FUTURA

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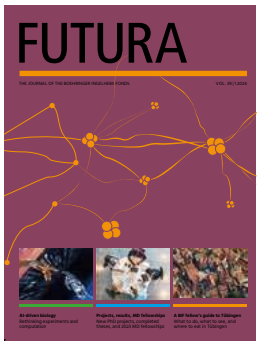
AI-driven biology
Rethinking experiments and computation

Projects, results, MD fellowships
New PhD projects, completed theses, and 2023 MD fellowships

A BIF fellow's guide to Tübingen
What to do, what to see, and where to eat in Tübingen



The cover illustration symbolizes the merging of AI and biomedical research as envisioned at the AITHYRA institute in Vienna, Austria. Its approach goes far beyond sleuthing through large amounts of data to find hidden correlations.

FACTS

AI-DRIVEN BIOLOGY: RETHINKING EXPERIMENTS AND COMPUTATION

FELLOWS

NEW PHD PROJECTS, FIRST ROUND 2023

NEW PHD PROJECTS, SECOND ROUND 2023

NEW PHD PROJECTS, THIRD ROUND 2023

NEW PHD PROJECTS, FIRST ROUND 2024

NEW PHD PROJECTS, SECOND ROUND 2024

NEW PHD RESULTS

MD PROJECTS

FOUNDATION

PAPERS IN THE SPOTLIGHT

PUBLISHING INFORMATION

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THOUGHTS ON HOW TO LEAD WELL

Marc Wittstock (l) and Dr Stephan Formella (r)

People who talk loudly and with conviction are often perceived as leaders, but an effective leader needs to know how to listen well. In this issue, we reprint, with the kind permission of *EMBO Reports,* an interview conducted with DeepMind Professor Michael Bronstein, who directs the AITHYRA Institute in Vienna. Founded this September, the institute has merged AI and biomedical research from the outset and aims to forge ties with other European hubs and propel Europe into a leading position in this field. Based at the Austrian Academy of Sciences, it was launched at the initiative of BIF's sister foundation, the Boehringer Ingelheim Stiftung.

In the interview, Michael describes the inclusive atmosphere he seeks to create by allowing the different approaches of biomedical and AI researchers to merge and give rise to something new. He also explains how listening to people from different disciplines has enriched his scientific work. Often, questions from people outside his field have given him a fresh perspective and pushed his thinking in new directions. This willingness to listen and adapt one's thinking is not only a prerequisite for being a good scientist, but also a sign of a good leader. As research becomes ever more cross-disciplinary, listening with the intent to understand, rather than with the intent to reply, is a valuable skill. Adopting this mindset is especially helpful when dealing with opposing positions or different perspectives.

> "Listening with the intent to understand, rather than with the intent to reply, is a valuable skill."

