FUTURA

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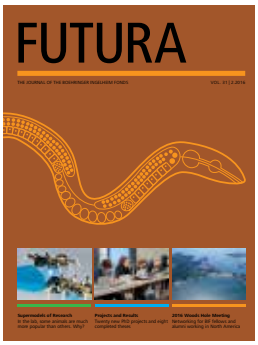
Supermodels of Research
In the lab, some animals are much more popular than others. Why?

Projects and Results
Twenty new PhD projects and eight completed theses

2016 Woods Hole Meeting
Science and more for BIF fellows and alumni in North America



The cover illustration shows a simplified model of a nematode or roundworm. There are more than 25,000 known nematode species, adapted to almost all ecosystems. One of them, *Caenorhabditis elegans*, is widely used in biological research. It is also the first multicellular organism whose genome was sequenced completely. Read more about animals in research on page 8.

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BALZAN PRIZE FOR REINHARD JAHN

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NOBODY IS AN ISLAND

"Science can be and has often been a place where co-operation, trust, and human relationships can thrive."

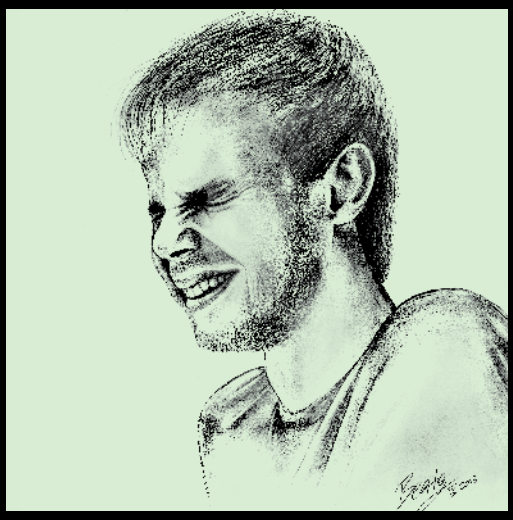
Early in the morning after the Brexit vote, the ripples of world politics were once again felt at BIF: an anxious young scientist enquired whether he could still apply for one of our travel grants. His e-mail was only the first of many such enquiries. We, as well as other funding bodies, reacted by sending out statements to all our fellows and alumni in the UK on how Brexit might affect eligibility to funding programmes.

At BIF it does not, since we define Europe geographically, not politically. And the British Islands will most likely not change their position on the map within BIF's lifetime. We will go on supporting Europeans worldwide and young scientists from overseas working in Europe – including scientifically significant islands. However, given the plunge the British pound has been taking, we had to adjust the stipend amount for the UK.

In view of the way the EU dealt with Switzerland, it seems fair to assume that it will find a way to continue to support scientists and projects in the UK after Brexit. Many of the more troubling aspects of recent developments are less tangible, but hurt the international endeavour of research nonetheless. Many of our fellows and alumni report a "climate change" in attitudes towards foreigners – not in their institutes but outside. There is also rising insecurity about one's future as a scientist and citizen in a non-EU Britain. Thus, many of them are reconsidering their plans and beginning to look for opportunities elsewhere.

The new American administration has also caused waves with its entry bans. Time will tell which measures will survive and how these will affect international exchange. In the past, and not only in the aftermath of 9/11, BIF fellows from certain nations already had difficulties obtaining visas for travelling not only to the USA, but also to European countries. Sometimes it just meant more time and effort for us and the fellows: extra paperwork, having to pass a personal interview at the embassy, etc. Sometimes efforts were in vain and fellows were not able to participate in our seminars or travel to scientific conferences. In times like these, it also means that fellows dare not leave the country for work or to see family, for fear they will have trouble coming back. PhDs and postdocs who need to succeed and build the basis for their next career step in a short amount of time can ill afford such a risk or lengthy and often costly appeals. Countries that offer such rough sailing should not be surprised if bright minds seek more pleasant shores and calmer waters. But no matter what happens, BIF will continue to support its fellows the best it can in choppy seas.

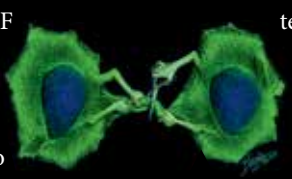
On a comforting note, science can be and has often been a place where cooperation, trust, and human relationships can thrive, even under adverse global politics. While Russia and Ukraine fought for land below them, astronauts from both countries together explored the space above in quest of new knowledge.

WHEN DEVOTION BEGETS EMOTION

By Beata Mierzwa, Institute of Molecular Biotechnology (IMBA), Vienna, Austria; beatascienceart.com

These portraits of PhD students were part of Beata's contribution to the Art & Science contest at the Vienna Biocenter in 2013. Her first scientific illustration (small inset in the centre) was a gift for the BIF team at a Hirschegg seminar – but Beata soon realized that showing the drawing in scientific presentations sparked interest and stayed in people's minds. Since then, she has combined her love for both science and art in an effort to communicate science in an unconventional way.



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.

ONE SMALL STEP FOR A GENE, ONE GIANT LEAP FOR MANKIND

It's true, we call them our closest cousins, but who would have thought that a crucial difference between man and ape stems from a tiny change? A single base pair substitution in the human-specific *ARHGAP11B* gene is what allowed humans to develop their superior cognitive abilities, according to researchers at the Max Planck Institute of Molecular Cell Biology and Genetics. This gene is only found in humans and in our closest relatives, the Neanderthals and Denisovan humans, but not in chimpanzees. It induces an increase in a specific subpopulation of brain stem cells called basal progenitors, which have been implicated in neocortex expansion – the part of the brain that is responsible for cognitive functions like speaking and thinking. But *ARHGAP11B* arose about five million years ago, whereas neocortex growth in our human ancestors only started much later. How, then, could this gene *ARHGAP11B* possibly be a key player in increasing neocortex size, researchers wondered. The answer to this question: a point mutation, a single C-to-G base substitution in the gene, which happened sometime between 1.5 million and 500,000 years ago. The mutation causes the elimination of 55 nucleotides from the gene's messenger RNA, leading to a 47 amino acid sequence in the resulting protein that is human-specific – which in turn leads to bigger brains. A tiny change, given the three billion base pairs of the human DNA, but one with truly life-changing consequences.

REFERENCE

Florio M, Namba T, Pääbo S, Hiller M, Huttner WB (2016) A single splice site mutation in human-specific ARHGAP11B causes basal progenitor amplification. *Sci Adv*, DOI: 10.1126/sciadv.1601941

FLOUNDERING NO MORE

It's a question that had even Charles Darwin flummoxed, but finally, after years of floundering, scientists have solved the evolutionary mystery: why are flounders flat? Of course flatfish, like the flounder, are not actually born flat. Flatfish larva start life fully symmetrical, but undergo a puzzling metamorphosis as juveniles whereby their eyes shift to one side of the head and they turn to spend the rest of their lives on one side. Moreover, as they move to their new home on the bottom of the seabed, the downward-facing side loses pigment. By comparing the genomes of two related fish species, the Japanese flounder (*Paralichthys olivaceus*) and its distant relative, the tongue sole (*Cynoglossus semilaevis*), an international team led by the University of Würzberg unlocked the mystery with the identification of a key development trigger: retinoic acid. Focusing on the genes that were active during the metamorphosis, the researchers found that retinoic acid leads to changes in the skin pigments of flounders and interacts with a thyroid hormone, causing their eyes to shift. Light also plays a central role in this process as the same pigments that capture light in the eye are expressed in the skin of the flounder larvae. The fish sense differences in brightness to adjust the concentration of retinoic acid, which in turn affects the thyroid hormone and promotes asymmetry generation.

REFERENCE

Shao C, Bao B, Xie Z, Chen X, Li B, Jia X *et al* (2017) The genome and transcriptome of Japanese flounder provide insights into flatfish asymmetry. *Nat Genet* **49:** 119–124

Photo: MPI f. Molecular Cell Biology and Genetics (top right); I, Luc Viatour/www.lucnix.be (bottom right)

THE GREEN PAGES

Spanning over 1,100 pages with some 14,000 entries, a new directory, thicker than a phone book, reveals a who's who of the botanical world. Instead of names and telephone numbers, the directory from Berlin's Botanical Gardens lists plants, mosses, fungi, lichens, and algae (living and extinct) named after people (real and fictional). Entries include, for example, the flowering plant *Goethea*, named after Johann Wolfgang von Goethe, and the woody plant *Napoleonaea*, which was first described as a genus in 1804, the same year its Corsican namesake crowned himself emperor of the French. The plants reveal an incredible passage of botanical research and world history, through the diverse names in science, politics, and culture after which they are named. The directory is the result of several years of work by Lotte Burkhardt, who for the first time links botanical, historical, and biographic research into one single book, for scientists and plant lovers alike.

Download: http://bit.ly/2k8UNUZ

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TRILLION TONS

This is the estimated weight of the physical structure of planet Earth's technosphere, which is comprised of all of the structures that humans have constructed to keep them alive on the planet – from houses, factories, and farms to computer systems, smartphones, and the waste in landfills.

