 1.3 million are alive who probably would have succumbed to the disease. Smallpox has been eradicated, and polio is banished from all but two countries. However, humanity is still a long way from conquering infectious and parasitic disease. Big global killers such as tuberculosis, HIV, and malaria still evade our efforts to produce effective vaccines. Infections such as Ebola, SARS, and new strains of influenza challenge our ability to develop, test, and supply vaccines quickly enough to prevent epidemics or even pandemics. And the problems are not purely biological: vaccines needing specialized storage or medical expertise to be administered are difficult to deploy in developing countries and disaster zones. To be truly successful, a vaccine must not only be medically effective, but also logistically feasible.

Back to the beginnings: Eight-year-old James Phipp went down in history for receiving the first recorded successful human vaccination from Edward Jenner in 1796. Jenner took the gamble of using fresh cowpox pus to inoculate the boy and then exposing him to the much more severe smallpox. His reasoning was that milkmaids who had had cowpox never got smallpox. He may also have heard of others who had used this technique before, like Peter Plett, a teacher in northern Germany. The gamble paid off because – unbeknownst to Jenner – the two pox viruses are similar enough that the antibodies the body produces following exposure to cowpox are effective against smallpox as well.

Over the next 100 years bacteria and viruses were identified as disease-causing agents and the study of immunology took off. Louis Pasteur found that weakening or killing bacteria or inactivating viruses and bacterial toxins – called attenuation – reduced the rates of infection while achieving immunization in a large number of cases. Today, many of the names from this research era, such as Robert Koch, Richard Pfeiffer, Emil von Behring, and Paul Ehrlich, are known even outside medical circles. Together with many less-known scientists, they developed vaccines against such diseases as rabies, cholera, typhoid, diphtheria, tetanus, and tuberculosis in infants. Cholera, typhoid, and diphtheria are caused not by the infecting bacterium itself, but by the toxin it produces. Therefore the immunization agent is called a toxoid, as it is produced from a harmless version of the corresponding toxins.

The second half of the twentieth century saw enormous leaps in the way vaccines were designed and produced. Already in 1949, advances in cell culture methods made it possible to grow many viruses *in vitro* for the first time, paving the way for new or more effective vaccines against hepatitis A, mumps, rubella, measles, rotaviruses, varicella, and smallpox. The first of these live savers were the vaccines against polio designed by Hilary Koprowski, Jonas Salk, and Albert Sabin. Salk's vaccine used three inactivated →

Photo: Jovana Rikalo/Stocksy



HERD EFFECT

For diseases such as measles that are communicated from person to person, vaccination lowers the chance of infection and thus protects individuals for whom vaccines do not work as well as unvaccinated people. To stop epidemics from spreading, there needs to be a specific percentage of vaccinated and naturally immune people in the population. This percentage depends on such factors as the contagiousness and transmission of the infection. For airborne measles and pertussis, the value lies at around 95%; for ebola, which is transmitted by bodily fluids, at 33 to 60%.

strains of polioviruses. He showed that he could reliably inactivate this dangerous virus for use in his injectable vaccine. Separately, Koprowski and Sabin both used attenuated, but live viruses, which were more dangerous, but more practical, because they could be given orally. Sabin's concoction was the vaccine of choice for the longest time, especially in developing countries. Only now is it being phased out by the WHO in favour of Salk's vaccine, because with polio on the decline, the danger from the vaccine exceeds the danger of catching polio naturally.

The next step in priming the immune system against invaders was to use not the whole pathogen, but only parts of it – such as the polysaccharide chains present on the surface of capsulated bacteria. Such subunit vaccines can protect against several strains at once by incorporating molecules from different strains. In this way researchers designed a vaccine effective against 23 strains of pneumococcus as well as vaccines against meningococcus and *Haemophilus influenzae* type B. These vaccines were the last to use the empirical approach that started with Jenner and Pasteur: isolating and inactivating the disease-causing agent and then injecting it whole or in parts into the healthy body.

The 1970s saw the development of technologies that would not have been possible with this approach. Take hepatitis B. In the mid-1970s Maurice Hilleman developed an effective vaccine by purifying and inactivating the virus-like particles extracted from the blood of infected people. These particles were a potentially dangerous and severely limited resource, but the method was necessary because the virus resisted culture *in vitro*. In the early 1980s, Bill Rutter and Pablo Valenzuela changed the production of this and many other vaccines through recombinant DNA technology. They inserted certain genes of hepatitis B into yeast cells, kidnapping their genetic machinery to produce an antigen analogous to the one Hilleman had used. For the first time, a vaccine could be produced without growing the organisms causing the disease, obliterating this bottleneck. Today, it is not only yeast cells but also bacteria and viruses that are engineered for recombinant vaccine production. With their help, we can make vaccines against pathogens that cannot be grown in the lab, such as human papillomavirus, which can cause cervical and other cancers. Recombinant DNA technology was also used to change the pathogens themselves, so that, for instance, the pertussis bacterium produced only a harmless variety of the toxin causing the disease, resulting in a better vaccine.

The polysaccharide vaccines had been a great step forward, but they could not protect those most at risk: children within their first years of live. Because T cells cannot bind to polysaccharides, these vaccines need a strong response from the B cell arm of the immune system, which is not yet fully developed in children. To activate both, researchers hitched the bacterial polysaccharides to specific carrier proteins that can be recognized by T cells. In this way, a lasting immunization involving memory B and T cells is formed. Today, these so-called conjugate vaccines protect all age groups as well as people with weak immune systems against *Haemophilus influenzae*, pneumococcus bacteria, and meningococcus types A, C, Y, and W135.

However, meningococcus type B (MenB), which causes half of all meningitis cases globally, was a tougher nut to crack. Its capsular polysaccharide is identical to the one present in human tissues and therefore cannot invoke an immune response. In addition, there are many different MenB strains, and engineering a host cell to create sugars identical to the pathogen is not yet feasible. The

Illustration: CagleCartoons.com

challenge was to find one or a few antigens as targets for the immune response that elicit a reliable response in all age groups and offer long-lasting protection against as many strains as possible. In the late 1990s, using a new approach called reverse vaccinology, Rino Rappuoli together with Richard Moxon and Craig Venter started to develop exactly that. Rappuoli and his team analysed the genomes of *Neisseria meningitidis* in order to identify new antigens that enable the immune system to recognize and fight as many strains of the pathogen as possible. Out of the 600 candidates they found, they selected four proteins that are effective against *N. meningitidis* and common to many MenB strains. Their new MenB vaccine was approved for use in the EU in 2013 and in the US in 2015 (it had already been administered in a 2014 outbreak of MenB at Princeton University). It prevents one of the most common diseases in infants and children in the developed world, which kills between 5 and 15% of infected patients and severely mutilates up to a quarter of its survivors.

Reverse vaccinology takes its name from the fact that it does not start with growing pathogens in the lab, but with the computer analysis of data about them. This way, we can identify motifs common to all or most of the relevant strains, gaining a number of potentially protective antigenic targets for vaccine development. The method has yielded impressive results, as it provides access to the whole repertoire of bacterial or parasitic antigens. This more rational approach to vaccine design makes it possible to search for vaccines against diseases we could not previously tackle due to factors such as the large variability of the pathogenic strains causing them or a limited immune response to the known antigens.

Once again, it is Craig Venter who is going even further by designing synthetic vaccines, which he hopes can turn the tables during an epidemic. Normally, virus material is collected from patients and sent to specialized centres for multiplication. Collecting enough material can take weeks. However, with the sequencing capabilities we have nowadays, one sample is all it takes. The information can then be used to build a synthetic virus, containing, for example, the "backbone" of a common flu virus plus the specific genes for the epidemic strain. During the H7N9 avian flu outbreak in 2013, it took only a few days to get from sequence data to a virus growing in culture and a vaccine in the form of a purely synthetic, self-amplifying mRNA system ready to deliver the antigen sequence to mice via lipid nanoparticles. From the first report of the virus, it took only eight days to prepare for experimental animal immunization. But so far these are only hopes.

Despite all these advances, there is still a group of big killers out there that have so far evaded all our vaccination efforts because, amongst other factors, they are so changeable. Among them are HIV, malaria, and tuberculosis, with death tolls in 2014 of 1.2 million, 0.5 million, and 1.5 million respectively, according to the WHO. HIV changes its "face to the customer" almost daily and thereby escapes detection by the immune system. In addition, it weakens the defence mechanisms of its host by killing and hiding within the cells of the immune system.

This last group of pathogens usually has two types of surface epitopes – the sites on the antigen to which antibodies bind. One set is visible to the immune system and is attacked by it, but varies frequently across strains. The other set is ignored by the immune system, although stable over long periods and common to many strains. For HIV, the body's immune response is directed almost exclusively towards the variable epitopes, not an efficient strategy.

We know that there are also antibodies against the stable epitopes which can protect against infection, at least in animal models. However, we have not yet figured out how to induce the body to produce them effectively. It is as if these stable epitopes are invisible to the immune system. A promising way of solving this puzzle is to look not only at the gene sequences of antigens, but also at their 3D structure, alone and bound to antibodies. By comparing "visible" and "invisible" epitopes, we should be able to engineer antigens that the immune system can recognize effectively.

Another hot topic in vaccine design does not concern the antigen itself, but everything else that a vaccine contains. Already in 1926, Glenny used aluminium salts as a so-called adjuvant (from the Latin *adiuvare* meaning to help) to increase the body's immune response to diphtheria and tetanus toxoids. Today, almost 80 years later, aluminium salts are still the most widely used adjuvant. It was not until 1997 that a second one, the oil-in-water emulsion MF59, was approved for human use. Many others have been tried but →

Despite all these advances, there is still a group of big killers out there that have so far evaded all our vaccination efforts because, amongst other factors, they are so changeable. Among them are HIV, malaria, and tuberculosis.

found to be too toxic for humans or hard to produce. Adjuvants increase the effectiveness of vaccines by protecting more people with less vaccine and fewer booster shots against a wider variety of strains for a longer time. For example, MF 59 doubles the percentage of people protected by the seasonal influenza shot from 43 to 86%. We still understand little about how adjuvants work and consequently about how to best select or even design them. We do know that there are several aspects at work. Adjuvants can act by slowly releasing antigens over time. Or they can involve the innate immune system by triggering toll-like receptors, situated on the surface of many immune cells. These receptors recognize pathogens that evolution has "hard-wired" into our genes, because they change so little. They also stimulate dendritic cells, a class of immune cells that activate T cells by presenting antigens to them. We also know that cells contain natural adjuvants that are released when they die. Together with other factors, such intrinsic adjuvants can provoke an immune response. This comes into play not only during normal immune responses, but also when the body rejects a transplant, fights a tumour, or attacks itself in autoimmune diseases.

One of the most efficient vaccines we have is the yellow fever vaccine 17D, which Max Theiler developed in 1937 by attenuating the live virus: one shot confers life-long immunity. It is still used in what is essentially its original form. But it took almost 80 years to find out what makes it so effective: Bali Pulendran from the Vaccine Center at Emory University in Atlanta, USA, discovered that it activates four toll-like receptors on different types of dendritic cells. The live-attenuated virus in effect works as its own adjuvant, as is the case in many other vaccines that include the whole pathogen. In contrast, vaccines containing only parts of the microbe or virus are usually less effective, but have a better risk profile, as there are less side effects and no danger of inducing the disease one is trying to prevent. In a 2007 article in *The Scientist*, Pulendran writes, "If we could only make something that would work as well as this [17D] against malaria or tuberculosis, for example, we wouldn't be here. We'd close up shop and go home."