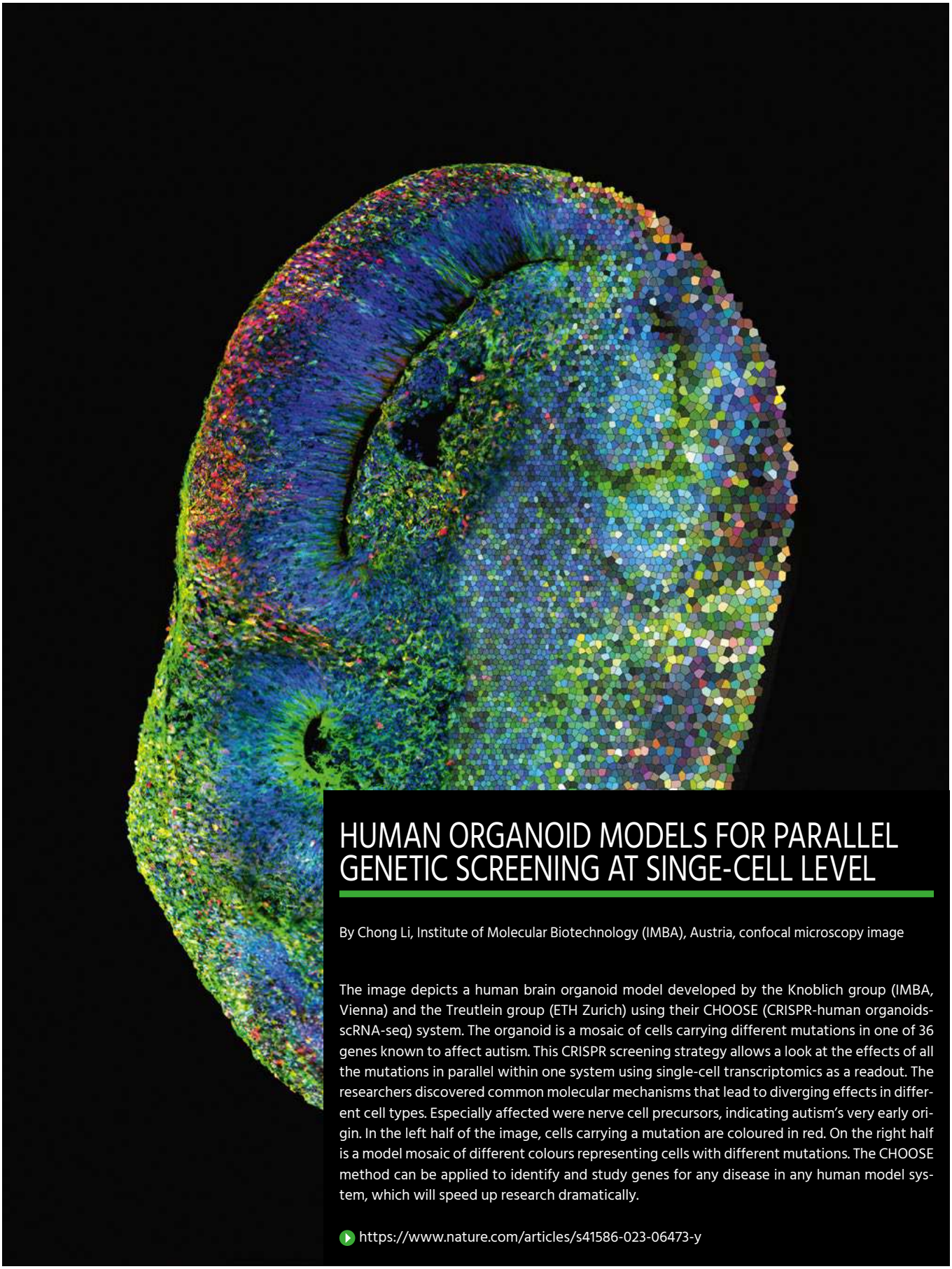
This is one of the tips we would like to offer those fellows of ours who are taking on a leadership role for the first time. This can be daunting, but it also brings the great reward of seeing people prosper under one's guidance. It is our opinion that the purpose of leadership, especially in science, is to produce more leaders, not more followers. While many things can be mastered from textbooks or videos, for some things we need real-life examples and experience, and leadership is among them. We therefore encourage our fellows to take the first steps towards leadership as early as possible – for example, by mentoring junior students.

About a sixth of our fellows have already assumed academic leadership positions (plus many in non-academic settings), a fact we note with satisfaction. There are also several BIF alumni who are or have been supervisors to BIF fellows themselves, including one supervisor who has already spawned the "third generation" of BIF fellows. At our 2025 alumni seminar "Leaders and Followers", we plan to delve deeper into the topic.

As a leader, it is your responsibility to create conditions conducive to success. In addition to research, you will probably be tasked with managing conflicts between members of your group while juggling many other, often unfamiliar, demands on your time. You will be well served by being a good project manager, clear communicator, and active listener who understands the context-related and individual needs of your group members.

So, as you consider your own leadership journey, ask yourself: What kind of leader do I want to be? What skills do I wish to master? And how can I inspire others to lead by being a good role model?