Source: Zalasiewicz J, Williams M, Waters CN, Barnosky AD, Palmesino J, Ronnskog AS *et al* (2016) Scale and diversity of the physical technosphere: a geological perspective. *The Anthropocene Review*, DOI: 10.1177/2053019616677743

WILD THINGS

The Serengeti in North Africa is famed for its wide open plains and dizzying array of wildlife, including lions, wildebeest, and zebras. But almost 25 years ago, the lion population took a severe hit with a particularly fatal outbreak of the canine distemper virus (CDV), which has, until now, puzzled scientists. Highly contagious, CDV has been present in the Serengeti since the 1960s. A spread through lions and spotted hyenas in Tanzania's Serengeti National Park in 1993/1994 was unexpectedly lethal, reducing the lion population by around 30 % and killing many spotted hyena juveniles. Since then, there have been numerous CDV outbreaks that have killed wild canids such as the bat-eared fox or African wild dog, but none have since proved fatal to lions and hyenas. Why was the outbreak in 1993/1994 fatal to lions and hyenas, while later outbreaks weren't? Genetic analysis of the different strains from 1993 to 2012 has shown that the strain of the 1993/1994 epidemic had rare mutations in two viral proteins: the CDV-H protein, which binds to the host cell receptor and therefore plays an important role in facilitating viral entry into host cells, and the CDV-V protein, which enables the virus to manipulate the innate immune response of the host. This made it strongly distinct from those in domestic dogs and wild canids and increased its ability to invade lion cells. These mutations suggest that this particularly lethal strain evolved from a strain that was especially well adapted to non-canid species such as lions and hyenas, and that it did not spill over from domestic dogs to lions and hyenas.

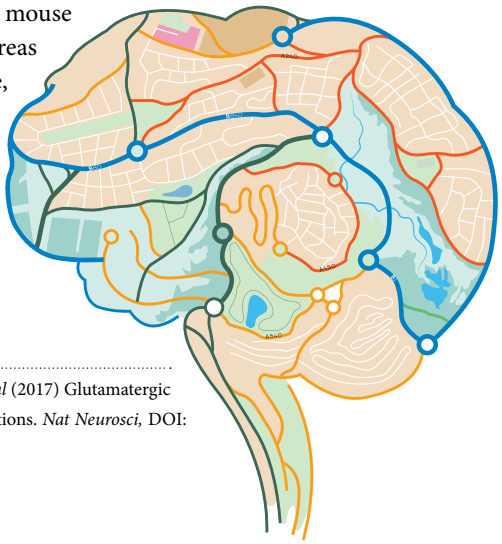
REFERENCE

Nikolin VM, Olarte-Castillo XA, Osterrieder N, Hofer H, Dubovi E, Mazzoni CJ *et al* (2016) Canine distemper virus in the Serengeti ecosystem: molecular adaptation to different carnivore species. *Mol Ecol*, DOI: 10.1111/mec.13902

Despite its name, the canine distemper virus can also infect lions and hyenas.

OUR INBUILT SPEEDOMETER

In order to navigate correctly through our environment, we not only need to know where we are going, but also how fast we are going. How does the brain achieve this? Researchers at the German Center for Neurodegenerative Diseases (DZNE) have now tapped into the secrets of this inbuilt speedometer, identifying a signal pathway in mice that delivers this information to the brain's own navigation system. During the study, specific areas in the brains of mice were stimulated while researchers recorded the brain activity evoked. By doing so, the researchers determined a neural pathway, uncovering specific cells in the medial septum that increase their firing rate as the mouse moves faster. The neurons are connected to other areas of the brain, including the brain's navigation centre, the entorhinal cortex. For humans, who share similar neural pathways, the research is key to better understanding Alzheimer's disease, which frequently impairs spatial orientation due to degeneration of these pathways in the early onset of the disease.



REFERENCE

Justus D, Dalügge D, Bothe S, Fuhrmann F, Hannes C, Kaneko H *et al* (2017) Glutamatergic synaptic integration of locomotion speed via septoentorhinal projections. *Nat Neurosci,* DOI: http://dx.doi.org/10.1038/nn.4447

Colored transmission electron micrograph showing *Treponema pallidum* (green) in penile skin (brown).

THE GREAT IMITATOR STRIKES BACK

Known as the "great imitator" (as it can cause symptoms similar to other diseases), syphilis has plagued humans for centuries. Though infection rates started to decrease dramatically following the availability of penicillin treatments, the disease has recently been re-emerging around the world, with more than 10 million cases now reported annually. Unfortunately, little is known about the patterns of genetic diversity in current infections or the evolutionary origins of the bacterium causing syphilis, *Treponema pallidium*. Because clinical samples only contain low quantities of bacterial DNA and the pathogen is difficult to culture in the laboratory, researchers from the University of Zurich decided to apply DNA capture and whole-genome sequencing techniques to ancient DNA samples. Their genomic analyses show that all syphilis strains from modern patient samples share a common ancestor from the 1700s. They also show the emergence of a pandemic cluster named SS14-Ω, which is present in contemporary infections around the globe. This cluster developed in the mid-20th century – after the discovery of antibiotics. It is highly resistant to azithromycin, a second-line drug that is widely used to treat sexually transmitted infections. There are, however, no known resistances to penicillin, the first-choice treatment.

REFERENCE

Arora N, Schuenemann VJ, Jäger G, Peltzer A, Seitz A, Herbig A *et al* (2016) Origin of modern syphilis and emergence of a pandemic *Treponema pallidum* cluster. *Nat Microbiol*, DOI: 10.1038/nmicrobiol.2016.245

Photos: www.gartenpraxis.de (top left); Kirsten Achenbach (top middle); shutterstock (top right); Biomedical Imaging Unit/Science Source (bottom right)

Although we have come a long way in copying nature, animal research is still crucial for many endeavours, such as understanding how the brain works.

THE **SUPERMODELS** OF BIOLOGICAL RESEARCH

By Mitch Leslie

Much of the knowledge that fills biology textbooks has emerged from work on only a few creatures. The nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, baker's yeast, and a handful of other species are the model organisms that scientists have studied intensively to try to determine how living things work.

Baker's yeast, *Saccharomyces cerevisiae*, doesn't develop cancer. Yet labs around the world that investigate cancer mechanisms have chosen to work on yeast. The decision is sensible, says Phil Hieter, a professor of medical genetics at the University of British Columbia in Vancouver, Canada, who runs one of those labs. The underlying genetic and molecular similarities between yeast and humans make it a good stand-in for tumour cells. "Just about anything having to do with the workings of a cell can be modelled in yeast," he says.

Hieter's lab has exploited the overlap between humans and fungi to pinpoint genes that enable a mitotic cell to parcel its chromosomes into different daughter cells. Failure of this mechanism can precipitate chromosome instability, which leads to cells losing or gaining whole chromosomes or portions thereof, one of the key characteristics of cancer. "It's an early event that leads to cells that accumulate mutations," says Hieter.

His decision to focus on yeast has paid off. After screening mutant yeast for almost a decade, he and his colleagues have compiled a list of more than 900 genes that promote chromosome instability when they are mutated or overexpressed. At least some of them also appear to play a role in cancer. In 2013, for instance, Hieter's team sequenced 21 of the genes in endometrial tumours and found three that were mutated in some patients and therefore might serve as cancer drivers. Although some of the genes he and his co-workers have identified in yeast don't seem to be involved in cancer, he says that "it's more amazing how often they are directly relevant."

The justified hope that findings will be relevant beyond a single species is why Hieter and other researchers focus on organisms like yeast in the first place. The idea that studying certain organisms can reveal general principles about life dates back at least as far as the ancient Greeks – although the term "model organism" didn't become common until the 1980s. These go-to organisms are now indispensable for biological research, as Sydney Brenner of the Salk Institute in La Jolla, California, acknowledged in his 2002 Nobel Prize lecture. Brenner, who shared the Nobel Prize in Physiology or Medicine with two other researchers, noted that "without doubt the fourth winner of the Nobel Prize this year is *Caenorhabditis elegans*; it deserves all of the honour but, of course, it will not be able to share the monetary award." The same could be said of the more than 20 different model organisms, which, according to the Foundation for Biomedical Research and the Nobel Prize itself, were involved in all but four of the Nobel Prizes for Physiology or Medicine of the last 50 years. Quite a performance, as the Nobel Prize honours "the most important discoveries →

within the domain of physiology of medicine" with "the greatest benefit to mankind".

Today scientists can choose among several dozen model organisms, depending on what questions they want to ask. The slithering slime mold *Dictyostelium discoidum*, for instance, has been a staple for researchers interested in differentiation, cell movement, and signal transduction. Scientists investigating fertilization and early stages of development have often turned to the purple sea urchin, *Strongylocentrotus purpuratus*.

Even in the select group of model organisms, a few species stand out because they garner most of the attention from researchers. Besides baker's yeast, *C. elegans*, and *Drosophila*, the eukaryotic supermodels include the house mouse *Mus musculus*, the zebrafish *Danio rerio*, the mouse-ear cress *Arabidopsis thaliana*, and the African clawed frogs in the genus *Xenopus*. Among prokaryotes and viruses, *Escherichia coli*, phage lambda, and SV40 are stalwarts.

Why labs around the world teem with roundworms but not earthworms, and zebrafish but not zebra spiders, comes down to a few factors. Apart from being suited to the question and allowing transfer of the results, practicality is one of the main criteria for a good model organism. The most used species are easy to raise and keep under lab conditions, able to reproduce rapidly, and comparably cheap to house and feed. "The more exotic an animal is, the harder it is to develop as a model," says Robert Grainger, a professor of biology at the University of Virginia School of Medicine in Charlottesville.

Nematodes, for example, don't require elaborate living quarters or fancy food. The worms prosper in "small agar-filled petri dishes at room temperature," says Ann Corsi, an associate professor of biology at the Catholic University of America in Washington, D.C., who uses nematodes for her research on how the mesoderm, the middle layer in the embryo, develops into distinctive cell types. "We can grow hundreds of them on the disc-shaped surface of the dish that is 60 mm in diameter," she says. For food, the worms only require a sprinkling of yeast. And unlike mice, nematodes can survive being frozen and thawed, making it easier to store them for long periods of time.