One big hope was pinned on DNA vaccines to achieve exactly that. Instead of presenting the pathogen itself – whole or in pieces – DNA vaccines contain only the genes coding for the antigen(s) embedded in a plasmid. The plasmid is injected directly, for instance, into the muscle, where it is supposed to induce the body's cells to produce the antigen for a given time. Ideally, the antigen is recognized as foreign, eliciting an immune response. Potential advantages of this radically new approach are the stimulation of B and T cells, while no infectious agents are present, and the easy large-scale production of a stable vaccine. As rapidly as the field is growing, it has yet to produce hard results. Several trials have shown that it works in theory, but the generated immune response has not been large enough to confer adequate protection. One possible way to improve on this may be to use not DNA, but RNA.

Traditionally, the feasibility of an antigen for use in a vaccine has been evaluated by looking at the body's production of the antibodies capable of neutralizing or destroying the disease-causing agent. The more we learn about the immune system, the more we realize that that is only part of the story. But we have no other measure to screen for efficient antigens. Systems biology could fill this gap by helping to identify the molecular networks that need to be activated within the immune cells to achieve protection. It has already shown us new links between immunization and metabolism and that different vaccine types induce specific changes in gene expression in certain cells of the immune system. It could also enable us to engineer antigens, adjuvants, and vectors of delivery with better efficiency. The promise of systems biology also encompasses new biomarkers to test for vaccine efficacy, making it possible to reduce trial size and length. But so far it is exactly that: only a promise.

Four of the big diseases for which we have not found effective vaccines are HIV, malaria, tuberculosis, and influenza. HIV and influenza are characterized by their enormous variability in antigens. The plasmodium parasite causing malaria passes through different stages while in the human host, with each stage showing large antigen variability. Tuberculosis presents a different problem: while the vaccination with the bacillus Calmette-Guerin – developed in the early twentieth century from the bovine tuberculosis bacteria – protects many infants against the disease, it does not kill all the bacteria. A chronic infection remains, which may turn into full-blown tuberculosis later in life. Nor does the vaccine protect adults. In addition, its efficiency seems to vary with geography for reasons we do not understand – like much about this vaccine.

It has been 120 years since Jenner's first vaccination. During this time, vaccines have proven to be the single most effective health measure ever introduced, preventing more than 100 million cases in the USA alone. Due to vaccines, cancer and cardiovascular disease have far surpassed infectious disease as the number one killers in industrialized countries. However, according to the WHO, there are globally still about 1.5 million deaths each year that could be prevented by vaccines already available. To save even more lives, we need to continue down the road leading to new and better vaccines, along which researchers already see signposts not just for preventing infections, but also for therapeutic vaccines against cancer and other diseases. But we also need to deliver existing as well as new vaccines quickly, cheaply, and efficiently to people in all parts of the world.

ADDITIONAL READING AND SOURCES

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De Gregorio E, Rappuoli R (2014) From empiricism to rational design: A personal perspective of the evolution of vaccine development. *Nat Rev Immunol* **14:** 505–514

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 denoted with a *.

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RETROTRANSPOSABLE SEQUENCES ARE DOCKING SITES FOR RNA-BINDING PROTEINS

cf. BIF FUTURA, VOL. 27 | 1.2012

JAN ATTIG
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Supervisor: Prof. Jerne Ule

Almost half of our genome is composed of retrotransposable elements (RTEs). Of these, the long interspersed elements (LINE) and Alu families, with over one million copies each, are the most common. Thousands of RTEs are transcribed as part of pre-mRNA transcripts, but whether they are passive bystanders or functional RNA elements remains unclear. The expression of all classes of RNAs is extensively regulated by RNA-binding proteins (RBPs). To survey the binding of RBPs to RTEs, I have taken advantage of binding data from individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) experiments for more than a dozen RBPs. I found that RBP-binding regions are generally scarce in RTEs; however, some RBPs specifically bind to individual RTE families. Using siRNA knockdown experiments, I addressed the role of these binding events in the processing of both protein-coding mRNAs and long non-coding RNAs. For mRNAs, I found that the interactions between RBPs and RTEs are important for mRNA processing. In the absence of the dedicated RBP, exons were included into the spliced mRNAs arising from or close to RTE sequences. These sequences were generally not found as part of the spliced mRNA in any human tissue, suggesting that they were erroneously included in the knockdown experiments. Hence, RBPs perform a proof-reading function on RTEs in pre-mRNA processing. In addition, I found that RTEs within non-coding transcripts were covered by RBPs. Using a 3'-end sequencing protocol, I demonstrated that RBPs mask poly(A) sites within these transcripts, which allows transcription to extend for several thousand kilobases; conversely in the absence of the RBP, the non-coding transcripts were much shorter and used a premature poly(A) site. Hence, binding to RTEs in non-coding regions prevents misprocessing of the non-coding RNAs, which subsequently leads to reduced expression. Altogether, the results of my PhD show that RTE sequences affect the expression of both protein-coding and non-coding transcripts. The specificity of RTE:RBP interactions suggest that individual RBPs have evolved to maintain accurate processing of RNA transcripts that have been invaded by RTE sequences.

PUBLICATIONS

Coelho MB, Attig J, Bellora N, König J, Hallegger M, Kayikci M *et al* (2015) Nuclear matrix protein Matrin3 regulates alternative splicing and forms overlapping regulatory networks with PTB. *EMBO J* **34:** 653–668

THE ROLE OF INTRACELLULAR TRANSPORT IN VISUAL SYSTEM DEVELOPMENT IN ZEBRAFISH

cf. BIF FUTURA, VOL. 26 | 3.2011

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Discipline: Developmental Neurobiologist, MSc
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Supervisor: Dr Filippo Del Bene

The development and function of polarized cells such as neurons depend on microtubule-associated intracellular transport. However, little is known about the role of specific molecular motors in the formation of synaptic connections. The first aim of my PhD was therefore to develop genetic tools for the in-depth analysis of motor function *in vivo*. Using the CRISPR/Cas9 system, we established a new method that enables the insertion of donor plasmids at defined genomic loci in the zebrafish genome. This allows not only the generation of loss-of-function alleles but also more advanced genomic engineering such as the insertion of reporter cassettes that reproduce the endogenous expression pattern of a target gene while disrupting its function. Building on these techniques, we investigated the role of the anterograde motor protein kinesin I heavy chain, Kif5aa, during the formation of the zebrafish retinotectal circuit. This circuit is formed by retinal ganglion cells (RGCs), which grow long axonal projections from their cell body in the retina to connect to cells in the visual processing centre in the optic tectum. Targeted disruption of Kif5aa does not affect RGC differentiation, and retinal axons reach their topographically correct targets, albeit with a delay. Using *in vivo* dynamic imaging, we found that anterograde transport of mitochondria in RGCs is impaired, as is synaptic transmission. Strikingly, this disruption of presynaptic activity upregulates the growth factor neurotrophin-3 (Ntf3) in postsynaptic tectal cells. This in turn promotes filopodial extensions and exuberant branching of retinal axons by signalling through the TrkC receptor. Thus, through the disruption of the molecular motor Kif5aa, we have uncovered an activity-dependent, retrograde signalling pathway that homeostatically controls axonal branching. We speculate that this pathway might play a role in other systems to coordinate connectivity and growth between different brain areas.

PUBLICATIONS

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MOLECULAR MECHANISM OF SECRETORY PROTEIN TRANSLOCATION BY THE SECA-ATPASE

cf. BIF FUTURA, VOL. 27 | 2.2012

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Institute: Howard Hughes Medical Institute and Department of Cell Biology, Harvard Medical School, Boston, MA, USA
Supervisor: Prof. Tom Rapoport

About 30% of bacterial cellular proteins are translocated through the SecY channel in the plasma membrane to the extracellular space or integrated into membranes. During post-translational translocation, the ATPase SecA associates with SecY and uses cycles of ATP hydrolysis to move its substrates through the channel. SecA is responsible for the translocation of around 400 secretory proteins and some membrane proteins, all with highly divergent amino-acid sequences. It has therefore been a long-standing question how SecA can interact with such a large number of different substrates and how conformational changes during the ATPase cycle are coupled to translocation. In my PhD, I showed that SecA uses a "push and slide" mechanism to move polypeptide substrates through SecY: protein translocation is largely driven by diffusion when SecA is in the predominant ADP-bound state, and infrequent power strokes by SecA upon ATP binding confer directionality of transport. This mechanism allows SecA to translocate polypeptide segments with which it interacts only weakly and explains how it can translocate its numerous substrates in a sequence-insensitive manner. Next, I applied single-molecule Förster resonance energy transfer to directly visualize conformational changes in SecA. I developed a method for the purification of an active intermediate of the translocation reaction, in which SecA is labelled with donor and acceptor fluorophores. When reconstituted into artificial membranes, I could show that the "two-helix finger" domain, which contacts the polypeptide chain during translocation, moves in an ATP-dependent manner between multiple conformations. Together, my data contribute to our molecular understanding of the reaction catalyzed by SecA and pave the way for a more detailed study of how SecA couples ATP hydrolysis to conformational changes.

PUBLICATIONS

Chen Y, Bauer BW, Rapoport TA, Gumbart JC (2015) Conformational changes of the clamp of the protein translocation ATPase SecA. *J Mol Biol* **427:** 2348–2359

Junne T, Wong J, Studer C, Aust T, Bauer BW, Beibel M *et al* (2015) Decatransin, a new natural product inhibiting protein translocation at the Sec61/SecYEG translocon. *J Cell Sci* **128:** 1217–1229

Bauer BW, Shemesh T, Chen Y, Rapoport TA (2014) A "push and slide" mechanism allows sequence-insensitive translocation of secretory proteins by the SecA ATPase. *Cell* **157:** 1416–1429

MECHANISMS OF MOLECULAR QUALITY CONTROL IN THE ENDOPLASMIC RETICULUM

cf. BIF FUTURA, VOL. 26 | 3.2011

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Secretory and transmembrane proteins play key roles in cellular communication and immune defence. Before leaving the endoplasmic reticulum (ER) after biosynthesis, each of these proteins must adopt its native structure. Molecular chaperones determine whether a protein may leave the ER for its final destination or is incompletely folded and must be degraded. The goal of my PhD project was to discover how chaperones assess the folding state of a protein. Two members of the Hsp70 chaperone superfamily reside in the ER: the conventional Hsp70, immunoglobulin binding protein (BiP); and the large Hsp70, glucose-regulated protein of 170 kDa (Grp170). I showed that Grp170 directly binds to incompletely folded proteins, but – unlike BiP – remains bound to its substrate in the presence of ATP. My results further demonstrate that chaperone:substrate interaction is differentially regulated for large and conventional Hsp70s. To understand how members of the ER Hsp70 superfamily recognize their respective substrates, I developed an intracellular peptide screen. This tool allowed me to determine the binding sites of the Hsp70 chaperones and the ERdj co-chaperones, which support them in their functions. Whereas BiP binds many sites that are spread throughout the substrates, Grp170 recognizes a few sites that coincide with those of the structurally unrelated ERdj4 and 5. All Grp170, ERdj4, and 5 binding sites are buried upon substrate folding or assembly, suggesting that biosynthesized proteins have evolved specific motifs to signal their folding state to the cell. My work provides a means to delete or introduce chaperone binding motifs at positions critical for folding and thereby influence a protein's fate in the ER. In addition, this knowledge is important to the understanding of protein misfolding diseases.