Photo: BIF, C. Costard



HUMAN ORGANOID MODELS FOR PARALLEL GENETIC SCREENING AT SINGE-CELL LEVEL

By Chong Li, Institute of Molecular Biotechnology (IMBA), Austria, confocal microscopy image

The image depicts a human brain organoid model developed by the Knoblich group (IMBA, Vienna) and the Treutlein group (ETH Zurich) using their CHOOSE (CRISPR-human organoids-scRNA-seq) system. The organoid is a mosaic of cells carrying different mutations in one of 36 genes known to affect autism. This CRISPR screening strategy allows a look at the effects of all the mutations in parallel within one system using single-cell transcriptomics as a readout. The researchers discovered common molecular mechanisms that lead to diverging effects in different cell types. Especially affected were nerve cell precursors, indicating autism's very early origin. In the left half of the image, cells carrying a mutation are coloured in red. On the right half is a model mosaic of different colours representing cells with different mutations. The CHOOSE method can be applied to identify and study genes for any disease in any human model system, which will speed up research dramatically.

https://www.nature.com/articles/s41586-023-06473-y

THE ANT WITH EFFICIENT MEDICAL CARE

Because its prey knows how to defend itself, the African ant species *Megaponera analis* has developed an efficient way to save nestmates injured during raids on the mounds of termites, their sole diet. The ants even have a special protocol to treat infected wounds – the only species other than humans known to do so. Researchers have now revealed the ants' secret: they "smell" the infection via changes in the hydrocarbons on the exoskeleton of the injured ant. The nursing ants clean such wounds even more carefully than usual and apply an antimicrobial cocktail they secrete from a gland behind their head. With this care, 93 % of infected ants recover; without it, only 7 % survive. A further testament to the effectiveness: about a fifth of the ants taking part in the raid on termite mounds lack one or two limbs (those with more limbs missing are left behind).

The scientist found that the ants' antimicrobial cocktail contains 112 chemical compounds and 41 proteins, half of which have – or are at least strongly suspected to have – antimicrobial or wound healing properties. However, the properties of the most abundant protein, an evolutionarily young one, are unknown. This might be a promising candidate for developing new, urgently needed antibiotics to combat the rise of multi-resistant bacteria, especially since the culprit in ants is *Pseudomonas aeruginosa* – a particularly nasty bug that has repeatedly developed antimicrobial resistance and accounts for upwards of 50,000 healthcare-associated infections a year in the US alone (15 % of them caused by multi-resistant strains). And just as in ants, it is often found in battlefield wounds in humans.

REFERENCE

Frank ET, Kesner L, Liberti J, Helleu Q, LeBoeuf AC, Dascalu A *et al* (2023) Targeted treatment of injured nestmates with antimicrobial compounds in an ant society. *Nat Com* **14**: 8446