Other species entered the stable of model organisms because their characteristics made them advantageous for particular fields. Large *Xenopus* eggs, which the female lays rather than gestating inside her body, are easier to observe and experiment with than the eggs of a mouse, notes Grainger. They have been invaluable for developmental biologists studying questions such as how the eye forms. Other amphibian species also produce large, external eggs, Grainger says, but a quirk of *Xenopus*' reproductive biology gave it an advantage as a model organism. Unlike these other amphibians, *Xenopus* females can lay eggs throughout the year if they receive the right stimulation. This ability came to light in the 1920s when a South African endocrinologist injected some of the frogs with urine from pregnant women. It contains the hormone chorionic gonadotropin, which stimulates egg-laying by the frogs. The experiment led to the first pregnancy test, but it also showed researchers how to produce large numbers of *Xenopus* eggs on their own schedule (although researchers now buy chorionic gonadotropin from a supplier rather than collecting urine from pregnant women).

Technological changes also influence which organisms scientists choose as models. If you walked into a lab that was studying rodents in the 1950s or 1960s, you probably would have found cages with rats, not mice. Yet today, researchers publish more than three times as many papers on mice as on rats. One innovation explains this shift. In the 1980s, scientists developed techniques to produce transgenic mice, either by knocking out or adding genes, making it possible to study everything from cancer to memory. However, these techniques failed in rats. Researchers didn't produce transgenic rats until more than 20 years later.

Chance also plays a role in which species become model organisms, says Jill Keeney, professor of biology at Juniata College in Huntingdon, Pennsylvania. Take zebrafish. Until the late 1960s, these 4cm-long fish, native to India and nearby countries, were more interesting to the aquarium enthusiast than to the geneticist. George Streisinger of the University of Oregon in Eugene was both. Like many researchers at the time, he wanted to move beyond the bacteriophages he had been studying for more than a decade and tackle more complex genetic questions, such as how genes shape the development of the nervous system. Because of his interest in tropical fish, Streisinger was already familiar with *D. rerio*, and he realized its potential. For one thing, the fish's embryos are transparent, allowing researchers to easily observe developmental changes resulting from mutations. Streisinger was hooked, and in the 1970s his lab pioneered the use of these fish as models.

When Streisinger decided to adopt zebrafish as a model organism, he didn't have to apply for permission. No agency or organization decides if a species does or doesn't qualify. Instead, "it's the [research] community that makes a model organism," says Keeney. A few scientists may start studying a species, and if it shows potential, it will start to attract more interest. Use of the organism can then snowball as the community expands and researchers develop new techniques for investigating its biology. The most successful research communities – such as the groups that elevated the formerly obscure organisms *D. melanogaster* and *C. elegans* into laboratory stars – share some similarities, says Alejandro Sanchez Alvarado of the Stowers Institute for Medical Research in Kansas City, Missouri. They single-mindedly focused on going after the biggest question that the organism could help them answer.

A prime example, says Sanchez Alvarado, is the work of Thomas Hunt Morgan of Columbia University in New York City

and his students, who in the early 1900s cemented *Drosophila's* role as a genetic model. They tackled one of the most compelling mysteries of the day – how traits are inherited – and by carefully crossing flies demonstrated that genes are located on chromosomes. These pioneering researchers made another smart choice, he says. They did not try to make the fly a model for studying development, which would have been much more difficult to untangle at the time. Not until the late 1980s were researchers ready to tackle the question of how genes shape development, and Christiane Nüsslein-Volhard shared the 1995 Nobel Prize in Physiology or Medicine with two other researchers for helping decipher the process in fruit flies.

Focusing on a few species established in research has produced plenty of important answers and has been crucial for our progress in medicine. Some researchers argue, however, that the stable of model organisms is too small and furnishes a limited view of how living things function. Of the 9 million species of organisms on Earth, only a handful account for most scientific research, Sanchez Alvarado says. "The statistical chances of every aspect of biology being represented in that percentage is very small." This group of research favorites isn't just small, it's arbitrary, he says. Scientists chose the species mainly for convenience, "not because they occupy an interesting position in the tree of life."

Introducing a new model organism can be difficult, as Sanchez Alvarado's experience illustrates. At the beginning of his career in the mid-1990s, he decided he wanted to study the molecular mechanisms of regeneration, and he quickly concluded that the existing model organisms weren't going to be helpful. "*Drosophila* can't grow back a wing, and if you cut a *C. elegans* in half, it's toast," he says. Planarians or flatworms have impressive regeneration abilities – a small piece can regrow into an entire animal – but he had to decide which of the 8,000 species would be the best candidate. On paper, a species from Europe and North Africa, *Schmidtea mediterranea*, looked promising. Like most other animals, it is diploid and has an embryo with three germ layers. These similarities would increase the odds that any discoveries he made would apply to other species.

Convinced they had the right animal, Sanchez Alvarado and his postdoc obtained a batch of the worms from another scientist. The planarians promptly died. If they had been working on a conventional model organism, they would have ordered replacements from a biological supply company, Sanchez Alvarado says. Instead, tracking down more of the planarians turned into an adventure. The location where the researchers eventually found the worms in the wild was a fountain in a park in Barcelona, Spain. "At the time it was a park heavily used by drug dealers," Sanchez Alvarado recalls. "We had to get out of there before dusk or we'd be in trouble." With chunks of liver as bait, he and his postdoc managed to capture some of the worms, which survived the trip back to the United States and became the basis for his studies. Now, he says, more than 150 other scientists are also working on *S. mediterranea*.

A new development could revolutionize the role of model organisms. The CRISPR/Cas9 system for gene editing can potentially target any gene in any creature, potentially allowing the use of model organisms for a broader range of questions and expanding the list of organisms that can serve as models. For example, some of the zebrafish's popularity for developmental studies has come at the expense of *Xenopus*, which is harder to study genetically. Grainger's lab has, however, made a variety of specific alterations in *Xenopus* genes with CRISPR/Cas9, so the technique might allow the frogs to regain some of their lost popularity, he says.

Even with CRISPR/Cas9, the traditional model organisms still have an important place in research, scientists say. Hieter notes that some cancer researchers have argued that CRISPR/Cas9 might allow labs to jettison yeast in favour of human cell lines. But he also notes that cultured cells can't match yeast for convenience, ease of genetic manipulation, and reproducibility of results. The findings obtained with cell lines "are not as clear and rigorous as with a model organism," he says.

The groups that elevated the formerly obscure organisms *D. melanogaster* and *C. elegans* into laboratory stars, single-mindedly focused on going after the biggest question that the organism could help them answer.

Zebrafish embryo receiving an injection. Zebrafish are popular laboratory animals, e.g. to study development, because their embryos are transparent.

LET'S TALK ABOUT ANIMAL RESEARCH – INTERVIEW WITH KIRK LEECH

Questions by: Kirsten Achenbach

Is research with animals really still necessary? Yes, says Kirk Leech, executive director of the European Animal Research Association (EARA) in an interview with FUTURA. Although there are alternatives at hand, animals are crucial for answering many questions in medical science.

What do you say when you are asked why animal testing is necessary?
Medical research has saved and improved the lives of millions of people. Animals have benefited too. Today's medicines and surgical techniques could not have been discovered without better understanding disease and the way the body works – the result of basic research programmes across the world. Pharmaceutical companies take these insights forward to develop new medicines which doctors and vets use to treat their patients. Most of the medicines we have come from animal research. Often science doesn't need to use animals and has developed a wide range of experimental techniques which are used in preference to animals, but for many key questions in medical science animals are crucial. These studies offer hope to millions who suffer from serious conditions such as cystic fibrosis, Alzheimer's disease, stroke, spinal cord damage, and infections like malaria which are rife in tropical regions.

Kirk Leech is the executive director of EARA. Previously, he worked in government affairs for the Association of the British Pharmaceutical Industry and for the British advocacy group Understanding Animal Research.

Which recent example highlights the necessity for and success of animal testing and research best?
The Ebola outbreak in West Africa. It was the worst the world has ever seen with almost 29,000 infections and more than 11,000 deaths, with Guinea, Sierra Leone, and Liberia among the worst hit. With a mortality rate between 50 % and 90 %, the virus spread through the human population with devastating consequences. The experimental vaccine which was rushed to Liberia would not have been possible without animal research. In a 2016 trial, it was found to be 100 % effective and offers fresh hope to control the disease and prevent further outbreaks. The vaccines were first tested on non-human primates, who developed both antibody and T cell responses that protected them against disease when they were later challenged with the Ebola virus. Coupled with eduction programmes and the build-up of medical infra- →

THE EUROPEAN ANIMAL RESEARCH ASSOCIATION

The European Animal Research Association, EARA (www.eara.eu), was established in 2014, in part because of a falling acceptance of animal research in Europe, as evinced for example by successful campaigns to stop transport companies from shipping laboratory animals. At the same time, the European research community was reluctant to engage with the media and public about the benefits of animal research, partly due to the legacy of animal rights extremism. EARA is funded by around 60 public and private research organizations in Europe. It aims to help create an accepting climate for animal research. It does so, for instance, by encouraging European researchers to openly talk about their research, supporting local advocacy groups, engaging politicians, and informing the public. As the only pan-European organization, EARA works closely with national organizations in Germany, UK, Holland, France, and Italy, the majority of which are represented on EARA's board. EARA has, for example, helped to create the Italian Research4Life advocay group (http://www.research-4life.it/), to co-ordinate the first Belgian pro-research statement for "World Day for Lab Animals" in April 2016, and to launch a paper with a set of standards in which more than 100 Spanish research institutes commit to talking openly about their animal research in December 2016. Recently, Germany's major research institutions have also launched the national initiative "Tierversuche verstehen – Understanding Animal Research" to foster acceptance and understanding.