PUBLICATIONS

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INVESTIGATING THE LINKS BETWEEN LOCAL CHROMATIN STATES AND TRANSCRIPTIONAL ACTIVITY

cf. BIF FUTURA, VOL. 28 | 1.2013

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Supervisor: Prof. Lars Steinmetz

Eukaryotic gene transcription is a highly regulated process in which molecular machineries interact with chromatin and transiently disrupt its local architecture. This interaction is partly mediated by post-translational modifications (PTMs) of histone proteins. Much of the dynamics of the interplay between the transcription machinery and modified histones, as well as the chain of causality in the resulting molecular events, remain elusive. During my PhD, I studied the deposition of histone PTMs during transcription in strains of the model organism *Saccharomyces cerevisiae* in which genes with a chromatin-related function were deleted. To quickly obtain genome-wide maps of histone PTMs in multiple strains, I first developed a protocol to markedly increase the throughput of chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) experiments. Using ligation of DNA barcodes, my colleagues and I generated the equivalent of 90 ChIP-seq datasets in a few days without the use of a robotic device. Bioinformatics analysis of these data showed that deletion of the histone methyltransferase Set2 (*set2*Δ) results in altered distribution of the trimethylation of lysine 4 of histone 3 (H3K4me3), which is not targeted by this protein. Using an atlas of transcription initiation sites in the *set2*Δ mutant, we were able to associate changes in the distribution of H3K4me3 with the emergence of new promoters within coding sequences of a subset of genes. In contrast to this gene-specific effect, we observed increased levels of acetylation on histone 3 across the entire *set2*Δ genome when compared to the wild-type strain. Our findings elaborate on the role of chromatin modifiers during transcription, showing that while they may affect the distribution of some histone PTMs, they can also specifically target subsets of genes to maintain local chromatin states. In order to broaden the impact of our methods, we are now applying some of our integrative approaches to the study of transcription initiation in the malaria parasite *Plasmodium falciparum*.

PUBLICATIONS

Chabbert CD, Adjalley SH, Klaus B, Fritsch ES, Gupta I, Pelechano V, Steinmetz LM (2015) A high-throughput ChIP-Seq for large-scale chromatin studies. *Mol Syst Biol* **11:** 777

Fritsch ES, Chabbert CD, Klaus B, Steinmetz LM (2014) A genome-wide map of mitochondrial DNA recombination in yeast. *Genetics* **198:** 755–771

ROLE OF microRNAS IN DEVELOPMENT AND FUNCTION OF INNATE LYMPHOID CELLS

cf. BIF FUTURA, VOL. 26 | 1.2011

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Supervisor: Prof. Andreas Diefenbach

Innate lymphoid cells (ILCs) are a newly discovered type of immune cell with roles in worm expulsion and the defence against intracellular pathogens and intestinal bacterial infections. Although several ILC functions, such as differentiation and cytokine production, depend on environmental cues, it is not clear how ILCs adapt to their environment. One of the key tools that cells use to respond to changes in their surroundings are small cellular RNAs called microRNAs (miRNAs). The goal of my PhD project was to investigate the impact of miRNAs on ILC development, survival, and function. The three known ILC subgroups are divided according to their expression of a lineage-defining transcription factor. For my analysis, I focused on group 3 ILCs (ILC3s), which express RORγt. Most abundant in the small intestinal lamina propria, ILC3s not only have important functions in the defence against attaching-and-effacing bacteria, but are also crucial mediators of lymphoid organ development. During foetal development, ILC3s are required for the formation of lymph nodes and Peyer's patches. After birth, ILC3s are critical for the development of organized lymphoid follicles in the gut wall, called cryptopatches. I used Dgcr8$^{\Delta ILC3,T}$ mice to show that deletion of miRNAs in ILC3 severely reduced the number of ILC3s. However, lymph nodes appeared to be unaffected and Peyer's patches were found in normal frequency and size. By contrast, cryptopatches were absent from the intestines of adult mice. Thus, prenatal lymphoid development was intact whereas postnatal lymphoid clusters were absent. By extension, we can assume that ILC3s depend mostly on miRNAs for postnatal functions. The results of my work contribute to our understanding of this enigmatic population of ILCs. Further investigation of the miRNAs and mRNAs expressed by ILC3s at different stages of development is necessary to determine the factors contributing to ILC3's dependency on miRNA. Due to the crucial functions of ILCs in organ homeostasis and defence, a better understanding of how these cells work might contribute to the discovery of novel approaches to target a variety of diseases.

PUBLICATIONS

Flach M, Diefenbach A (2015) Development of gut-associated lymphoid issues. In Mucosal Immunology, Mestecky J, Strober W, Russell M, Cheroutre H, Lambrecht BN, Kelsall B (eds) pp 31-42. Oxford: Elsevier, 4th edn

THE NUCLEAR EXPORT OF ACTIN: A BIOCHEMICAL AND STRUCTURAL PERSPECTIVE

cf. BIF FUTURA, VOL. 27 | 2.2012

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Actin participates in many cellular processes including muscle contraction, cell motility, and cytokinesis. It is predominantly localized in the cytoplasm as a result of active nuclear export via the nuclear export receptor exportin 6 (Xpo6), which recognizes the actin–profilin complex as a cargo together with RanGTP. The actin nuclear export pathway is conserved in vertebrates and insects, and the loss of Xpo6 function results in the nuclear accumulation of actin. We believe that disruptions in this pathway underlie the intranuclear rod myopathies, which are muscle disorders caused by mutations in skeletal α-actin and are characterized by rod-like accumulations of actin in the muscle cell nuclei. As there is no structural information available for Xpo6 or its complexes, the aim of my project was to use X-ray crystallography to understand the molecular interactions involved in actin nuclear export. I first isolated actin from tissue extracts whereas Xpo6, profilin, and RanGTP were recombinantly expressed and purified from *Escherichia coli*. After developing a single-step protocol for the purification of profilin–β/γ-actin complexes from cytoplasmic extracts, I was able to show that non-muscle β- and γ-actin isoforms have a higher affinity for the export complex than muscle α-actins. Interestingly, Xpo6 forms a stable complex with RanGTP in the absence of cargo, which is unusual for nuclear export receptors. I also showed that the profilin–actin complex is extremely sensitive to salt, particularly to chloride ions. I have optimized buffer conditions and complex formation strategies to assemble a stoichiometric and pure actin export complex. So far, I have successfully crystallized the actin export complex and the cargo-free Xpo6, the latter of which currently diffract to 7.4 Å. I am further optimizing crystals of both Xpo6 and the actin export complex for structure determination. With the structural information provided by the actin export complex, we hope to be able to understand whether a disruption of the interaction between actin and Xpo6 underlies the intranuclear rod myopathies. Ultimately, my studies may lead to the design of Xpo6 mutants that can recognize and export the actin mutants that lead to disease.

PUBLICATIONS

The results of this project have not yet been published.

CHROMOSOME OSCILLATIONS ARE INDISPENSABLE FOR CELL DIVISION

cf. BIF FUTURA, VOL. 26 | 1.2011

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Supervisor: Prof. Patrick Meraldi

Mitosis results in the equal division of parental genetic material between two daughter cells. Microtubules of the mitotic spindle attach to chromosomes, drive their alignment onto the metaphase plate at the spindle equator, and cause their segregation into two nascent cells. On the metaphase plate, chromosomes undergo highly regular oscillations along the axis of the bipolar mitotic spindle. The importance of chromosomal alignment onto the metaphase plate and the oscillatory motion remains a mystery. Despite no direct evidence, they are believed to be crucial for proper chromosome segregation in anaphase. The aim of my PhD project was to understand what regulates chromosome oscillations and their importance for cell division. I created a viable human cell line that had attenuated and irregular chromosome oscillations, suggesting that the oscillations are not necessary for cell division and survival. I then developed an assay whereby manipulating the culturing temperature produced cells in which chromosomes oscillate with reduced frequency and prolonged amplitude and do not align precisely in the middle of the mitotic spindle, giving rise to a wide metaphase plate. Using high-resolution live-cell imaging, I showed that these cells nevertheless progressed to anaphase. Although the cells divided with more chromosome segregation errors, the errors did not correlate with wide metaphase plates. I conclude that chromosomal alignment onto a narrow strip precisely at the spindle equator is not necessary for accurate chromosome segregation. I also found that the two half spindles in dividing cells carry small but detectable differences in the stability of their microtubules. Such variations cause the microtubules to grow and shrink at different rates, leading to an imbalance in the forces exerted on chromosomes. Rather than being essential for cell division, chromosome oscillations may therefore be a mechanism to balance out the forces within the mitotic spindle. This new knowledge expands our understanding of how the mitotic spindle works.

PUBLICATIONS

Gasic I, Nerurkar P, Meraldi P (2015) Centrosome age regulates kinetochore-microtubule stability and biases chromosome mis-segregation. *eLife* **4:** e07909

Vladimirou E*, Mchedlishvili N*, Gasic I*, Armond JW, Samora CP, Meraldi P, McAinsh AD (2013) Nonautonomous movement of chromosomes in mitosis. *Dev Cell* **27:** 60–71

MODELLING INTRACELLULAR TRANSPORT IN *DROSOPHILA* OOCYTES

cf. BIF FUTURA, VOL. 26 | 3.2011

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Discipline: Physicist, Diploma
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Supervisor: Prof. Raymond E. Goldstein

In *Drosophila*, the head-to-tail body axis is established by the localization of *bicoid* and *oskar* mRNAs to opposite ends of the oocyte. This is thought to occur mainly through motor-driven transport on a weakly polarized microtubule (MT) cytoskeleton. However, a clear description of the three-dimensional (3D) organization of the cytoskeleton is lacking and the roles of other transport mechanisms such as diffusion and cytoplasmic flows are poorly understood. Flows are driven by active transport on the cytoskeleton; thus, motor- and flow-mediated transport are two coupled representations of the same underlying network. Based on computer simulations, the aim of my PhD was to study intracellular transport by combinations of diffusion, transport on the cytoskeleton, and flows. I first defined a theoretical model in which motor-driven and flow-mediated transport are coupled. Surprisingly, simulations showed that a randomly oriented cytoskeleton with only a weak directional bias can be optimal for cargo localization to a target zone because dispersive circulatory fluid flows are minimized while on-average directed cytoskeletal transport is preserved. Thus, the presence of flows can, in principle, rationalize anti-intuitive cytoskeletal organizations. Next, I developed two theoretical models for the MT cytoskeleton in the 3D geometry of a *Drosophila* oocyte. Constrained by experimental data, both models show a compartmentalized organization of the cytoskeleton, suggesting that it is more ordered than previously thought. Computing cytoplasmic flows and simulating cargo transport in 3D accurately reproduces *oskar* and *bicoid* mRNA localizations in wild type and in various polarity mutants, thus validating the predicted cytoskeletal organization. The models reveal that underlying the polarity phenotypes is a bifurcation transition between different cytoskeleton topologies. In summary, by substantially modifying historically held views of the MT cytoskeleton, my findings shed new light on mRNA patterning in *Drosophila*.

PUBLICATIONS

Khuc Trong P, Doerflinger H, Dunkel J, St Johnston D, Goldstein RE (2015) Cortical microtubule nucleation can organise the cytoskeleton of *Drosophila* oocytes to define the anteroposterior axis. *eLife* **4:** e06088

Khuc Trong P, Guck J, Goldstein RE (2012) Coupling of active motion and advection shapes intracellular cargo transport. *Phys Rev Lett* **109:** 028104

A NEURAL CIRCUIT FOR GRADIENT CLIMBING IN *CAENORHABDITIS ELEGANS*

cf. BIF FUTURA, VOL. 25 | 3.2010

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Discipline: Biologist, Diploma
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Supervisor: Dr Cori Bargmann

Animals use environmental cues for navigation but it is not known how neural circuits collect information to control behaviour. To gain insights into these pathways, I studied odour-evoked behaviour in *Caenorhabditis elegans*, an organism with just 302 neurons of known connectivity. First, I developed a method to image neural activity in freely moving animals under microfluidic stimulation. This approach increases precision and throughput of calcium imaging in this model organism and allowed me, for the first time, to relate odour-evoked neural responses to behaviour. I found that although sensory neurons always responded to odours, the behavioural output was highly variable, suggesting that variability is a result of neural processing rather than sensory failure. I next studied a sub-circuit of neurons driving navigation behaviour in response to a buttery odour that signals food to *C. elegans*. I found that the circuit transmits a simple message: "Keep going, food odour increases." This selectivity is achieved by efficient response adaptation and subsequent normalization. Behaviour driven by this circuit mirrors its response asymmetry: animals actively suppress changes in direction when odour concentration increases but not when it decreases. This "optimist strategy" repeated over and over eventually leads animals to the odour source. I used optogenetic stimulation and genetic silencing tools to show the necessity and sufficiency of this circuit in regulating the odour response. Finally, using genetic analysis, I found two molecular regulators of cellular odour-response adaptation: the loss of CHE-3 (a cytosolic dynein heavy chain) disrupted olfactory cilium morphology and, unexpectedly, caused stronger, non-adapting odour responses; conversely, loss of inositol polyphosphate 1-phosphatase (INPP1) caused hyperadaptation. My results therefore provide an example of a behavioural computation and its implementation at the level of genes and cells in a neural circuit.