STEM CELL TRANSPLANT: HAVING THE GUTS FOR SUCCESS

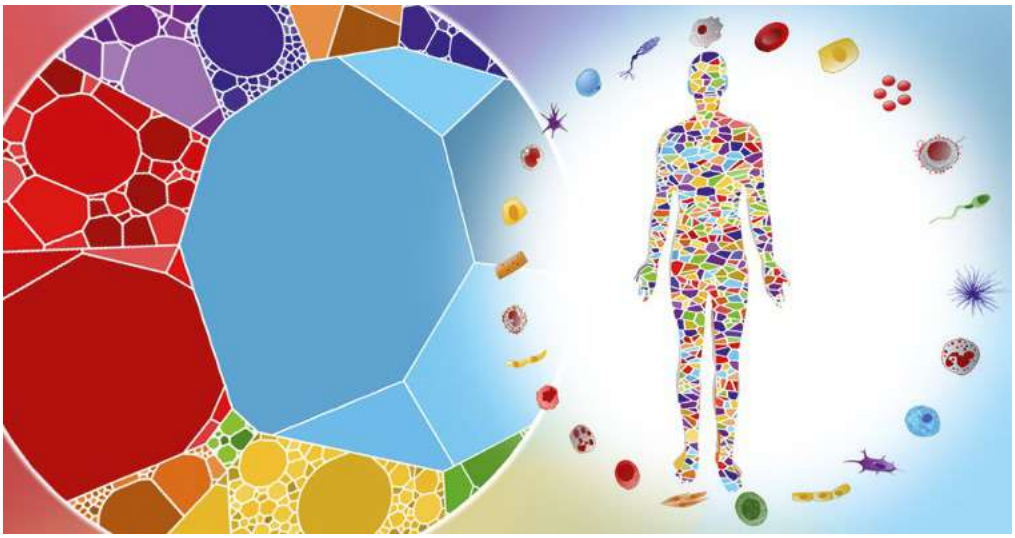
Today, we can cure many people with blood cancer with a stem cell transplant. But in 40 to 60 % of the cases, transplanted immune cells attack tissues of the host they are supposed to save, especially the skin, liver, and gut. About 40 % of the affected patients – 15–25 % of all transplant patients – with the so-called graft versus host disease die. Researchers have now found an important puzzle piece on how to prevent this. They followed 78 patients for two years after their procedure and dove deep into the metabolic footprint of the microbes in stool samples to unravel how to predict and even prevent transplant rejections. They unveiled a complex interplay in the gut microbiome influencing transplant outcomes: not just the bacteria and the substances they produce play a role, but also bacteriophages – viruses that prey on bacteria – and their metabolic byproducts. Together, they influence not just whether the transplanted cells stay friendly, but also how well the patients can recover from the disease. From their data, the researchers formulated a risk index which they hope to use to predict who is most at risk from a relapse or an attack by the donor stem cells. Armed with this prediction, they want to intervene early. By transferring the right kind of bacteria and bacteriophages, they hope to give patients a much better chance to recover. In trials with mice, they have already had success with preventing graft versus host disease or a recurrence of the cancer and are now ready to start human trials.

Stem cell therapies can cure disease, but come with risks. Having the right gut microbiome might lower that risk.