Photos: private (top); shutterstock (left)

structure, vaccines and an effective treatment are hoped to halt the rapid spread of the disease in West Africa. However, on the road to finding safe vaccines and treatments for Ebola or other diseases, there are currently no alternatives to animal research.

How many animals are used in research worldwide?
Trying to estimate that number is difficult because many countries do not provide comprehensive statistics. However, we know that the major centres for research are the USA (about 11–25 million animals), the EU including the UK (about 12 million animals), and Canada (about 3 million animals). Unfortunately, Australia, China, and Japan are the only countries heavily involved in research that do no report their research statistics.

How does public opinion on animal testing influence research?
That is a difficult question to answer. But if politicians and other decision makers only ever hear and see negative stories about animal research and the research and benefits are not shared, then we can't be surprised if politicians decide, in the absence of any other information, to limit animal research.

What can institutions or individual scientists do to sway public opinion?
I think openness and transparency is essential to both improve public understanding and acceptability of animal research, and deal with the all too common myths that are circulated everywhere. We think institutions need to be clear about when, how, and why they use animals in research. They need to enhance communications with the media and the public about research using animals. Finally, they need be proactive in providing opportunities for the public to find out about research using animals.

In your opinion, is it possible to convince extremists?
No. Our audience is the public, who have many genuine questions and concerns about the use of animals in research.

How does EARA support scientists and institutions?
We see our role as helping to establish local animal research advocacy groups and networks in countries where they don't exist. In too many European countries the public only hear negative arguments about animal research. We think they deserve a balanced picture. EARA helps institutions and researchers make the case for the continued use of animals in science with the public. We also help the research community engage with national and EU decision makers on issues surrounding the regulation of research.

What are EARAs recommendations for scientists working with animals?
As the recent undercover investigation at the Vrije Universiteit Brussel in Belgium showed, animal research is still viewed by the public as secretive. Research institutions have a responsibility toward the public to be open about the animal research they carry out and to highlight the role research using animals plays in biomedical research.

Mr. Leech, thank you very much for the interview.

ANIMAL RESEARCH IN THE EU

One of the myths surrounding animal research is that it is cheaper and easier than alternative methods. However, animals need to be housed, fed, cared for, and as living beings, are less predictable than a cell in a petri dish. Also, there is a lot more paperwork involved in meeting the legal requirements for animal research. In the EU, each and every experiment involving vertebrates and cephalopods must either be declared to or authorized by the appropriate authorities. For experiments required by law, such as toxicity tests or efficiacy of new medicines, a declaration is sufficient. In Germany, this accounts for about a quarter of all experiments. Before scientists can start any research involving such animals, they have to file an application. In it, they need to describe the experiments in detail, set forth what they intend to learn and why it is important, that it can only be learned by this method, that there is no other or less burdensome method, that they have the respective training, and that their institute has the manpower and facilities to care appropriately for the animals. A single application can encompass 50 to 60 pages and take several months. This is why, wherever possible, researchers prefer to use alternative methods.

Mice are the most widely used vertebrates in reasearch – the organization of their DNA and their gene expression is similar to humans.

Further information:
www.eara.eu, www.understandinganimalresearch.org.uk
www.tierversuche-verstehen.de

Photo: iStock

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 an *.

REGULATION OF HOST–PATHOGEN INTERACTIONS BY THE UBIQUITIN SYSTEM

cf. BIF FUTURA, VOL. 26 | 3.2011

EVGENIJ FISKIN
Discipline: Biochemist, Diploma
Institute: Institute of Biochemistry, Goethe University Frankfurt, Frankfurt, Germany
Supervisor: Prof. Ivan Dikic

Covalent attachment of ubiquitin (Ub) is one of the most prevalent post-translational modifications of eukaryotic proteins. The last step of protein ubiquitination, in which one or more Ub molecules are added to create a distinct chain, is catalysed by E3 ligases. Whereas eukaryotes use E3 ligases to stimulate immune defence programs, many prokaryotic pathogens use them to counteract Ub-dependent immunity. Bacteria, which lack the Ub system, have evolved virulence-promoting E3 ligases that exploit the host's ubiquitination machinery to modify specific targets. Using the enteritis-causing pathogen *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), the goal of my PhD thesis was to characterize E3 ligase-driven events in the host and pathogen during infection. First, I developed a method to isolate and quantitatively analyse ubiquitinated proteins from infected human epithelial cells. I showed that infected cells detect the presence of *Salmonella* virulence factor SopE by activating the linear ubiquitin chain assembly complex (LUBAC). I found that LUBAC-mediated modification of proteins with linear Ub chains is required to mount a pro-inflammatory response to *Salmonella* invasion. Using an antibody against linear Ub chains, I immunopurified known and previously uncharacterized LUBAC substrates involved in *Salmonella*-driven inflammation. Next, I examined targets of the inflammation-promoting *S.* Typhimurium E3 ligase SopA. By comparing ubiquitination events in cells infected with wild-type or SopA-deficient *Salmonella*, I discovered two host substrates for SopA-driven immune modulation. Given the challenges posed by the emergence of multi-resistant pathogens, my data suggest that manipulating bacterial infection-driven ubiquitination might represent a viable therapeutic alternative.

PUBLICATIONS

Fiskin E*, Bionda T*, Dikic I, Behrends C (2016) Global analysis of host and bacterial ubiquitinome in response to *Salmonella typhimurium* infection. *Mol Cell* **62**: 967–981

van Wijk SJ, Fiskin E, Dikic I (2013) Selective monitoring of ubiquitin signals with genetically encoded ubiquitin chain-specific sensors. *Nat Protoc* **8**: 1449–1458

van Wijk SJ, Fiskin E, Putyrski M, Pampaloni F, Hou J, Wild P *et al* (2012) Fluorescence-based sensors to monitor localization and functions of linear and K63-linked ubiquitin chains in cells. *Mol Cell* **47**: 797–809

THE ROLE OF CULLIN-3-BASED UBIQUITIN LIGASES IN ENDOCYTIC TRAFFICKING

cf. BIF FUTURA, VOL. 26 | 2.2011

MICHAELA GSCHWEITL
Discipline: Molecular Biologist, Diploma
Institute: Institute of Biochemistry, ETH Zurich, Zurich, Switzerland
Supervisor: Prof. Matthias Peter

Ubiquitination is an essential post-translational modification that has a role in a plethora of intracellular pathways. The largest family of ubiquitin ligases functions by recruiting the ubiquitination machinery to a Cullin protein backbone to form modular Cullin-based E3 RING ligase (CRL) complexes. My PhD project focused on Cullin-3 (CUL3) and CRL3 complexes, which are responsible for the ubiquitination of an ever-growing number of substrates. The depletion of CUL3 in human cells disrupts influenza A virus (IAV) trafficking and also the endocytic trafficking of endogenous cargo such as epidermal growth factor (EGF), reflecting a global perturbation of the endocytic system and endosome maturation. However, how the complex has such a widespread effect is not known. The first part of my project involved searching for substrate-specific CUL3 adaptor proteins that account for these functions. Through a series of siRNA screens, I identified the Speckle-type POZ protein-like (SPOPL) protein as a CRL3 adaptor that directly influences endocytic trafficking. Furthermore, I showed that EPS15 is a ubiquitination substrate of CUL3–SPOPL *in vivo* and *in vitro*. EPS15 associates with endosomal sorting complexes required for transport (ESCRT)-0 and is involved in the formation of intraluminal vesicles (ILVs), which are essential for endosome maturation. I demonstrated that EPS15 is highly stable upon SPOPL depletion in cells and that it is ubiquitinated in a SPOPL-dependent manner via a *bona fide* SPOPL-binding motif, which results in its proteasomal degradation. Overall, my data establish that CRL3 complexes directly regulate ILV formation at endosomes and that these processes are essential for faithful endogenous cargo trafficking and degradation, and for IAV entry into host cells. This will ultimately help us to understand the role of post-translational modifications such as ubiquitination in cargo sorting at endosomes and other membrane systems within a cell.