PUBLICATIONS

Larsch J, Flavell SW, Liu Q, Gordus A, Albrecht DR, Bargmann CI (2015) A circuit for gradient climbing in *C. elegans* chemotaxis. *Cell Rep* **12:** 1748–1760

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CHARACTERIZATION OF A NOVEL IMMUNE EVASION MECHANISM OF INFLUENZA A VIRUS

cf. BIF FUTURA, VOL. 26 | 1.2011

MARKUS LESCH
Discipline: Infection Biologist, MSc
Institute: Max Planck Institute for Infection Biology, Berlin, Germany
Supervisor: Prof. Thomas F. Meyer

The influenza A virus (IAV) is responsible for 250,000 to 500,000 deaths each year – depending on the virulence of the prevailing IAV strain – and thus represents a major public health burden. My group recently discovered that IAV infection of human cells *in vitro* leads to a strong upregulation of the cellular microRNA-141 (miR-141). This in turn causes post-transcriptional silencing of the interferon-induced anti-viral MxA protein (encoded by myxovirus resistance gene A), which enables enhanced viral replication. The aim of my PhD project was to characterize this novel immune evasion strategy at the molecular and the evolutionary levels. In particular, I sought to determine the molecular mechanisms underlying IAV-induced miR-141 upregulation and whether the mechanism is conserved in mice. Using several virological and cell biological techniques, I showed that miR-141 is induced only by replication-competent viruses and not by non-infectious virus particles, viral metabolites (such as proteins or RNA), or a mini-replicon system. Furthermore, I used computational promoter analyses, short interfering RNA, and pharmacological inhibitors to show that induction is partially driven by the cellular transcription factor c-Jun. Quantitative real-time polymerase chain reaction revealed that miR-141 is induced in murine cells. However, it does not silence the murine MxA homologue, myxovirus resistance gene 1, implying the presence of additional virus-relevant miR-141 targets. My findings shed light on the complex mechanism of miR-141's proviral activity and add another piece to the complex picture of immune evasion by IAV. As IAV is used as a model system for other negative sense-strand RNA viruses, my work will likely impact on research on other viruses with high medical and socio-economic impact, such as Ebolavirus or Hantavirus. My discovery of c-Jun as target for medical intervention is also of special interest. There are concerns of new pandemics – for example, of the avian IAV subtypes H5N1 and H7N9 – for which no vaccine stocks exist. Furthermore, resistance to directly acting anti-viral agents such as oseltamivir (Tamiflu) is spreading. Inhibitors of c-Jun, either small molecules or short interfering RNA, could be useful as anti-IAV drugs that hamper viral defence mechanisms.

PUBLICATIONS

The results of this project have not yet been published.

STRUCTURAL STUDIES OF COMPLEMENT ACTIVATION CASCADES IN INNATE IMMUNITY

cf. BIF FUTURA, VOL. 27 | 3.2012

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Discipline: Biochemist, Diploma
Institute: Aarhus University, Aarhus, Denmark
Supervisor: Prof. Gregers Rom Andersen

The human complement system is a central part of innate immunity. Complement recognizes pathogen- or danger-associated molecular patterns, which triggers activation cascades that eventually result in host defence strategies. Both underactivation and overactivation of complement lead to severe diseases, such as rheumatoid arthritis and systemic lupus erythematosus. Comprehensive structural information on one activation cascade, the so-called alternative pathway (AP), has improved our understanding of the functions of the complement system as well as the development of therapeutics for related diseases. However, there was little information about the two other cascades, the classical (CP) and lectin (LP) pathways. The aim of my PhD studies was to structurally characterize the main proteins of these two pathways: complement components C4b, C2, C3 proconvertase complex C4b2, and C3 convertase complex C4b2a. I obtained a crystal structure of C4b using macromolecular crystallography and a model of C4b using small-angle X-ray scattering (SAXS) in solution. Both structures have high similarity to the C4b paralogue from the AP and reveal many C4b-specific details of functional importance. Using SAXS, I also obtained models of the other target proteins. Comparison of the C2 and C4b2 models with their paralogues from the AP suggested that the CP/LP and AP pathways have different activation and control modes. Diversity in the way in which complement is activated and controlled implies that the system is capable of dealing with different types of danger with specific, finely tuned mechanisms. C4b2a, the convertase produced in the final step of CP/LP activation, showed very high structural similarity to the AP convertase, which was expected given their homologous functions. My results demonstrate that different pathways for triggering the complement system lead to the formation of structurally and functionally identical proteins, C3 convertases, which act to induce host defences. This sheds light on how the human immune system deals with diverse dangers as well as on probable evolutionary paths for the complement system.

PUBLICATIONS

Mortensen S, Kidmose RT, Petersen SV, Szilágyi Á, Prohászka Z, Andersen GR (2015) Structural basis for the function of complement component C4 within the classical and lectin pathways of complement. *J Immunol* **194:** 5488–5496

THE ROLE OF POLYCOMB REPRESSIVE COMPLEX 2 IN MOUSE PREIMPLANTATION DEVELOPMENT

cf. BIF FUTURA, VOL. 25 | 3.2010

PETER NESTOROV
Discipline: Biochemist, Diploma
Institute: Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland
Supervisor: Prof. Antoine Peters

Pluripotency is the ability of a cell to differentiate into any of the three embryonic germ layers, and is therefore referred to as the ground state of development. The pluripotent state represents a bridge between generations: it is initiated by the fusion of the two gametes from the previous generation and gives rise to the germline of the next generation. Studies in model systems suggest that pluripotency is governed by a core transcriptional network that arises during mammalian preimplantation development in a process accompanied by dynamic changes in chromatin organization, histone modifications, and DNA methylation. In my PhD project, I addressed the developmental and regulatory roles of the evolutionarily conserved Polycomb repressive complex 2 (PRC2) at the interface between two generations in the oocyte and in the preimplantation embryo. PRC2 regulates cellular "memory" by ensuring proper differentiation even in the absence of the original signal. I demonstrated that genetic ablation of core members of PRC2 early during mouse oogenesis reduces the trimethylation of lysine 27 on histone 3 (H3K27me3) *in vivo* and leads to a developmental and transcriptional response in late oocytes and early embryos. Furthermore, the mutant phenotypes revealed a dose-dependent requirement for PRC2 in the preimplantation embryo. I further described the transcriptional dynamics during early embryonic development of genes encoding chromatin modifiers. This experiment highlighted the existence of maternal and embryonic variants of the major chromatin-modifying complexes. In summary, my work reveals an important role for chromatin-based regulation in the preparation and acquisition of pluripotency *in vivo*. The requirement for PRC2 in the oocyte and in early embryos supports the potential role of histone modifications like H3K27me3 in the epigenetic inheritance of traits between generations.

PUBLICATIONS

Nestorov P, Tardat M, Peters AHFM (2013) H3K9/HP1 and Polycomb: Two key epigenetic silencing pathways for gene regulation and embryo development. *Curr Topics Dev Biol* **104:** 243–291

Nestorov P, Hotz HR, Liu Z, Peters AH (2015) Dynamic expression of chromatin modifiers during developmental transitions in mouse preimplantation embryos. *Sci Rep* **5:** 14347

EXAMINING THE REGULATION OF THE ANAPHASE-PROMOTING COMPLEX AT THE SINGLE-CELL LEVEL

cf. BIF FUTURA, VOL. 26 | 2.2011

ANDREJ ONDRACKA
Discipline: Biochemist, Diploma
Institute: The Rockefeller University, New York, NY, USA
Supervisor: Prof. Fred Cross

Cell-cycle transitions are driven by oscillations in the activity of cyclin-dependent kinases (CDKs) and their associated regulatory cyclin. These oscillations can occur as a result of cyclin degradation following its ubiquitylation by the anaphase-promoting complex (APC). The APC itself requires the activator Cdh1 and the APC–Cdh1 complex is inhibited through the CDK-mediated phosphorylation of Cdh1 at multiple sites. This important but poorly understood regulatory step leads to mitotic entry. In my PhD project, I analysed the regulation of APC–Cdh1 in the budding yeast *Saccharomyces cerevisiae*. First, I developed and characterized a fluorescent biosensor to measure the dynamics of APC–Cdh1 activity by quantitative time-lapse microscopy. I found that the timing of APC–Cdh1 inactivation in cells is relatively constant compared with other cell-cycle events, which often occur with substantial cell-to-cell variability. This suggests that a mechanism has evolved to specifically buffer any fluctuations in APC–Cdh1 inactivation. To investigate this further, I next determined the role of the multiple Cdh1 phosphorylation sites in the generation of such a robust mechanism. The complete disruption of Cdh1 phosphorylation leads to persistent APC–Cdh1 activity, which results in cell-cycle arrest before mitotic entry. I showed that the phosphorylation of Cdh1 is highly redundant: partial removal of sites can be tolerated, and no single phosphorylation site is necessary for inactivation. However, at least two phosphorylation sites must be present for APC–Cdh1 inactivation. Furthermore, incomplete phosphorylation of Cdh1 produces a highly variable phenotype in cell-cycle progression at the single-cell level. As a result of the partial but not completely restrained activity of APC-Cdh1, some of the cells are in cell-cycle arrest but they occasionally complete later cell-cycle events albeit with a delay and in the incorrect order. In addition, I showed through genetic interactions that the phosphorylation sites on Cdh1 can be modified by multiple cyclin–CDKs, and that competitive inhibition by a stoichiometric inhibitor Acm1 also contributes to APC–Cdh1 inactivation. Taken together, my results reveal that multiple highly redundant mechanisms regulate APC–Cdh1 activity to ensure a robust and timely progression through the cell cycle.

PUBLICATIONS

The results of this project have not yet been published.

NOVEL INSIGHTS INTO THE CATALYTIC REPERTOIRE OF NON-LTR RETROTRANSPOSONS

cf. BIF FUTURA, VOL. 26 | 1.2011

ANNA MILENA SCHNEIDER
Discipline: Biologist, Diploma
Institute: Max Planck Institute for Developmental Biology, Tübingen, Germany
Supervisor: Dr Oliver Weichenrieder

Non-LTR retrotransposons (NLR) are mobile genetic elements that replicate by a copy-and-paste mechanism via an RNA intermediate. The dominant NLR in humans is LINE-1 (L1), which is frequently activated in cancers and has massively contributed to the shape of our genome. NLRs generally contain two open reading frames (ORF1 and ORF2) and are characterized by a unique genomic integration mechanism known as target-site primed reverse transcription (TPRT). The L1ORF2 protein (L1ORF2p) contains an endonuclease domain (L1-EN) that initiates TPRT by nicking one strand of the genomic DNA. However, it is not clear which enzyme nicks the second DNA strand and what determines the nicking position. Furthermore, the ORF1p is highly diverse among NLRs and as well as containing RNA-binding domains, some of these proteins also include an esterase-like fold that may have a functional role. I combined X-ray crystallography, biochemistry, and cell biology to investigate these aspects of NLR biology. First, I showed that the L1-EN is also able to nick the second DNA target strand in a structure-specific manner to create the characteristic L1 target-site duplications. I found that this process depends on the size and conformation of the protruding hairpin loop of the L1-EN, which probably probes the malleability of the DNA minor groove. Second, I determined crystal structures and the enzymatic activity of the esterase-containing ZfL2-1 ORF1p from *Danio rerio*, which suggest that this protein can accommodate long fatty-acid chains. Furthermore, the ZfL2-1 esterase and also a human L1ORF1p construct could bind negatively charged lipids. These properties hint at lipid and membrane binding as novel and analogous functions among ORF1ps, next to their known activities in binding and organizing NLR RNA substrates. Taken together, my results indicate that NLRs are more autonomous than previously thought: TPRT can be completed by the activity of the self-encoded endonuclease and lipid binding by the ORF1p could target NLRs to membranes for assembly or could enable NLRs to cross cell and species barriers.