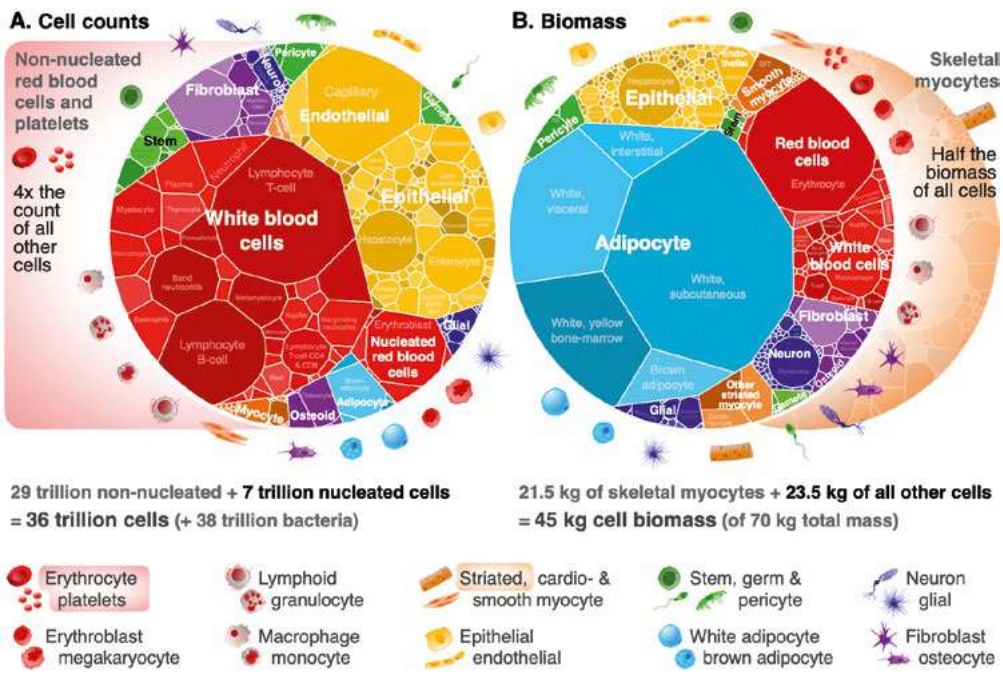
REFERENCE

Thiele Orberg E, Meedt E, Hiergeist A, Xue J, Heinrich P, Ru J, Ghimire S (2024) Bacteriophage consortia are associated with protective intestinal metabolites in patients receiving stem cell transplantation. *Nat Can,* DOI: https://www.doi.org/10.1038/s43018-023-00669-x

Photos: Erik Frank; CDC/Unsplash



Cell numbers and cell size correlate inversely in the human body.



CELL SIZE AND NUMBER: THE SMALLER THE MORE

What size are human cells in relation to their number? While cell size and number are tightly regulated and related to their respective function, this question has only now been answered for the first time. An international team used a classical cell biology approach integrating decades worth of histological and anatomical data on the size, mass, and numbers of about 1,200 cell types from 60 tissue systems. Collating about 1,500 references, they estimated these numbers for an average adult man and woman and a 10-year-old.The resulting index showed that the bigger a cell, the fewer there are and vice versa. Across the whole body, this inverse relationship is followed so closely that if you weighed all the cells of a certain size class

and compared the absolute weights of the different classes, all would contribute about the same amount to overall body mass. The international team also found that different cell types hardly vary in how the single cells stray from the "normal" cell size for the type. This, they conclude, points to a universal mechanism regulating cell size over seven orders of magnitude from blood to muscle cells. Understanding the mathematical rules behind these patterns could reveal key principles of cell growth mechanisms, which in turn are relevant in cancer, ageing, etc. The data, including methods and sources, is available in an interactive online tool that makes it possible to explore cell parameters at different levels across types and tissues.

REFERENCE

Hatton IA, Galbraith ED, Merleau NSC, Shander JA (2023) The human cell count and size distribution. *PNAS* **120**: e2303077120. Link: https://doi.org/10.1073/pnas.2303077120

https://humancelltreemap.mis.mpg.de

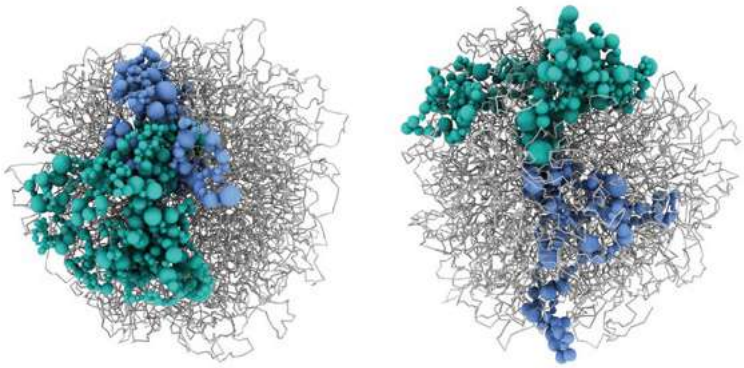
The microscopy technology pMINFLUX enables 1 nm resolution at sub-nanosecond resolution for several dyes simultaneously. Researchers at LMU Munich achieved this by measuring how long it takes the different dyes to light up after they were excited.

Source: Cole F, Zähringer J, Bohlen J, Schröder T, Steiner F, Pfeiffer M *et al* (2024) Super-resolved FRET and co-tracking in pMINFLUX. *Nat Photon* **18**: 478–484

https://doi.org/10.1038/s41566-024-01384-4

Graphic: MUI/Institute for Neuroscience

THE GENETIC ARCHITECTURE OF INTELLIGENCE



The 3D models showcase how DNA is organized within neurons, comparing two conditions: one with the presence of the protein SATB2 (left) and one without SATB2 (right). Chromosome 4 is represented in blue, Chromosome 14 in green.