PUBLICATIONS

Gschweitl M, Ulbricht A, Barnes CA, Enchev RI, Stoffel-Studer I, Meyer-Schaller N *et al* (2016) A SPOPL/Cullin-3 ubiquitin ligase complex regulates endocytic trafficking by targeting EPS15 at endosomes. *eLife* **5**: 13841

Huotari J, Meyer-Schaller N, Hubner M, Stauffer S, Katheder N, Horvath P *et al* (2012) Cullin-3 regulates late endosome maturation. *Proc Natl Acad Sci USA* **109**: 823–828

MITOPHAGIC SIGNALLING PATHWAYS IN MITOCHONDRIA

cf. BIF FUTURA, VOL. 27 | 3.2012

MARIIA LEVCHENKO
Discipline: Biochemist, BSc
Institute: Institute for Cellular Biochemistry, Göttingen, Germany
Supervisor: Prof. Peter Rehling

Mitophagy is a cellular quality control pathway that removes defective mitochondria. Receptors on the mitochondrial outer membrane label damaged organelles and target them for degradation within the lysosomal lumen. In the yeast *Saccharomyces cerevisiae*, this receptor function is attributed to autophagy-related protein 32 (Atg32), but the signalling pathways that are activated by mitochondrial malfunction are not yet fully defined. Therefore, the aim of my PhD project was to isolate Atg32-associated complexes from *S. cerevisiae* mitochondria and determine their composition. Atg32 is rapidly degraded upon cell lysis, so I used yeast mutants deficient in various degradation pathways to stabilize the protein. Interestingly, in the absence of lysosomal protease Pep4, mitophagy induction resulted in the accumulation of a modified Atg32, whereas the unmodified Atg32 was degraded. The nature of this modification is yet to be determined. To assess the signalling complexes that form during mitophagy, we devised an extraction procedure from yeast cell powder and isolated Atg32 together with its binding partners. We observed a 230 kDa complex that dissociated upon mitophagy. This could suggest that Atg32 is inhibited by its binding partners under physiological conditions to prevent excessive mitochondrial degradation. Mitophagy induction would then lead to the dissociation and de-repression of Atg32, allowing it to interact with downstream autophagy components. Further analysis of the composition and dynamics of these various complexes under different conditions is a focus of my ongoing work. In summary, my PhD studies suggest a model of the yeast mitophagic process in which under physiological conditions, Atg32 is sequestered into a mitochondrial complex to prevent mitophagy initiation. Upon a mitophagic stimulus, Atg32 is released from this inhibitory complex, modified by an unknown mechanism, and then delivered to the lysosome together with its mitochondrial cargo. Meanwhile, organelles that are spared from degradation remove Atg32 from their surface. The results of this study have therefore uncovered novel mechanisms of the mitophagy pathway and provided directions for future research.

PUBLICATIONS

The results of this project have not yet been published.

IMAGING AND RECONSTRUCTING THE BEATING HEART AND CARDIAC MORPHOGENESIS

cf. BIF FUTURA, VOL. 27 | 1.2012

MICHAELA MICKOLEIT
Discipline: Biologist, MSc
Institute: Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Dresden, Germany
Supervisor: Dr Jan Huisken

Life-threatening congenital heart disease affects 1 in 100 newborns and a thorough understanding of cardiac morphogenesis is fundamental to treating this condition. Currently, our knowledge is limited by a lack of high-resolution images of the heart, which are difficult to capture *in vivo* because of the continuous and rapid cardiac motion. The aim of my PhD thesis was to overcome this limitation by combining physiological, high-speed microscopy with dedicated synchronization algorithms. I first captured three-dimensional (3D) cardiac dynamics in zebrafish embryos with selective plane illumination microscopy (SPIM). I reconstructed the 4D structure of the beating heart with a customized JavaScript, which detects a specific cardiac phase using image correlation (retrospective gating). In a complementary approach, I used optogenetics to briefly stop the heart and obtain static high-resolution reconstructions. I also performed ultra-high-speed volume scanning with a liquid lens to resolve non-periodic phenomena such as blood circulation and arrhythmic cardiac activity. By extending my cardiac imaging technology to long-term experiments and developing innovative sample mounting techniques, I reconstructed the first time-lapse images of cardiac development in a living vertebrate embryo, which allowed me to follow morphogenetic changes *in vivo*. I quantified the cell rearrangements during cardiac looping and found that this process occurs in two characteristic steps: tube elongation and chamber formation. Throughout these steps, the heart poles undergo substantial rearrangement with convergent extension at the inflow and cell addition to the outflow region, while the myocardial body remains structurally unchanged. I also established that tube elongation depends on cardiac activity, whereas chamber formation is partially intrinsic. The insights gained from my project tremendously broaden our understanding of cardiac looping in zebrafish and will form the basis for the quantitative characterization of models to help combat congenital heart disease.

PUBLICATIONS

Kaufmann A*, Mickoleit M*, Weber M*, Huisken J (2012) Multilayer mounting enables long-term imaging of zebrafish development in a light sheet microscope. *Development* **139**: 3242–3247

Mickoleit M, Schmid B, Weber M, Fahrbach FO, Hombach S, Rieschauer S *et al* (2014) High-resolution reconstructions of the beating zebrafish heart. *Nat Methods* **11**: 919–922

ESCRT-III POLYMER DYNAMICS IN CYTOKINETIC ABSCISSION

cf. BIF FUTURA, VOL. 28 | 2.2013

BEATA MIERZWA
Discipline: Molecular Biologist, MSc
Institute: Institute of Molecular Biotechnology (IMBA), Vienna, Austria
Supervisor: Dr Daniel Gerlich

Cytokinesis is the process by which dividing cells are separated after chromosome segregation. First, a cleavage furrow ingresses the plasma membrane to partition the cytoplasm between the two spindle poles, which gives rise to an intercellular bridge between the emerging daughter cells. During subsequent abscission, machinery involving the endosomal sorting complex required for transport (ESCRT)-III splits the plasma membrane to physically separate the cells. ESCRT-III assembles into polymers that mediate membrane deformation and fission not only during cytokinesis but also in various other cellular processes, including nuclear envelope sealing, multivesicular body formation, and virus budding. Prevailing models of ESCRT-III function postulate that persistent filaments change their curvature to deform membrane tubes, followed by binding of the ATPase vacuolar protein sorting 4 (VPS4) to initiate filament disassembly. However, whether ESCRT-III polymers exchange their subunits with soluble cytoplasmic pools – similar to other force-generating filament systems such as actin and tubulin – is not known. Using live cell microscopy and photobleaching assays in mammalian cells, I found a rapid and continuous turnover in ESCRT-III polymers, about two orders of magnitude faster than the net rate of polymer growth. Unexpectedly, VPS4 also accumulated simultaneously with growing ESCRT-III polymers. RNAi depletion experiments revealed that VPS4 is essential for ESCRT-III dynamics and membrane constriction during abscission. Furthermore, the rate of ESCRT-III accumulation at the intercellular bridge was substantially reduced in VPS4-depleted cells. Through collaborations with Aurélien Roux's and Simon Scheuring's laboratories, we confirmed by *in vitro* reconstitution using yeast proteins that Vps4 mediates ESCRT-III turnover by inducing dynamic filament rearrangement, and revealed that Vps4 promotes polymer growth by counteracting growth-inhibitory subunits. Taken together, our observations show that VPS4 sustains the growth of ESCRT-III polymers by continuous turnover. Such dynamic remodelling might facilitate polymer shape adaptation to variable geometries during membrane constriction, which has broad implications for a variety of cellular processes.

PUBLICATIONS

Mierzwa B, Gerlich DW (2014) Cytokinetic abscission: molecular mechanisms and temporal control. *Dev Cell* **31**: 525–538

STRATEGIES FOR ENGINEERING SENSORY PHOTORECEPTOR CHIMERAS

cf. BIF FUTURA, VOL. 27 | 3.2012

ROBERT OHLENDORF
Discipline: Biophysicist, MSc
Institute: Humboldt University of Berlin, Berlin, Germany
Supervisor: Prof. Andreas Möglich

Throughout nature, sensory photoreceptors mediate diverse responses to ambient light, from phototropism in plants to vision in humans. Optogenetics uses these light-switchable proteins to accurately control cellular processes with minimal invasiveness. Often distinct sensor and effector modules in the protein are coupled by helical linkers and enable light perception and biological output function, respectively. Rewiring different sensor and effector modules into photoreceptor chimeras allows light-mediated control of target cellular processes for therapy or analysis. However, a major challenge is fusing module linkers in a way that preserves signalling within the chimera. My PhD project tackled this issue by exploring ways to efficiently engineer photoreceptor chimeras. The scarce knowledge of signalling mechanisms between protein modules hampered my initial rational-design approach, which was guided by homology of the parent proteins. Therefore, I developed a brute-force method, termed PATCHY (primer-aided truncation for the creation of hybrid enzymes), which circumvents the problem by generating a complete library of all possible linker fusions and then uses high-throughput testing to isolate functional light-regulated chimeras. Screening libraries of a blue-light sensor coupled to a histidine-kinase effector in *Escherichia coli* yielded light-induced and light-repressed chimeras with periodic linker lengths of 7n and 7n+1 residues, respectively. Increments of seven residues maintain the relative angular orientation of sensor and effector and conserve the polarity of the chimera's response to light. In contrast, one additional residue changes the orientation and inverts the response, suggesting that sensor–effector signalling occurs via an angular reorientation. The small fraction of linker combinations that yielded light-regulated chimeras revealed a delicate fine-tuning of linker sequence and protein function. Thus, systematic testing of linker variants with PATCHY not only enables the development of novel photoreceptor chimeras for cellular manipulation, but also reveals the general rules determining module compatibility and signalling in modular signal receptors.