PUBLICATIONS

Schneider AM, Schmidt S, Jonas S, Vollmer B, Khazina E, Weichenrieder O (2013) Structure and properties of the esterase from non-LTR retrotransposons suggest a role for lipids in retrotransposition. *Nucleic Acids Res* **41:** 10563–10572

IDENTIFICATION OF GLIAL FUNCTIONS MODULATING MOTOR CO-ORDINATION IN *DROSOPHILA*

cf. BIF FUTURA, VOL. 25 | 3.2010

SILKE THOMAS
Discipline: Molecular Biomedic, MSc
Institute: Institute of Neurobiology, University of Münster, Münster, Germany
Supervisor: Prof. Christian Klämbt

Complex behavioural tasks are computed by neurons. Neuronal network function is dependent on glia, which have various roles such as the regulation of neuronal excitability by recycling neurotransmitters or regulating extracellular ion homeostasis. To decipher the underlying mechanisms, I studied the role of *Drosophila* glia in motor co-ordination. In an RNA interference (RNAi)-based screen, my colleagues and I identified genes that induced locomotion defects in adult flies when silenced in all glial cells. We then focused on those genes that induced locomotion phenotypes upon silencing in ensheathing glial cells but not in other glial subtypes. Ensheathing glial cells are closely associated with neurons and thus more likely to directly modulate neuronal functions than more distant glia. After analysing 6,000 genes, I identified six such genes. For more detailed analyses, I established a larval tracking tool allowing simultaneous recording of many crawling larvae at very high resolution. Silencing of one gene, which I named *rumpel*, leads to paralysis of larvae at high temperatures or when confronted with mechanical stress. Analyses of promoter fragments and tissue-specific RNAi experiments indicated that *rumpel* is required in glia closely associated with the neuropil, the synapse-dense area in *Drosophila*. As *rumpel* encodes a sodium/solute transporter, it may be needed to enable energy-demanding processes such as neurotransmitter recycling. Silencing of another gene, which I named *schlaflos*, did not affect larval crawling but did lead to reduced sleep in flies, which nevertheless maintained their circadian rhythm. Analyses of *schlaflos* promoter fragments showed broad expression, including glial cells close to neurons associated with sleep control. Interestingly, *schlaflos* encodes a GABAA receptor and GABA has been linked to sleep control. My work implies a novel role for glia in modulating sleep and underlines the functional relevance of neuron–glia interaction in establishing co-ordinated motor programs.

PUBLICATIONS

Risse B*, Thomas S*, Otto N, Löpmeier T, Valkov D, Jiang X, Klämbt C (2013) FIM, a novel FTIR-based imaging method for high throughput locomotion analysis. *PLoS ONE* **8:** e53963

Schmidt I, Thomas S, Kain P, Risse B, Naffin E, Klämbt C (2012) Kinesin heavy chain function in *Drosophila* glial cells controls neuronal activity. *J Neurosci* **32:** 7466–7476

THE ROLE OF THE ARYL HYDROCARBON RECEPTOR IN B-CELL IMMUNITY

cf. BIF FUTURA, VOL. 26 | 3.2011

MATTEO VILLA
Discipline: Medical Biotechnologist, MSc
Institute: MRC National Institute for Medical Research, London, UK
Supervisor: Dr Brigitta Stockinger

The aryl hydrocarbon receptor (AhR) is a cytosolic ligand-activated transcription factor that mediates the toxicity of dioxins. Its physiological roles, particularly in the immune system, are increasingly being recognized. For example, the receptor is known to influence the function of interleukin 17-producing cells, which are key for the immune response at barrier organs such as the gut and the skin. B cells, which produce antibodies to protect the host against microbial threats, also express AhR. The aim of my PhD project was to investigate the role of AhR in shaping the B-cell response. B cells upregulate *Ahr* upon activation of the B-cell receptor (BCR). To first show that the AhR pathway is functional in B cells, I added FICZ – a natural ligand that induced the translocation of AhR to the nucleus and the transcription of the target gene *Cyp1a1*. I next assessed the effect of AhR deficiency in B cells using a B cell-specific *Ahr*-deficient mouse model generated in our laboratory using the Cre-LoxP technology. AhR-deficient ($Ahr^{-/-}$) B cells proliferated less than AhR-sufficient ($Ahr^{+/+}$) cells following *in vitro* BCR activation. However, I found that $Ahr^{-/-}$ cells that overcame the proliferative block divided to the same extent as $Ahr^{+/+}$ cells. This suggests that AhR modulates the activation threshold of B cells rather than their intrinsic ability to proliferate. I corroborated these *in vitro* data with *in vivo* experiments showing that in mice, $AhR^{+/+}$ B cells proliferate more and outcompete $Ahr^{-/-}$ B cells. Furthermore, when challenged with the influenza virus, AhR-deficient mice produced fewer antibodies. With regards to the underlying mechanism, we propose that the proliferative defect in $Ahr^{-/-}$ B cells is due to a reduced cyclin O level in these cells. This lesser-known cyclin family member is encoded by the *Ccno* gene, which is a direct target of AhR. Cyclin O is thought to be involved in the G_1–S phase transition. Altogether, my results suggest that AhR has a role in the proliferation of B cells. This occurs through a mechanism that may involve cyclin O and the correct functioning of this pathway ultimately ensures an effective antibody response.

PUBLICATIONS

The results of this work have not yet been published.

THE REGULATION OF PP1 PHOSPHATASES DURING EPITHELIAL REMODELLING

cf. BIF FUTURA, VOL. 26 | 3.2011

YANXIANG ZHOU
Discipline: Biologist, MRes
Institute: Cancer Research UK London Research Institute, London, UK
Supervisor: Dr Nicolas Tapon

Epithelial tissues are one of the basic building blocks of animals. Intercellular contacts between neighbouring cells in epithelia allow them to withstand mechanical forces and to communicate with each other. During development, remodelling of epithelia is necessary for the correct formation of organs and involves the reversible phosphorylation of many intracellular protein complexes. Although the phosphorylation of these complexes has been well described, the dephosphorylation step is far less understood. In my PhD project, I investigated these dephosphorylation reactions using *Drosophila* as a model system. I focused on three scaffold proteins: the apoptosis-stimulating protein of p53 (ASPP), Ras-association domain family 8 (RASSF8), and coiled-coil domain-containing 85 (Ccdc85). Previously, our group has shown that ASPP and RASSF8 play a role in junction maintenance. I further established that ASPP forms a complex with phosphoprotein phosphatase 1 (PP1) and acts as its regulatory subunit. PP1 alone is promiscuous and can dephosphorylate many proteins; ASPP defines a narrower range of substrates. In collaboration with Stephane Mouilleron at the London Research Institute, we co-crystallized human ASPP2 and PP1 and the resulting structure suggests a novel binding mechanism for PP1s. PP1 binds to the SH3 domain of ASPP2 via its C terminus and this is important for the function of ASPP *in vivo*. Expressing a mutant form of ASPP that cannot bind to PP1 was unable to rescue defects caused by *ASPP* loss-of-function. Furthermore, I showed that RASSF8 and Ccdc85 can associate with the ASPP/PP1 complex. I also generated a ccdc85 mutant, the phenotype of which was similar to *ASPP* and *RASSF8* mutant flies, suggesting that all three proteins act together *in vivo* to regulate PP1. Finally, I established an *in vitro* dephosphorylation assay to test substrates of the ASPP–PP1 complex. In collaboration with Bram Snijders at the London Research Institute, we established methods for the identification of putative dephosphorylation sites using mass spectrometry. Taken together, this work describes the importance of new PP1 regulators *in vitro* and *in vivo* and lays the foundation for the discovery of new substrates of these phosphatase complexes.

PUBLICATIONS

Zaessinger S, Zhou Y, Bray SJ, Tapon N, Djiane A (2015) *Drosophila* MAGI interacts with RASSF8 to regulate E-Cadherin-based adherens junctions in the developing eye. *Development* **142:** 1102–1112

THE CO-RELEASE OF GLUTAMATE AND GABA FROM SINGLE VESICLES

cf. BIF FUTURA, VOL. 26 | 1.2011

JOHANNES ZIMMERMANN
Discipline: Neurobiologist, Diploma
Institute: NeuroCure, Charité Berlin, Berlin, Germany
Supervisor: Prof. Christian Rosenmund

Until about a decade ago, scientists thought that each neuron in the brain released only one type of neurotransmitter, which thus defined its mode of action. However, recent results suggest that the co-release of two or more classical neurotransmitters from the same neuron is quite common throughout the nervous system. The reasons for this are not well understood but there is evidence that co-storage of two different neurotransmitters in the same synaptic vesicle can increase the filling state – or capacity – of the vesicle. Neurons can even release both glutamate and GABA, the two major excitatory and inhibitory neurotransmitters, respectively: in inhibitory neurons of the auditory system, for example, the co-release of glutamate is required to refine an inhibitory map. However, the details of this mechanism have not been thoroughly determined on a vesicular level. Using patch-clamp recordings, I analysed whether vesicular glutamate transporter 3 (VGLUT3) expression induces the co-release of glutamate and GABA from murine striatal interneurons in culture. As the kinetics of glutamatergic and GABAergic responses are different, they can easily be distinguished. I found that action potentials in GABAergic neurons expressing VGLUT3 evoked mixed postsynaptic currents (PSC) mediated by both GABA and glutamate release. By analysing the fusion of single synaptic vesicles, I determined that the quantal events underlying the evoked mixed PSC included vesicles containing both glutamate and GABA. I next examined the postsynaptic receptors that mediate the glutamatergic response and found that glutamate release from GABAergic neurons did not alter the overall expression level of postsynaptic AMPA glutamate receptors. Interestingly, however, synaptic input of "purely" glutamatergic neurons to striatal GABAergic neurons expressing VGLUT3 impeded the detection of glutamate co-release, probably by drawing away AMPA receptors to glutamatergic synapses. Taken together, the results of my PhD are the first direct electrophysiological proof of glutamate/GABA co-release from single vesicles and suggest that the broader neuronal network participates in the detection of co-release.

PUBLICATIONS

Zimmermann J, Herman MA, Rosenmund C (2015) Co-release of glutamate and GABA from single vesicles in GABAergic neurons exogenously expressing VGLUT3. *Front Synaptic Neurosci* **7**: 16

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.

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PROFILES

Dr Sarah Teichmann,
The European Bioinformatics Institute (EMBL-EBI), Hinxton, UK
Fellowship: 1997–1999

Dr Edward Lemke,
European Molecular Biology Laboratory (EMBL), Heidelberg, Germany
Fellowship: 2003–2005

Edward Lemke and Sarah Teichmann, both with the EMBL, have received ERC Consolidator Awards. These grants come with up to 2 million euros and are awarded to talented researchers with proven potential. Edward is working on next-generation single-molecule protein fluorescence at EMBL Headquarters in Heidelberg, Germany. Sarah, who is stationed in Hinxton, UK, won the grant for her project "ThDefine: Re(defining) CD4+ T Cell Identities One Cell at a Time". Sarah has also been awarded the EMBO Gold Medal for the "use of computational and experimental methods to better understand genomes, proteomes, and evolution".