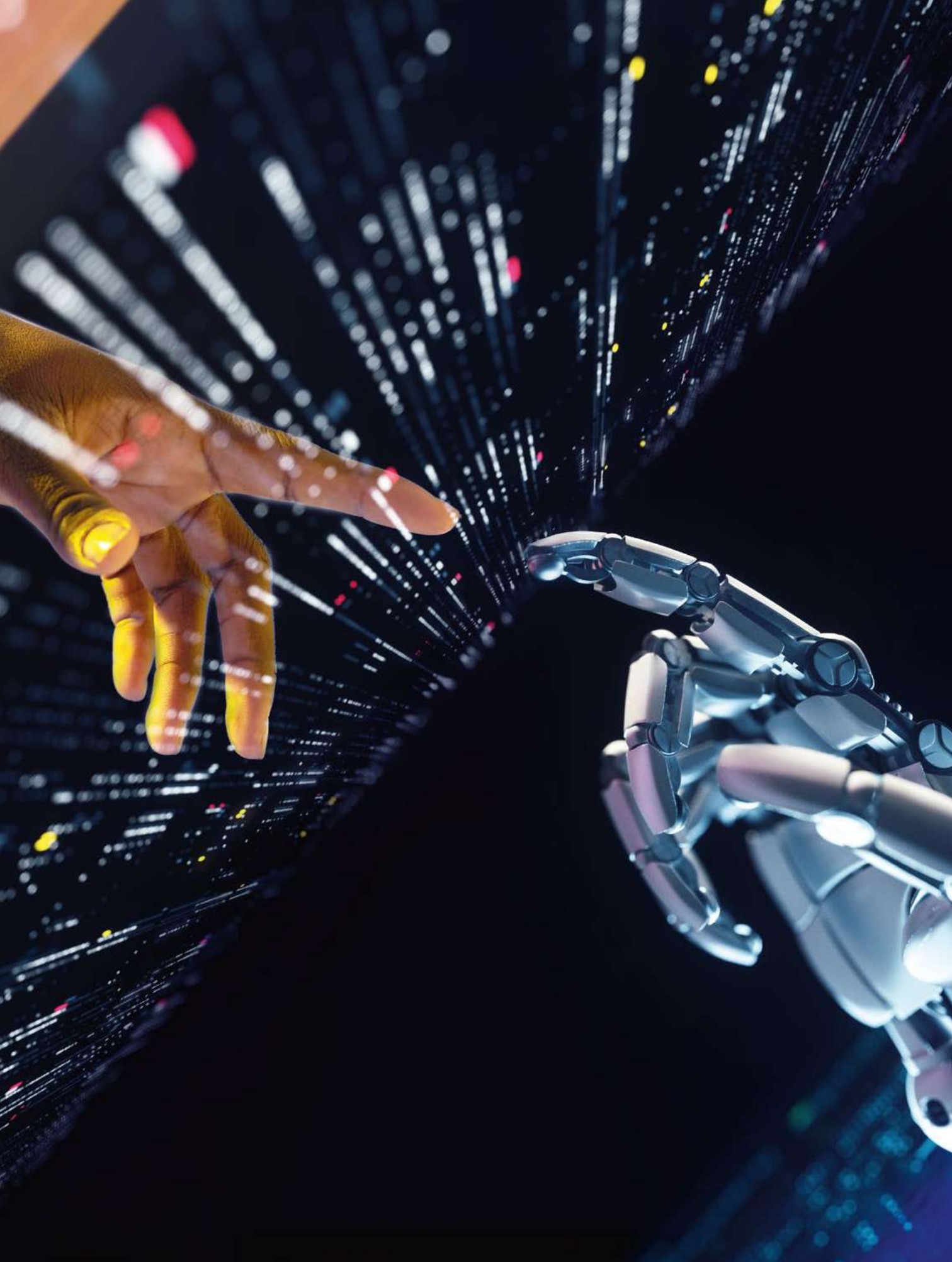
Having loopy DNA in your brain, it seems, is one prerequisite for being intelligent. Recent research has shown that the 3D architecture of genes – a complex mix of loops and origami-like folding – is highly specific for different cell types. It enables cells to access and activate the specific set of genes needed to fulfil their role. An international research team has now analyzed how DNA architecture is orchestrated by the protein SATB2. It is almost exclusively active in the pyramidal cells of our cortex, where our cognitive abilities preside. SATB2 binds to DNA and amongst others we know that a certain mutation of this protein lowers the intelligent quotient of a human to about 40. SATB has also stayed very much the same over time, showing little difference between mice and men.