PUBLICATIONS

Ohlendorf R, Schumacher CH, Richter F, Möglich A (2016) Library-aided probing of linker determinants in hybrid photoreceptors. *ACS Synth Biol,* DOI: 10.1021/acssynbio.6b00028

VISUAL LOOMING-EVOKED ESCAPE BEHAVIOUR AND ITS NEURAL PATHWAY IN LARVAL ZEBRAFISH

cf. BIF FUTURA, VOL. 28 | 2.2013

INCINUR TEMIZER
Discipline: Biomedical Physicist, MSc
Institute: Max Planck Institute of Neurobiology, Munich, Germany
Supervisor: Prof. Herwig Baier

A key function of an animal's visual system is extracting ecologically relevant information from the environment in order to initiate appropriate behaviour. Avoiding the strike of an approaching predator requires rapid visual detection of its looming image, followed by a directed escape maneuver. Although looming-sensitive neurons have been discovered in various animal species, the relative importance of the different features that the visual system extracts from the looming image is still unclear. The neural mechanisms that compute an object's approach are also largely unknown. To investigate the neural basis of visually evoked escape, I first established a new behavioural paradigm in head-restrained zebrafish larvae. I explored the previously unknown escape behaviour of the larvae in response to looming stimuli and found that a virtual looming stimulus, i.e. a dark expanding disk on a bright background, reliably elicited rapid escape movements. I characterized spatio-temporal features of the stimulus that are critical for eliciting escape. Furthermore, I demonstrated that a key determinant of escape initiation is the apparent size of the looming stimulus depending on the animal's viewing angle. My next goal was to identify the visual areas in the zebrafish brain that receive the retinal input generated by the looming stimuli. Using two-photon microscopy calcium imaging experiments in the axon terminals of retinal ganglion cells (RGC) during stimulus presentation, I identified three visual areas that responded robustly to looming stimuli. In only one of these areas, the tectum, I further identified selective responses to behaviourally relevant looming stimuli that elicited escape. Finally, I showed that targeted laser ablation of RGC axons in the optic tectum impaired the escape behaviour, establishing the importance of an intact tectum for looming stimulus-evoked escape. My results describe a novel visually evoked escape behaviour in zebrafish larvae that provides a powerful model for studying sensorimotor integration. In addition, this study implies that zebrafish larvae use neurobehavioural computations that are similar to those used across the animal phyla to gauge the approach of a threatening stimulus.

PUBLICATIONS

Temizer I, Donovan JC, Baier H, Semmelhack JL (2015) A visual pathway for looming-evoked escape in larval zebrafish. *Curr Biol* **25**: 1823–1834

PLASTICITY AND INTRINSIC PROPERTIES OF DISTINCT DORSAL HORN INTERNEURON POPULATIONS

cf. BIF FUTURA, VOL. 27 | 3.2012

CAROLIN VON SCHOULTZ
Discipline: Neuroscientist, MSc
Institute: Institute of Pharmacology and Toxicology, University of Zurich (UZH), Zurich, Switzerland
Supervisor: Prof. Hanns Ulrich Zeilhofer

The transmission of pain signals to higher brain areas relies on a balance between excitatory and inhibitory neurotransmission in the dorsal horn region of the spinal cord. Transgenic reporter mice that fluorescently label inhibitory interneurons are routinely used to investigate the functional properties of distinct interneuron subpopulations. However, a similar tool to define the role of excitatory (glutamatergic) interneurons in processing painful signals – a process called nociception – was missing. In my PhD project, I performed an electrophysiological and morphological characterization of this reporter mouse, which expresses an enhanced green fluorescent protein (eGFP) under the vesicular glutamate transporter 2 (vGluT2) promoter. I used whole-cell patch-clamp recordings to show that eGFP-positive superficial dorsal horn neurons in these mice have characteristic properties of spinal excitatory interneurons. This mouse strain is thus a valuable tool for investigating glutamatergic neurons in spinal nociceptive circuits. To study how nociceptor synapses modify information flow, I analysed the endocannabinoid (eCB) system, which evokes short- and long-term depression (STD and LTD) of neurotransmission in many brain areas. Using the vGluT2::eGFP mice and a glutamate decarboxylase reporter mouse line, I showed that cannabinoid receptor 1-dependent STD and N-methyl-D-aspartate receptor-dependent LTD take place at nociceptor synapses with glutamatergic target neurons, and both forms of plasticity are evoked by release of the major eCB 2-arachidonoylglycerol. Although nociceptors with GABAergic targets also undergo STD and LTD, the underlying mechanism is unknown. My findings contribute to a better understanding of the heterogeneity and cell type-specific plasticity of dorsal horn interneurons. This knowledge will help us to understand the mechanisms underlying nociceptive processing and may pave the way towards the development of new and better analgesics.

PUBLICATIONS

Punnakkal P, von Schoultz C, Haenraets K, Wildner H, Zeilhofer HU (2014) Morphological, biophysical and synaptic properties of glutamatergic neurons of the mouse spinal dorsal horn. *J Physiol* **592:** 759–776

Kato A, Punnakkal P, Pernia-Andrade AJ, von Schoultz C, Sharopov S, Nyilas R *et al* (2012) Endocannabinoid-dependent plasticity at spinal nociceptor synapses. *J Physiol* **590:** 4717–4733

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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A WHALE OF A GOOD TIME – 2016 WOODS HOLE MEETING

By Kirsten Achenbach

Every two years, BIF invites current fellows and alumni working in North America to gather for a scientific meeting, including networking and whale watching. This year's seminar was the 11th in the series and the 10th to take place at the Marine Biological Laboratory (MBL) in Woods Hole.

A breaching humpback whale – an amazing sight we were lucky enough to see.

1 **The participants** in the 2016 meeting.

2 **Members of a botany course** collecting specimens on the shore in Woods Hole in 1895. Today, more than 120 years later, almost 600 students from more than 40 countries take part in the MBL's world-famous courses every year. The focus of these graduate-level courses ranges from physiology (the oldest) and embryology to neurobiology, microbiology, imaging, and computation integrated with biological research.

3 **The first alumni meeting** in North America took place in Ridgefield, CT, in 1993 to mark BIF's tenth anniversary. Just compare the styles.

4 **Thomas Hunt Morgan** was awarded the Nobel Prize in 1933 for his discoveries concerning the role of the chromosomes in heredity.

5 **A view of Eel Pond** with the Marine Biological Laboratory – the site of BIF's meeting – in the background.

Woods Hole has attracted great minds since its beginnings in 1888, and they have laid the ground work for much of today's cell, developmental, and reproductive biology. There was Edwin Grant Conkin, who first showed that certain cytoplasmic regions of the egg are programmed to form specific tissues or organs; Thomas Hunt Morgan, who launched the field of experimental genetics through his work with fruit flies and was a MBL trustee for 50 years; and, more recently, the discovery of kinesin, a motor protein involved in mitosis, by Ron Vale, Michael Sheetz, and others during their summer research at the MBL. Since 1929, there have been 56 Nobel Prize winners who were scientists, course faculty, or students at the MBL. In 2008, our seminar basked in some reflected glory when the winner of the Nobel Prize in Chemistry was announced during the seminar. It was awarded for the discovery and development of green flourescent protein. "We woke up and the whole street was filled with news vans wanting to interview Osamu Shimomura," recalls Claudia Walther, BIF's managing director. Who knows – maybe there was a future Nobel Prize winner at the 2016 meeting.

Of this year's 70 participants, 25 current PhD fellows had to present their projects. Further talks included keynote lectures by alumni on topics such as the regeneration of hearts and limbs, the expression of proteins and genes, cell-fate programming, microtubule cytoskeleton dynamics, as well as epidemiology. Three career talks took a look behind the scences of the Howard Hughes Medical Institute and examined careers in biotech and science management. The talks generated lively discussions and not only the current fellows profited from the scientific exchange with the alumni. Stormy weather meant that the traditional Sunday whale-watching trip had to be moved to Monday. Although it meant much reorganization behind the scenes, it proved well worth it – as weather and whales put on a great show so that the seminar ended on a relaxed and beautiful note.

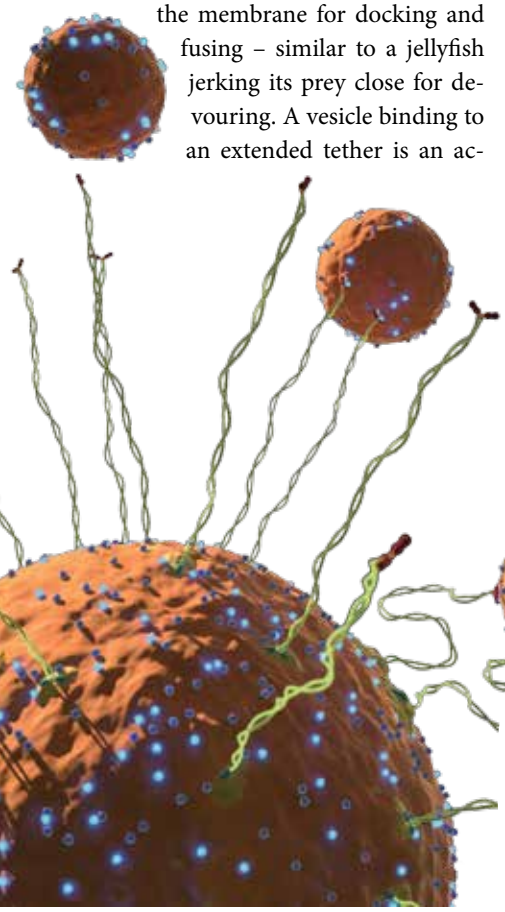
Photos: iStock (background); BIF (1+3); MBL Archives (2+4); Tom Kleindinst (5)

PAPERS IN THE SPOTLIGHT

In "Papers in the Spotlight" we present papers from current BIF fellows or recent alumni. The selection is based not only on scientific merit, but also on the general interest of the topic. If you would like to see your paper discussed here, send an e-mail to kirsten.achenbach@bifonds.de.