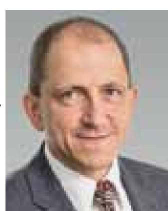
Prof. Michael Boutros,
German Cancer Research Center (DKFZ), Heidelberg, Germany,
Fellowship: 1997–1999

Michael Boutros has been named acting chairman and scientific member of the management board of the German Cancer Research Center (Deutsches Krebsforschungszentrum, DKFZ). He will be the temporary successor to Professor Otmar D. Wiestler. Boutros will lead the DKFZ jointly with its administrative-commercial director, Professor Josef Puchta.

Prof. Stefanie Dimmeler,
University of Frankfurt, Germany
Fellowship: 1991–1992

Prof. Artur Scherf,
Institut Pasteur, Paris, France
Fellowship: 1986–1987

In 2015, two of the 190 ERC Advanced Grants went to BIF fellows. Stefanie Dimmeler's project "Endothelial Long Non-Coding RNAs" studies how such RNAs influence our heart – especially its regenerative abilities – as well as their role in stroke and cancer cases. Artur Scherf was awarded the grant for his project "Exoribonuclease-Mediated Degradation of Nascent RNA in Malaria Parasites: A Novel Mechanism in Virulence Gene Silencing". In this first round of calls under the EU's Horizon 2020 programme, competition was exceptionally fierce, as the ERC stated in its press release.



Prof. Peter Kohl,
Research Centre for Cardiovascular Medicine (FKM), University of Freiburg, Germany
Postdoc Fellowship: 1992–1995

Peter Kohl now heads the newly founded Research Centre for Cardiovascular Medicine (FKM) at the University Heart Centre Freiburg-Bad Krozingen, Germany, a highly specialized hospital for heart and circulatory diseases. He took up the post in November 2015 and moved his labs from the Cardiac Biophysics and Systems Biology Chair at the National Heart Lung Institute, Imperial College London, UK, to Freiburg in February 2016.

Dr Stephanie Ganal-Vonarburg,
University of Bern, Switzerland,
Fellowship: 2009–2012

Stephanie Ganal-Vonarburg won the Cord Michael Becker Prize 2015, which is presented for outstanding PhDs in molecular medicine in Germany. It recognized her work on the role of the microbiome in the development of our immune system. Since 2013, the 5,000-euro prize has honoured the memory of Cord Michael Becker, a BIF alumnus who developed a study course concept for molecular medicine adopted at many German and international universities. It is awarded yearly by the Forschungsstiftung Medizin, a foundation at the University of Nuremberg-Erlangen, Germany.

Dr Grzegorz Sienski,
Whitehead Institute for Biomedical Research at MIT, Boston, USA
Fellowship: 2011–2013

Grzegorz Sienski received not just one but two prizes for his PhD project on the widespread influence of transposons and the piRNA pathway on chromatin patterns and gene expression at Vienna's Institute of Molecular Biotechnology (IMBA). He was awarded the PhD Prize of the Vienna Bio Center as well as the prestigious Kirsten Peter Rabitsch Award 2015 of the IMBA. This prize honours an IMBA student who was killed while travelling in South America. Last year, Greg started his second postdoc at MIT's Whitehead Institute for Biomedical Research, Boston.

Photos: EMBL, Photolab (Teichmann); EMBL (Lemke); DKFZ (Boutros); Goethe-Universität Frankfurt a. M. (Dimmeler); Artur Scherf; Britt Schilling/ Universitätsklinikum Freiburg (Kohl); Dr Stephanie Ganal-Vonarburg; Dr Grzegorz Sienski

WHO'S WHO AT BIF?

HIDDE PLOEGH, MEMBER OF THE BOARD OF TRUSTEES

Hidde Ploegh, born in 1953 in the Netherlands, has acheived a remarkable career without ever needing to apply for a position. He studied biology and chemistry at the University of Groningen and received his PhD from the University of Leiden. Right away, he was asked to lead a junior group at the University of Cologne, Germany. Hidde Ploegh held positions at a number of institutions, such as the Netherlands Cancer Institute and Harvard Medical School, before becoming a member of the Whitehead Institute in 2005. With more than 400 published papers, he has contributed to our understanding of how antigen processing works and how viruses evade our immune system.

Photo: Whitehead Institute/Chris Sanchez

Why did you choose a career in science?
I'm not even sure it was a conscious decision or even a choice. The longer I was exposed to science, the more I enjoyed it, and then I discovered that I could hold my own against some people I believed to be far more talented. So I stuck with it.

What fascinates you about doing research?
I've commented on this before. Like the properties of a stem cell, it is the self-renewing aspect of good research – it is endless in the positive sense of the word. Unexpected results – those that don't conform to received wisdom – can be the most fun.

What is your most remarkable BIF experience?
The fellowship application review meetings are intense, but fun. I particularly enjoyed our visit to Istanbul, the Golden Horn, the Blue Mosque … I probably would not have seen those sights if it weren't for the BIF meeting. The second memorable reason was the presence of the late Hermann Fröhlich, who was in exceptional form in his discussions with Herbert Jäckle and Benjamin Kaupp. Fröhlich was an original (and a Swabian).

What is your favourite activity?
That would have to be fishing for striped bass and bluefin tuna (in fact anything that bites) on the Atlantic Ocean with my friend Fred Alt.

What is your remedy for stressful situations?
A Martini (straight up, with a lemon twist) can help.

What fault in others can you tolerate best?
Mispronouncing my name comes to mind. Someone talking while chewing their food is at the other end of the spectrum.

Your advice to fellowship holders?
Be willing to take risks. Read, and then read some more (repeat), and then: remember what you read. Avoid big data like the plague if you cannot assess the quality of the data.

Which scientific achievement do you admire most?
In my own field, solving the atomic structure of the products of the major histocompatibility complex by Don Wiley and colleagues, and in immunology more generally: deciphering how antibody diversity is generated through somatic gene rearrangements. That was done by Susumu Tonegawa.

One thing you could not live without?
Oxygen.

BIF'S GUARDIAN IN THE MOUNTAINS

Hiking for hours in sunshine or rain to eventually reach a sometimes snow-covered Nebelhorn summit has been in the DNA of BIF's summer seminars in the Alps from their beginnings in the 1980s. Generations of BIF fellows, many of them newcomers to the mountains, proudly reached their first summit safely guided by Peter Schulze. Peter selected the best paths for the weather, coached anxious fellows across more precarious parts, motivated the tired ones, and had some extra sprints for the marathon runners. Not a man of many words, he led with natural authority and his exceptional judgement of people and groups. He became legendary among fellows not least for his stamina: there was hardly ever a fellow who could best him (and they tried!), even at the age of 65. He has now passed on responsibility to the next generation of mountain guides, who have big shoes to fill. Peter, all the best for your future and thank you very, very much for more than a quarter of a century of wonderful hiking and for bringing us home safely! Or as he would probably put it, "Hat scho passt", or "It's all good".

Peter during the first trip in Oberjoch in 1985.

PROFILES

Prof. Daniel Schmidt,
University of Minnesota, Minneapolis, USA
Fellowship: 2006–2009

In spring 2015, Daniel Schmidt accepted an assistant professorship at the University of Minnesota. His lab is part of the College of Biological Sciences and invents and applies protein-engineering technologies to study fundamental functional principles of natural and artificial living systems at a cellular level. In order to do so, the team seeks mechanistic explanations for how cells sense, integrate, and exchange information, and how pathologic changes in these processes relate to health and disease.

Dr Barbara Treutlein,
Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany
Fellowship: 2009–2010

Barbara Treutlein has been awarded a Max Planck Research Group Leader position and now heads a group at the MPI in Leipzig with a joint appointment at the MPI for Molecular Cell Biology and Genetics in Dresden. Her group uses single cell genomics data to reconstruct developmental pathways, lineage hierarchies, and tissue heterogeneity in human organs and organoids, placing particular focus on illuminating uniquely human biology.

Prof. Detlef Weigel,
MPI for Developmental Biology, Tübingen, Germany
Fellowship: 1987–1988

Detlef Weigel's "outstanding scientific work in the field of developmental and evolutionary biology" has been officially recognized by the Leopoldina, the German National Academy of Sciences, which presented him with the Mendel Medal. Weigel, who directs the MPI for Developmental Biology, has made several important discoveries concerning plant flowering and its timing as well as the health of hybrid plants. The medal honours pioneering work in the fields of general and molecular biology and genetics. It has only been awarded 24 times since its inception in 1965.

Photos: Christoph Engel (top); University of Minnesota (Schmidt); Barbara Treutlein; MPI-EB Tübingen (Weigel)

HOW TO FIND THE SHARPEST NEEDLES IN THE STACK

By Kirsten Achenbach

Many of the readers of this journal have undergone and passed it – the peer review process at BIF. But do you really know what is involved in selecting BIF fellows out of the yearly stack of close to 700 applications?

After each of the three deadlines per year, the BIF staff vets up to 260 applications within two weeks according to formal criteria such as the completeness of documents, topic (is it really basic biomedical research?), etc. When in doubt, the decision is taken in favour of the applicant. After clearing this hurdle, the documents are made available online to BIF's Board of Trustees. Each application is evaluated by two of the Board's scientists for BIF's three quality criteria: the applicant's achievements, the scientific quality of the project, and the scientific standard of the laboratory in which the applicant will be based. At the following board meeting, the scientists discuss their ratings and decide who will go on to the next stage – the final round of selection. Wherever possible, the approximately 50 remaining candidates are then invited to a face-to-face interview with either Dr Claudia Walter or Dr Carsten Lambert. In parallel, each application is sent to an external expert in the respective field for review – of course after qualified and willing scientists have first been identified. In the next step, the application, interview report, and expert review are analysed together by two of BIF trustees according to BIF's quality criteria. At the next meeting the board discusses each application individually, in great depth, and sometimes passionately, before making their final decision. In the end, around 15 students (less than 10%) are offered a BIF PhD fellowship. This very diligent – and yes – work-intensive process ensures the selection of the most talented young scientists working on the best projects in laboratories that have published excellent work in their fields. This in turn maintains the reputation the programme and our fellows enjoy.

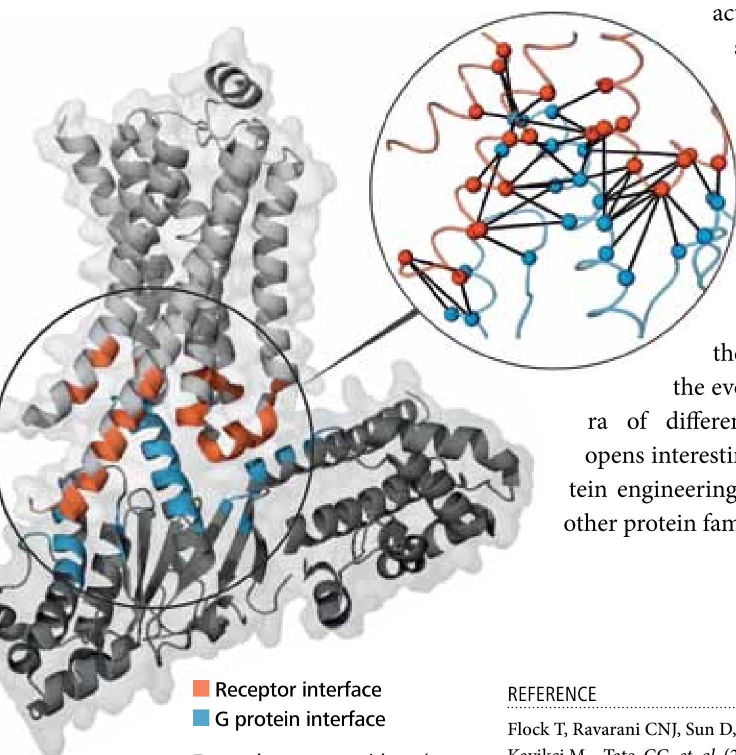
Photo: shutterstock (bottom)

PAPERS IN THE SPOTLIGHT

In "Papers in the Spotlight", we present papers from current or recent BIF fellows. 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 presented here, send an e-mail to Kirsten.Achenbach@bifonds.de.