The researchers analyzed the DNA architecture of mouse cells with and without SATB2 using Hi-C, a technique to detect which parts of DNA interact with each other. They found that SATB2 targets hundreds of genes associated with intelligence and learning, distributed over the whole genome. SATB2 tweaks the genetic 3D architecture at all levels to bring its target genes in position to be activated as needed while we think. The researchers took special note of the fact that SATB2 also affects gene loci associated with the risk of neuropsychiatric disease. The researchers postulate that a faulty DNA organization intensifies or might even cause such disease. This could widen available treatment options, targeting not just malfunctioning synapses or out of balance neurotransmitters, but also trying to untangle faulty loops of DNA.

REFERENCE

Wahl N, Espeso-Gil S, Chietera P, Nagel A, Laighneach A, Morris DW *et al* (2024) SATB2 organizes the 3D genome architecture of cognition in cortical neurons. *Mol Cell* **84**: 621–639.e9

https://doi.org/10.1016/j.molcel.2023.12.024



AI-DRIVEN BIOLOGY: RETHINKING EXPERIMENTS AND COMPUTATION

This interview was conducted by Thomas Lemberger, head of Open Science at EMBO.

An interview with Michael Bronstein, DeepMind Professor of Artificial Intelligence at the University of Oxford and Founding Scientific Director of the AITHYRA Institute of the Austrian Academy of Sciences.

EMBO report (ER): Michael, it's a great privilege to talk to you. We thought we might have a chat about fundamental research in machine learning and applications of AI in biology. Of course, we would like to learn more about the new institute you are about to direct in Vienna, and finish with your perspective on how AI may develop in the future.

AI has become popular everywhere and one of the inflection points was the release of ChatGPT and the rise of large language models. A factor of their success is the ability to build so-called foundation models, which are trained on very large amounts of data and acquire broad generalization capabilities. Is there anything similar in biology, in drug design, and how do we get there?

Michael Bronstein (MB): I think there is some broadness to the definition of what counts as a foundation model and, more generally, artificial intelligence. François Chollet posits that intelligence is not about how well a specialized model performs, but how good it is in learning new things, or in other words, how well it can generalize across tasks. In this sense, we are probably still far away from "general intelligence", and this is one of the reasons why I personally don't like the term "artificial intelligence", because we don't really understand and agree on what intelligence is. In large language models such as ChatGPT, the key to their success was the scale, being able to create very large models that can be trained on huge amounts of data. We don't yet have anything of comparable scale in biology, and probably the main limitation is the amount of data we have. It is very expensive to obtain experimental data. Maybe it will be possible to overcome the lack of experimental data with simulation and it's an interesting question how to combine simulated data with experimental data. However, the range and diversity of problems in biology is significantly bigger than in language, and it could be that in some applications we don't necessarily need the kind of scale we find in ChatGPT. There have recently been some publication claims about "foundation models", for example in chemistry, but I think we are still a few years away from them. →

"The range and diversity of problems in biology is significantly bigger than in language, and it could be that in some applications we don't necessarily need the kind of scale we find in ChatGPT."

Photo: Getty Images/Andriy Onufriyenko

ER: Do you think there is a strategy to scale up the production of data in a form that is immediately usable by AI models, and at the scale necessary for training foundation models?