ORGANELLES MAKE TETHERS SOFT TO CAPTURE VESICLES

In open water, a jellyfish trails its nettles to capture food. In a similar way, cell organelles extend stiff tethers from their surface into cellular space to capture vesicles transporting cargo between different compartments of the cell. Thanks to Marcus Jahnel and his colleagues, we now know that when a vesicle binds to such a tether, the tether turns from stiff to flexible and collapses. It thus pulls the vesicle close to the membrane for docking and fusing – similar to a jellyfish jerking its prey close for devouring. A vesicle binding to an extended tether is an active process involving a GTPase called Rab5. It induces a sudden structural change in the 200 nm long tether – the dimeric coiled-coil protein EEA1 – making it more flexible. However, for the flexible form of EEA1, the extended state is energetically unfavourable. It therefore collapses, generating a force of three piconewtons, enough to pull the bound vesicle 100 nm closer to the membrane. Marcus, from Stephan Grill's lab at the Biotechnology Center of the Technical University Dresden, and his colleagues measured this using optical tweezer and EM experiments. Their results also suggest that the tethers can cycle between rigid-extended without Rab 5 and flexible-collapsed when bound to Rab5. "This mechanism could explain how long tether proteins generate enough force to pull a vesicle towards a membrane. As a means of molecular force generation, an entropic collapse could also be at work in many other biological processes involving such long coiled-coil proteins," summarizes Marcus.



Active Rab5 (shiny blue particles) induces a change in flexibility of EEA1 (green filaments).

REFERENCE

Murray DH*, Jahnel M*, Lauer J, Avellaneda MJ, Brouilly N, Cezanne A *et al* (2016) An endosomal tether undergoes an entropic collapse to bring vesicles together. *Nature* **537:** 107–111

Marcus Jahnel, fellow 2008–2010

Shared first authorship is denoted with an *.

VIRUSES USE YOUR GENES

Viruses can be nasty. They invade you, make you sick, might even kill you, and to add insult to injury, they hijack your own genes to do so. A paper in *Nature* by BIF fellow Andreas Puschnik and others shows which genes are relevant to viruses of the *Flaviviridae* family and how to find the ones essential only to this family. The *Flaviviridae* encompass more than 100 members, many of which cause devastating mosquito-borne diseases such as dengue, West Nile, or yellow fever. Only against one of them – yellow fever – do we have an effective vaccine. At the lab of Jan Carette at Stanford University's Department of Microbiology and Immunology, Andreas used CRISPR to create a library of liver cells with a different gene knocked out in each cell. He then infected the cells with different *Flaviviridae* viruses. The host cell was resistant if it lacked a gene the virus needed to replicate. He discovered that the closely related mosquito-borne viruses hijack very similar host

Photos: EEA1 project cover A.Mario Avellandeda (bottom left); Andy Crump, TDR, World Health Organization/ Science Source (centre)

TO MAKE YOU SICK

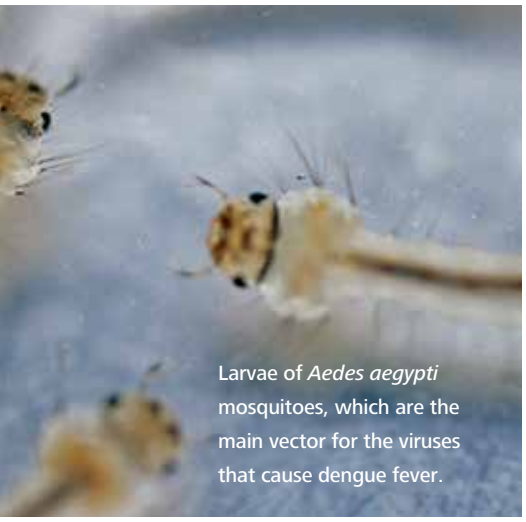
genes, but hepatitis C virus required a different set of genes. "Since we started out with cells that could thrive without the knocked-out gene, we could identify genes essential to the virus, but not the host. That means possible therapeutic approaches targeting these genes will prevent infection or disease, but hopefully do little to no harm to a patient's cells," Andreas says. "We also found a common set of genes essential for a group of *Flaviviridae* viruses encompassing Zika, yellow fever, and dengue. For these, there might even be a one-drug-fits-all solution."

Joshua Stokes, St. Jude Children's Research Hospital (right)

REFERENCE

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

Andreas Puschnik, fellow 2015–2016



Larvae of *Aedes aegypti* mosquitoes, which are the main vector for the viruses that cause dengue fever.

SPECIAL CHAPERONES SPOT DANGEROUS PROTEINS

Like the chaperones of yesteryear who prevented unwanted "couplings", a newly identified functional class of chaperones targets proteins which come with an especially high risk of causing protein clumping. A protein starts out as a linear strand of amino acids lined up like beads on a piece of string. Before it can do its job, it needs to fold into its 3D functional shape. Chaperones typically guide this process by binding to specific short amino acid sequences. Some also monitor protein quality, targeting a protein for degradation if it is folded wrong – for instance, by showing certain hydrophobic amino acid sequences on the outside that can cause clumping if not carefully tucked away inside. Julia Behnke from the lab of Linda Hendershot at the St. Jude Children's Research Hospital in Memphis, TN, analysed the target sequences of the Hsp70 chaperone family within the endoplasmic reticulum to find out how cells distinguish between harmless and potentially dangerous hydrophobic sequences. To do so, she created a novel *in vivo* reporter system to identify the sequences chaperones recognize. She found a hitherto unknown class of chaperones that specifically attaches to the rare, clumping sequences and marks them for destruction. Julia summarizes: "Our work shows for the first time how cells can sort out proteins showing specific clumping-promoting sequences on their outside."

Chaperones recognize amino acid sequences on the surface of proteins.

REFERENCE

Behnke J, Mann MJ, Scruggs F, Feige MJ, Hendershot LM (2016) Members of the Hsp70 family recognize distinct types of sequences to execute ER quality control. *Molecular Cell* **63:** 739–752

Julia Behnke, fellow 2011–2013

PROFILES

Professor Volker Bormuth,
Université Pierre et Marie Curie, Paris, France
Fellowship: 2005–2008

Professor Simon Elsässer,
Karolinska Institut, Stockholm, Sweden
Fellowship: 2008–2010

Benjamin Judkewitz,
Charité – University Hospital, Berlin, Germany
Fellowship: 2007–2008

Tim Nicolai Siegel,
University of Würzburg, Germany
Fellowship: 2005–2008

Marion Silies,
University Medical Center Göttingen, Germany
Fellowship: 2006–2008

Five BIF fellows have been awarded ERC Starting Grants for talented young research leaders, i.e. up to 1.5 million euros over five years each to set up their own labs: **Volker Bormuth** is working on multisensory signal processing and analysing brain-wide neuronal circuits to understand behaviour. **Tim Nicolai Siegel** is studying the role of three-dimensional genome architecture in antigenic variation. **Marion Silies'** project MicroCyFly aims to dissect the microcircuitry of the *Drosophila* visual system. **Benjamin Judkewitz** will use his ERC grant to develop noninvasive optical technologies to study deep brain tissues. In November, he was also the recipient of the one million euro Alfried Krupp-Förderpreis for young professors, one of the most renowned prizes for young academics in Germany. **Simon Elsässer** wants to resolve chromatin dynamics by rapid protein labelling and bioorthogonal capture. He also received two further grants for his work on polypeptides encoded by short open reading frames: a five-year Ragnar Söderberg fellowship in Medicine and the newly established Lau grant. The latter runs for three years and intends to support world-class research in regenerative medicine at the Karolinska Institutet and to strengthen research partnerships with top universities in Hong Kong and China.

Professor Michael Rape,
University of California, CA, Berkeley, USA
Fellowship: 2001–2002

In September 2016, Michael Rape was presented with the 2016 Blavatnik National Award for Young Scientists. Worth 250,000 USD, it is the largest unrestricted cash award given to early-career scientists. The Blavatnik Family Foundation states: "By deciphering the ubiquitin code, Dr. Rape's basic science work has opened the door to new and unique ways to manipulate ubiquitylation for next-generation therapies in oncology, immunology, and inflammation."

Lisa Traunmüller,
University of Basel, Switzerland
Fellowship: 2014–2016

The charitable foundation EMPIRIS has selected Lisa Traunmüller as the sole recipient of the 2016 Award for Research in Brain Diseases. This award comes with 10,000 Swiss francs and is awarded to young scientists for outstanding contributions to non-clinical basic research related to brain disorders such as Alzheimer's disease, Parkinson's disease, brain cancer, epilepsy, and depressive disorders.

Dr Florian Schmidt
University of Bonn, Germany
Fellowhip: 2008–2010

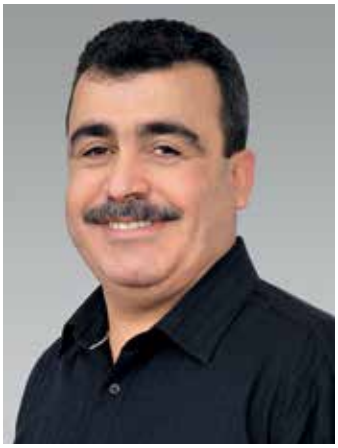
Florian Schmidt has been accepted in the Emmy Noether Programme of the DFG to start his own group at the Institute of Innate Immunity at the University of Bonn, Germany. He moved from Hidde Ploegh's lab at the Whitehead Institute to Bonn in February 2017. The focus of his new group will be the antiviral inflammatory response and mechanistic details of inflammasome activation, which he will study using alpaca antibodies, also called nanobodies. In January, he also received the Research Prize 2017 of the Peter and Traudl Engelhorn Foundation for "new findings in molecular biology of infection offering perspectives for therapy".

Suyang Zhang,
University of Cambridge, United Kingdom
Fellowship: 2014–2017

Suyang Zhang has been awarded the most prestigious student prize at the MRC Laboratory of Molecular Biology, the Max Perutz prize. She was commended for her "elegant analysis by biochemistry and electron microscopy of the mechanism controlling the key protein complex APC/C during cell cycles". The prize is given annually for outstanding work prior to the award of a PhD.