MANY LIGANDS, ONE ACTIVATION MECHANISM

Cells constantly need to signal information – to regulate immune response, convey sensory input, or release hormones. Cells heavily rely on G protein-coupled receptors (GPCRs) for these and many more processes, so heavily, in fact, that 30% of all prescribed drugs target this system. But we still do not know how a ligand binding to a GPCR outside the cell triggers the release of GDP from the G protein inside it. This is especially interesting, as humans alone developed around 800 different GPCRs and 16 Gα genes. Tilman Flock from the Babu group at the LMB's Structural Studies Division at the MRC in Cambridge, UK, discovered that despite the diversity of the different receptors, Gα proteins all share the same activation mechanism, which is spatially separated from the receptor itself. They created a cataloguing system to compare 80 known structures and nearly 1,000 sequences of Gα proteins. The catalogue – available online – allowed them to analyse how the residues along a sequence interact with each other and into which 3D shape they will change a protein. "When a ligand binds to a protein, it changes these interactions and thus its shape. In Gα proteins, this triggers a hinge which releases GDP, the universal signal for Gα proteins," says Tilman. "This mechanism has stayed the same throughout the evolution of a plethora of different receptors. This opens interesting avenues for protein engineering – maybe even for other protein families."



Forces between residues (see black lines in inset) determine protein shape.

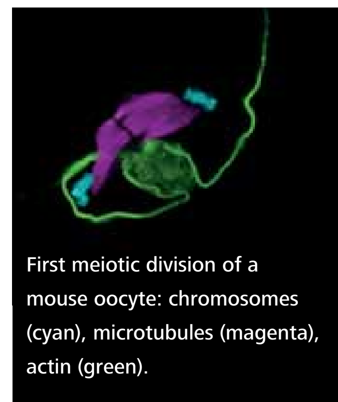
REFERENCE

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

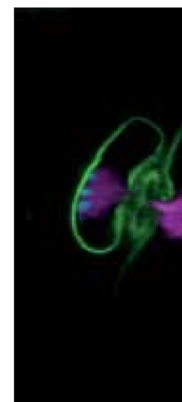
Tilman Flock, fellowship 2013–2015

HOW TO MAKE AN EGG

Errors during meiosis – cell divisions halving the chromosome set leading to haploid eggs and sperm – are the number one cause of miscarriages, infertility, and genetic disorders such as Down's syndrome. While the genes governing mitosis are well understood, the same is not true for meiosis – partly because the study of mammalian oocytes is hampered by slow genetic screens, scarce oocytes, and large protein content in the oocyte. Sybille Pfender and Michał Pasternak – equal contributing first authors working in the Schuh Lab at the MRC Laboratory of Molecular Biology in Cambridge – developed the first RNA interference screen in mammalian eggs. RNAi targets proteins for degradation via complementary siRNAs, effectively blocking protein expression. For their study, the authors identified 774 promising targets. "We microinjected siRNA into mouse oocytes enveloped in very small follicles and let them grow in vitro," says Sybille. "This way, we were able to block protein synthesis before the oocytes built up their protein store in ad-



First meiotic division of a mouse oocyte: chromosomes (cyan), microtubules (magenta), actin (green).



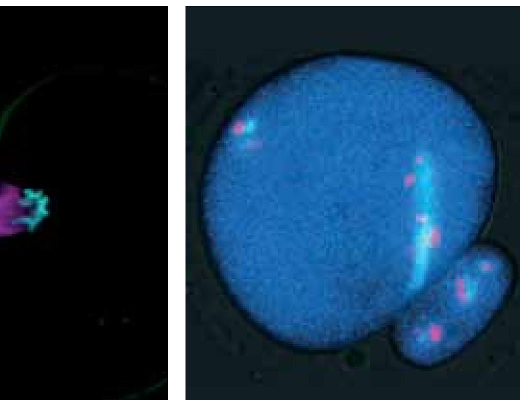
vance of meiosis," adds Michał. The authors followed 2,241 oocytes through meiosis with live confocal microscopy to elucidate the effects the target genes had on chromosome segregation. They described over 50 phenotypes and identified genes not yet associated with meiosis, gaining insight into how chromosome segregation errors arise. They also generated the largest data set on meiosis in mammalian eggs. The knowledge and data gained by the screen will drive our understanding of mammalian meiosis forward.

REFERENCE

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

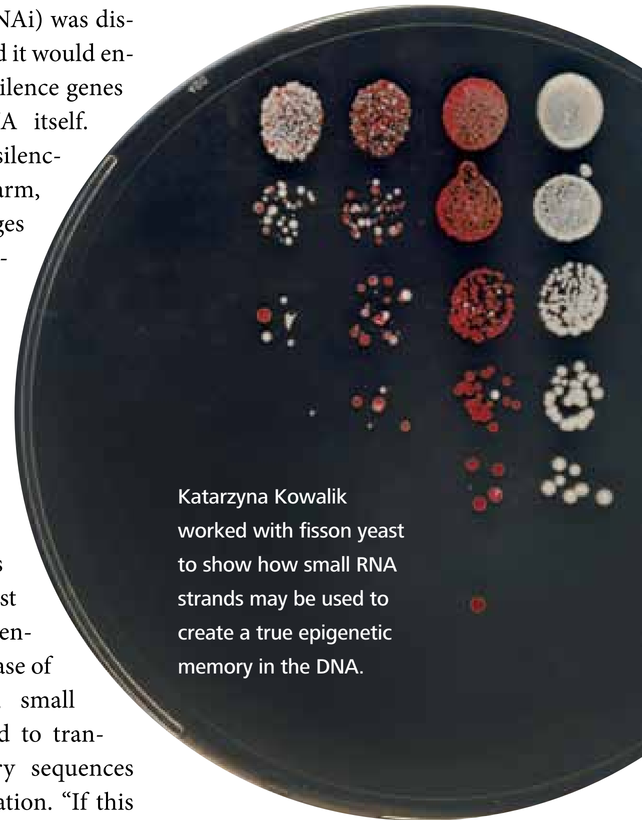
Michal Pasternak, fellowship 2013–2015
Sybille Pfender, fellowship 2010–2012

Photos: Flock (bottom left); Michał Pasternak, MRC Laboratory of Molecular Biology (middle); Katarzyna Kowalik (top right)



SMALL RNA STRANDS EFFECTIVELY SILENCE GENES

When RNA interference (RNAi) was discovered in 2002, it was hoped it would enable researchers to reliably silence genes without changing the DNA itself. While post-transcriptional silencing by RNAi works like a charm, heritable epigenetic changes are achieved only haphazardly. Katarzyna Kowalik, working with fission yeast in the Bühler lab at the Friedrich Miescher Institute for Biomedical Research in Basel, revealed why: She showed that the Paf1 complex, a part of the RNA polymerase, protects protein-encoding genes against silencing – accidental or intentional – by ensuring fast release of transcripts. During RNAi, small RNA strands (siRNAs) bind to transcripts with complementary sequences and target them for degradation. "If this happens before the transcript is released, an epigenetic mark is attached to the DNA which will be passed on during cell division", reports Katarzyna. The mark causes the DNA to coil itself into its heterochromatin form, denying the transcription engines access to the targeted gene and effectively silencing it. Katarzyna found mutated versions of the Paf1 complex with lower transcription and termination speeds. They stably silenced the targeted genes for at least 100 generations in the absence of triggering siRNAs, creating a true epigenetic memory. Through this, we are closer to harnessing the full potential of RNAi and, thus, a better understanding of epigenetic memory, advances in biotechnology, and new RNAi-based therapies.



Katarzyna Kowalik worked with fisson yeast to show how small RNA strands may be used to create a true epigenetic memory in the DNA.

REFERENCE

Kowalik KM, Shimada Y, Flury V, Stadler MB, Batki J, Bühler M. (2015) The Paf1 complex represses small-RNA-mediated epigenetic gene silencing. *Nature* **520:** 248–252

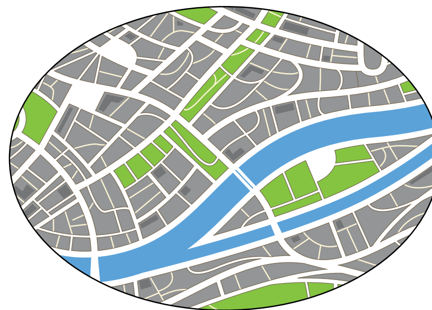
Katarzyna Kowalik, fellowship 2012–2014

BRAIN CLOCK KEEPS SPATIAL MEMORIES IN ORDER

Finding your way to class, the cafeteria, or through a maze if you are a lab rat is an important part of life. Magdalene Schlesiger found a clock in the brain that helps to store spatial memories in the correct order. Her work was done in the Stefan Leutgeb laboratory at the University of California San Diego in collaboration with Christian Leibold at the Ludwig Maximilian University in Munich. The clock is located in the so-called medial entorhinal cortex (MEC), which stores a map of our surroundings in its grid cells. These cells were thought to send spatial information to the place cells of the hippocampus, the learning centre of the brain. However, it turned out that the MEC transmits mostly temporal information towards their hippocampal targets. Magdalene found this out while analysing data from rats running back and forth along a straight line. She looked at the activity within the hippocampus in normal rats and rats with lesions within the MEC. "I found that the MEC input helps the hippocampus to time the firing of its cells so that memories can be stored in the correct order," says Magdalene. "Without this input, you would still know all the elements of the way, but not their order: instead of turning left, left, right you might turn left, right, left – and wind up at the park instead of at work." Many neurological conditions like Schizophrenia, stroke, or Alzheimer's impact a patient's navigational abilities. Setting the MEC clock to ensure precise timing of the place cells in the hippocampus could help to improve memory function.

REFERENCE

Schlesiger MI, Cannova CC, Boublil BL, Hales JB, Mankin EA, Brandon MP *et al* (2015) The medial entorhinal cortex is necessary for temporal organization of hippocampal neuronal activity. *Nat Neurosci* **8:** 1123-32.
Magdalene Schlesiger, fellowship 2011–2014

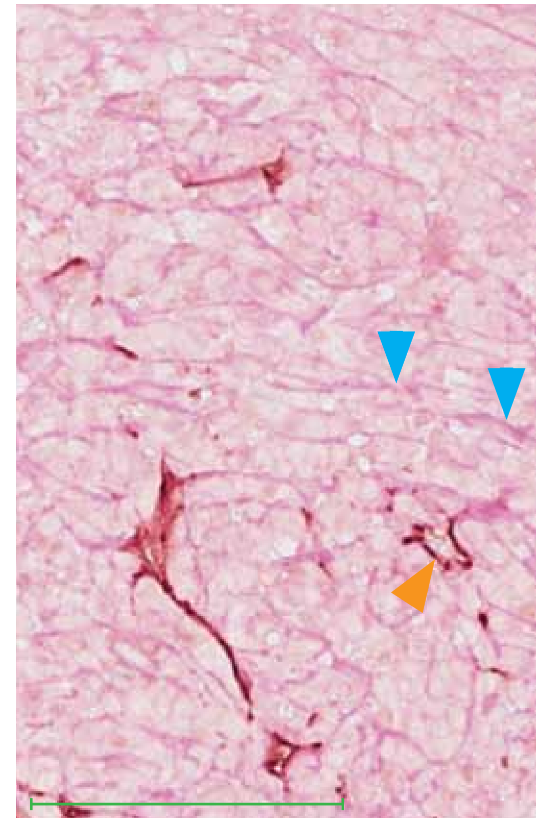


A clock in the so-called medial entorhinal cortex in the brain helps to store spatial memories in the correct order.