Photos: Bormuth: private; Elsässer: private; Judkewitz: PM Charite, Lisanne Schulze; Siegel: Universität Würzburg; Esässer: Karolinska Institutet; Silies: private; Professor Michael Rape: HHMI; Lisa Traunmüller: Universität Basel; Dr Florian Schmidt: private; Suyang Zhang: private

WHO'S WHO AT BIF?



SÜLEYMAN TANGÜLER

Süleyman Tangüler, born in Adana, Turkey, in 1961, became a master machinist before coming to Germany in 1991. He worked as a machinist in Gonsenheim, near Mainz, and continues to do so on a part-time basis. In 2001, he started at BIF working only two hours a day and gradually assumed responsibility for the care of the Schlossmühle and its garden as part of a full-time position. With the move to the gardenless offices in Mainz in 2012, his ride-on lawn mower had to go, but not Süleyman. Again part-time, he is still indispensable for the running of the BIF office. He takes care of our equipment, provides technical support for all seminars and conferences, and organizes the archive. He also assists in many secretarial and organizational matters, in particular during applications deadlines, for board and committee meetings, and is BIF's safety officer.

What do you like best about your work?
I like working, especially seeing results. Hence, I like preparing the seminars best.

What is your favourite pastime?
Spending time with my family and kids. Especially in summer, we often eat, play, and talk with a group of other families in our allotment garden along the River Rhine.

What part of Turkey would you like to visit?
Diyarbakir, in the east. It boasts one of the world's largest and best preserved ancient fortifications. They are five kilometers long, with 82 towers, and were erected in 349 by Emperor Constantinus II and later expanded by my namesake, Suleyman I. In 2015, they were declared a World Heritage Site.

What fault in others can you tolerate best?
Everybody is as he or she is, but that is easy for me to accept. My thoughts are – well, one can also be like this. Acting without thinking, however, is something I find difficult, as I myself like to plan first.

Which scientific field interests you most?
Since my father took me to tour an old castle when I was a child, I am fascinated by history and love to learn how people lived in the past. That is also why I want to see Diyarbakir.

For you, what is the biggest difference between Germany and Turkey?
I miss the more spontaneous way people interact in Turkey – in the afternoon, everybody puts on the kettle and offers small cakes to the neighbours – people walk from house to house and chat. In Germany, you have to make an appointment. Many want to be left alone on the weekends. However, now that I'm 55, I'm learning to appreciate a quiet Sunday myself.

One thing you could not live without?
Clearly my *aile*, my family, and my *arkadaş*, my friends.

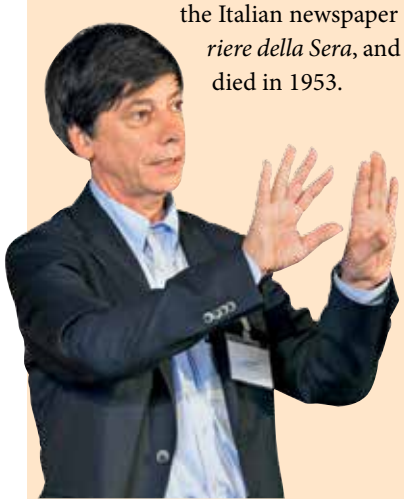
BALZAN PRIZE FOR REINHARD JAHN

Reinhard Jahn, member of BIF's Board of Trustees and director at the Max Plank Institute for Biophysical Chemistry in Göttingen, Germany, was honoured with the 2016 Balzan Prize. It is considered one of the most prestigious scientific awards worldwide and is endowed with 750,000 Swiss francs. Jahn is recognized for his pioneering studies on synaptic vesicles and exocytosis, essential for signal transmission in the nervous system.

In his acceptance speech Jahn said: "One should not forget that in my field, the research is carried out by teams composed mostly of young scientists ... who do the actual experiments. ... Personally, I consider it one of the most beautiful aspects of the Balzan Prize that part of the award will be used to support young scientists."

The Balzan Prize Foundation recognizes the outstanding achievements of people and organizations that foster culture and the sciences, as well as meritorious initiatives working for the cause of humanity, peace, and fraternity throughout the world. Awardees are nominated by internationally recognized cultural and scientific institutions and figures and selected by a committee of 20 eminent European scholars and scientists.

The foundation was established in 1956 by the daughter of Eugenio Balzan, who was a journalist and co-owner of the Italian newspaper *Corriere della Sera*, and who died in 1953.



Photos: Balzan Foundation, Carletti (bottom right); BIF

A BIF FELLOW'S GUIDE TO ...

STANFORD

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 Andreas Puschnik. He reports from Stanford, USA – not a true city in its own rights, but home to a world-famous university.

FACTS & FIGURES

Country: USA
Population: About 35,000
Area: 7 km²
Students: About 15,000
Famous for: Palm trees and tech startups
Website: www.stanford.edu

WHERE TO STAY

Stanford Terrace Inn: Laid-back option with a heated saltwater pool, plus free organic breakfast and shuttle service.
Stanford Park Hotel: Antique-filled option with a pool and gym, offering wine socials and iPads for guest use.
The Cardinal Hotel: 1924 building offering cozy, minimalist lodging, and an on-site cafe.

NIGHTLIFE

Nola's: New Orleans-themed spot offering Creole fare and cocktails in a space reminiscent of the French Quarter.
The Nuthouse: Divey local bar known for its affordable pitchers, free peanuts, pool, and table football.

RESTAURANTS

Oren's Hummus Shop: Mediterranean food in the heart of Palo Alto.
Fambrini's: Great sandwich shop hidden on the second floor of an office building.
Pizzeria Delfina: Artisanal, "hipster" pizza à la California.

ACTIVITIES

Stanford football: Visit a college football game during the fall season. 1
Bing concert hall: Enjoy world-class classical and contemporary music at the Bing.
Walk the dish: Ideal for a relaxed walk or run. On the peak of the hill you can see the "dish", a radio telescope that was used to communicate with satellites and spacecraft. 3

BEST SIGHTS

Main Quad and Memorial Church: The heart and oldest part of Stanford University. The church was built during the American Renaissance by Jane Stanford as a memorial to her husband Leland. 2
Hoover Tower: A 87 metre high structure on the campus with a great view. The tower houses the Hoover Institution Library and Archives, an archive collection founded by Herbert Hoover before he became president of the United States. 4

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

Andreas Puschnik

Name Andreas Puschnik
Nationality German
Age 28
University Stanford University
Supervisor Jan Carette, PhD

Photos: Andreas Puschnik

2016 HEINRICH WIELAND PRIZE FOR PETER SCHULTZ

BIF's SISTER FOUNDATIONS

Some 100 people flocked to Munich's Nymphenburg Palace on 13 October 2016 for the Heinrich Wieland Prize award ceremony for Peter Schultz and the attendant scientific symposium on synthetic biology. The Boehringer Ingelheim Foundation has honoured Schultz with the 100,000 euro prize for his "biologically inspired synthesis of new molecules and, in particular, for the expansion of the genetic code". Schultz, professor at the Scripps Research Institute in California, reprogrammed the cell's own construction machinery to incorporate amino acids beyond the common 20 into the proteins it builds at precisely defined positions. These artifical amino acids confer new chemical, physical, and biological properties on these proteins.

So far, Schultz's tools have enabled researchers to teach cells to insert more than 100 artificial amino acids with different functions into the proteins of bacteria, the individual cells of plants, and mammals, and even into entire organisms such as fruit flies and roundworms. His findings have already led to the development of new drugs – some approved, others in the clinical trial stage – against degenerative diseases, cancer, autoimmune disorders, and neglected diseases. "Peter Schultz has given us a wide array of invaluable tools that enable us to rationally design molecules and cells, to understand the processes of life, and to treat medical conditions," says Professor Wolfgang Baumeister, director at the Max Plank Institute of Biochemistry in Martinsried, Germany, and chair of the prize's selection committee. Baumeister left the board at the end of 2016, after having served the maximum time on the board under the prize's statutes. His successor will be Felix Wieland, grandson of the prize's namesake, Heinrich Wieland, and professor at the University of Heidelberg.

During Baumeister's tenure and especially his chairmanship, the prize has seen many changes. The Boehringer Ingelheim Foundation took over its sponsorship in 2011, its endowment was doubled to 100,000 euros, and it celebrated its 50th anniversary in 2014. Since then, a top-tier international scientific symposium has accompanied the award ceremony. The foundation would like to express its profound gratitude to Wolfgang Baumeister for his great dedication to the prize's development and his unwavering application of the highest quality criteria in every aspect.

Photo: Axel Griesch

Prof. Peter Schultz (centre) received the Heinrich Wieland Prize 2016. It was presented by Prof. Wolfgang Baumeister (left) and Christoph Boehringer (right) on 13 October 2016.

UPCOMING EVENTS

29 MARCH–2 APRIL 2017

115th International Titisee Conference

The meeting, titled "Evolutionary Mitochondrial Biology: Molecular, Biochemical, and Metabolic Diversity", will be chaired by Vamsi K. Mootha, Harvard Medical School, Boston, MA, USA, and Michael W. Gray, Dalhousie University, Halifax, Canada. It will bring together investigators studying mitochondria from a phylogenetically broad range of eukaryotes, with the goal of exploring the mechanistic basis and physiological consequences of their diversity.

Participation is by invitation only.

7–9 JULY 2017

Meeting of BIF's Board of Trustees

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

14–16 JULY 2017

European alumni seminar

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. The title of this year's seminar is "World of Sounds". Further details with the programme.

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

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