TWO CANCER GENES LET THE

Tumours can induce angiogenesis, the formation of new blood vessels to ensure their blood supply. However, using a novel approach, Elvin Wagenblast and his colleagues found two genes that help a tumour to tap the body's capillaries and colonize the body via an additional process called vascular mimikry. A tumour is made up of cells with subtly different abilities. Elvin, who works in the lab of Gregory Hannon at Cold Spring Harbour, New York, USA, created clones of different mouse breast cancer cells and tagged them with a barcode. He then injected them into mice. After 24 days, he

Photos: shutterstock, Magdalene (bottom left); Elvin Wagenblast (middle);

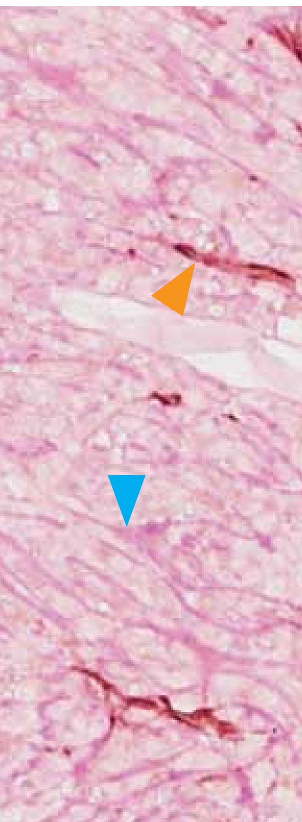


Mouse breast tumour tissue is stained for normal blood vessels (orange arrows) and vascular mimicry channels (blue arrows). Scale = 100 microns.

BLOOD FLOW

examined where the different clones had settled and profilerated and then analysed which cell genes they expressed. It turned out that the clones expressing SERPINE2 and SLPI were the ones most often found in metastases and in blood borne tumour cells. Their respective proteins Serpine2 and Slpi programme cells to act as endothelial cells and build pseudo blood vessels. Furthermore, both proteins work as anti-clotting agents, ensuring that blood will flow through the pseudo vessels towards the tumour and that – via the same route – cancer cells can enter the regular blood stream. These findings might explain why drugs trying to hinder the formation of new blood vessels have not been as effective as hoped. "It is not yet known how widespread these genes are in other tumours," says Elvin, "but at least these two genes are overexpressed in patients whose breast cancer has spread to the lungs, hinting at the relevance of the finding."

CC BY 3.0, Blausen.com staff. Blausen gallery 2014. Wikiversity Journal of Medicine. DOI:10.15347/wjm/2014.010. ISSN 20018762. (bottom right)



REFERENCE

Wagenblast E, Soto M, Gutiérrez-Ángel S, Hartl CA, Gable AL, Maceli AR *et al* (2015) A model of breast cancer heterogeneity reveals vascular mimicry as a driver of metastasis. *Nature* **520:** 358–362.

Elvin Wagenblast, fellowship 2010–2012

GUT MICROBES TUNE T CELLS AGAINST INFLAMMATION

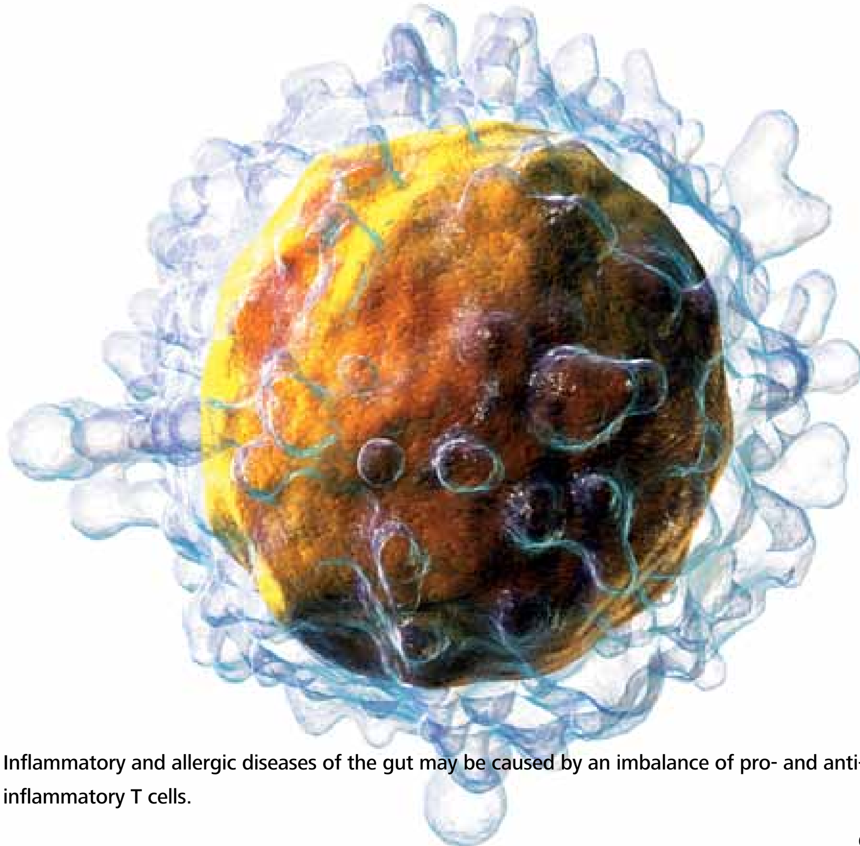
Our immune system needs to balance between attacking foreign cells and tolerating the body's own cells as well as benevolent foreign cells. These two roles are mediated by effector (pro-inflammatory) and regulatory (anti-inflammatory) T cells, respectively. Esen Sefik and David Zemmour, both working in the Benoist-Mathis Laboratory at Harvard University, found that benevolent gut microbes play an active part in keeping the balance between aggression and cooperation, at least in the colon. The study showed the microbes are necessary in order to induce a certain subset of Treg cells in the lining of the colon. "Surprisingly, these cells need both Rorg and Foxp3, two transcription factors *in vitro* studies showed to be antagonistic," says Esen, first author of the study. "Normally, Foxp3 is seen as promoting homeostasis and, therefore, acting anti-inflammatory while Rorg is pegged as inflammatory," adds David. In a mouse model of colitis, the presence of the commensals and the subsequent Rorg expression alleviated the symptoms, however. The findings imply that inflammatory and allergic diseases of the gut are due to an imbalance between different kinds of T cells, opening interesting avenues for intervention, especially as the identified Treg subset is also present in humans.

REFERENCE

Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D *et al* (2015) Individual intestinal symbionts induce a distinct population of RORγ* regulatory T cells. *Science* **349:** 993-997

Esen Sefik, fellowship 2012–2015

David Puyraimond-Zemmour, fellowship 2013–2015



Inflammatory and allergic diseases of the gut may be caused by an imbalance of pro- and anti-inflammatory T cells.

A BIF FELLOW'S GUIDE TO ...

LISBON

Travelling is fun – especially if you get insider tips from locals! In each edition of FUTURA, one fellow shows you around his or her city. In this edition, your guide is Catarina Nabais. She reports from Lisbon, Portugal's biggest city and capital.

FACTS & FIGURES

Country: Portugal
Population: About 550,000
Area: 100 km²
Students: About 113,400
Famous for: Fado music, wine, coffee, climate, and nightlife
Websites: www.visitlisboa.com, www.cm-lisboa.pt

WHERE TO STAY

Travellers House: Ranked as one of the best hostels in Europe, it is located in the very heart of Lisbon.
The Independente Hostel: Set on the border of the Principe Real and Bairro Alto district, this hostel is close to the most historical sites.

NIGHTLIFE

Bairro Alto: The nightlife centre of Lisbon, where one can find the best bars, clubs, and trendy shops.
Alcantara Docks: Old warehouses converted into bars and clubs with a great view over the Tagus river.

RESTAURANTS

Bacalhoeiro: A cosy restaurant where one can enjoy tasty Portuguese dishes.
Pastéis de Belém: Eat the most famous sweet pastries in Lisbon. 1
Clube de Fado: Enjoy your Portuguese meal while listening to traditional Fado music.
A Brasileira: One of the most iconic coffee shops, located in Chiado.

ACTIVITIES

Rooftop Bars: Enjoy a refreshing drink on one of the many rooftop bars with great views of the city. You will find several around the Bairro Alto district.
Enjoy the beach: The Lisbon coast offers amazing Atlantic sea beaches with white sands and warm waters for surfing or relaxing.

BEST SIGHTS

The Castle of São Jorge: A beautiful Moorish castle on the hilltop overlooking the historical Lisbon centre. 2
Belém: Located along the riverside, this is the neighbourhood with the largest number of monuments from the Portuguese voyages of discovery, including the Discoveries Monument 3 and Jeronimo's Monastery. 4 Truly worth visiting!
Terreiro do Paço: Here, spices and other goods arrived from all over the world.

Contributors wanted! If you would like to introduce your city to the readers of FUTURA, send an e-mail to Kirsten.Achenbach@bifonds.de

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Catarina Nabais

Photos: Catarina Nabais

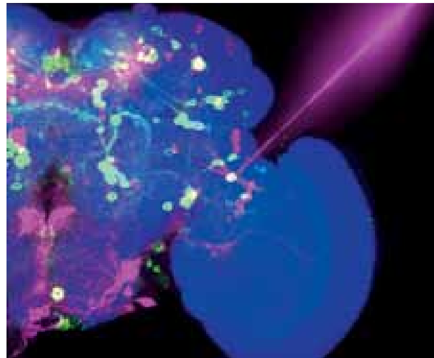
OPTOGENETICS PIONEER HONOURED WITH 2015 HEINRICH WIELAND PRIZE

BIF's sister foundation, the Boehringer Ingelheim Foundation, honoured Professor Gero Miesenböck from the University of Oxford with its 100,000-euro Heinrich Wieland Prize. The award was presented as part of a symposium on optogenetics at Nymphenburg Palace in Munich on 6 November.

Gero Miesenböck was the first to insert a light-controlled on-off switch into brain cells and thus pave the way to turning nerve cells on and off quickly, simply, and reliably. Since its first use in cell cultures in 2002, Miesenböck and other researchers have developed and improved upon the original idea and the pioneering approach has been used by groups all over the world.

Optogenetics has allowed brain researchers to make great advances by enabling them to study neural circuits in unprecedented detail, at the speed of thought, and in the living brain. It has already shown which nerve cells wake us up and has helped to clarify how cocaine and other drugs reprogramme the reward system of the brain. And in mice with Parkinson's disease, it has turned the typical shuffling gait into sure steps once again. In 2015, researchers used optogenetics to attempt to restore vision in blind people for the first time.

The speakers at the scientific symposium were, besides the awardee, Professor Christian Lüscher, University of Geneva, Switzerland, Professor Botond Roska, FMI Basel, Switzerland, and Professor Arthur Konnerth, TUM, Munich. The laudation was held by Nobel Prize Laureate Professor Bert Sakmann, TUM Munich, Germany.



Brain of a fruit fly: the nerve cells shown in magenta have been altered to respond to the light signal (shown in the same colour).

Otto Boehringer, then chairman of the Executive Committee of the Boehringer Ingelheim Foundation, presenting the 2015 Heinrich Wieland Prize to Gero Miesenböck, professor at the University of Oxford, UK.

Photos: Axel Griesch Fotografie (botom); A. Claridge-Chang, R. Roorda, and G. Miesenböck/University of Oxford (top)

UPCOMING EVENTS

1–3 JULY 2016

Annual meeting of former BIF PhD and MD fellows based in Europe

The seminar takes place at Gracht castle in Erftstadt/Liblar near Cologne, Germany. This year's title is "Bodies of the Future". Further details with the programme.

8–9 JULY 2016

Meeting of BIF's Board of Trustees in Boston, USA.

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

27 AUGUST–2 SEPTEMBER 2016

Seminar for current PhD fellows working in Europe in scenic Hirschegg (Kleinwalsertal), Austria

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

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

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