MB: Biotech and pharma companies are trying to do so, with success in many cases. One example is Recursion, which scaled up cell-painting technologies, making it possible to image hundreds of millions of cells and see what happens to the cells when you perturb them either chemically or genetically. What I would like to do is to take a step back and look at the next generation of data sources, where the consumer of the data will not be a human but a machine. In a recent blog post that I co-authored with Luca Naef, my colleague and co-founder of the start-up VantAI, we called it "black box data". What we mean by this is a type of data that might not necessarily be understandable by a human and would only make sense in conjunction with appropriate machine-learning algorithms. AlphaFold, for example, was trained on PDB, a dataset collected by structural biologists for structural biologists. This data is very expensive and takes a lot of time to acquire – sometimes years for a single protein structure. If we say that the data does not necessarily need to be viewed by human scientists, we can come up with completely new experimental data sources, or maybe repurpose existing technologies that have never been considered suitable for a particular problem. For example, VantAI is using structural proteomics, which is up to six orders of magnitude cheaper than cryoEM, but doesn't give the full structure of the molecules, only sparse interactions between them. But this is good enough with appropriate generative models to be able to generate new molecules, and we use such approaches to design molecular glues. This is something that has become possible only recently, when we had the right scale and the right machine-learning algorithms, like generative diffusion or flow matching. I think this conjunction of experimental technology and machine learning may potentially open an entirely new chapter in biological research.

ER: You are one of the pioneers in the field of geometric machine learning and have worked with graph neural networks. Why are these models so powerful? And what are the applications in biology and drug discovery?

"If we say that the data does not necessarily need to be viewed by human scientists, we can come up with completely new experimental data sources …"

MB: Geometric deep learning is based on a very old principle in science, which is symmetry and invariance. You can argue that, at some level, symmetry is the very foundation of science itself. Everything from the structure of space-time to the fundamental forces in the universe can be derived from corresponding invariances and symmetries. What we try to do in geometric deep learning is to use similar principles to study and design neural network architectures. Historically, some of the deep-learning architectures that have become successful in computer vision and image analysis in the past decade, such as convolutional networks, were already based on such geometric ideas.

While these principles are well known, I think it's fair to say that only recently they have become more of a conscious design rather than just a happy coincidence. In the biological field, and especially in biochemistry, geometric architectures became prominent maybe five years ago. You can represent molecules as graphs – nodes representing atoms linked by edges representing chemical bonds. You don't care about how you order the atoms in the molecule or the nodes in the graph. This is called permutation symmetry. For the prediction of many chemical and physical properties of the molecule, you also don't care about how it is positioned in space. If, for example, you rotate or translate the molecule, its properties remain unchanged. Graph neural networks make it possible to incorporate such symmetries. The most prominent example of such an architecture is AlphaFold 2, and I think it has been an important factor that has contributed to the success of geometric approaches in biochemistry and the fact that these architectures are now widely used in drug design and drug discovery.

ER: Do you think that a synthetic biology approach could be useful to explore protein design space? For example, synthesizing potential candidate protein domains, measuring their biochemical characteristics, and then improving models in cycles of training and new experiments?

MB: I would say that we should use all the data that is possible, and another avenue is simulation. As far as I know, companies such as Isomorphic Labs are working on simulated data and accelerating or even potentially replacing expensive simulations like molecular dynamics with generative machine-learning models. This is another interesting avenue that we would like to explore in the new institute, which, if successful, might bring a profound change to the ways that computational science is done. If you look at areas from weather forecasting and climate modeling to molecular simulations, they rely on computational models rooted in small-scale laws that we know and understand well and that we try to apply at large scales where the governing laws are much less understood. Such simulations are usually very computationally intensive and require fine spatial and temporal resolution to work well. It might be possible to accelerate or even entirely bypass numerical simulations altogether with appropriate generative machine-learning models. It would be very interesting, because it will probably be the first time we forego mathematically or phys-

ically principled models for something that is significantly faster and scalable but allows for mistakes in certain cases but overall makes sense.

"It might be possible to accelerate or even entirely bypass numerical simulations altogether with appropriate generative machine-learning models."

ER: Speaking of the new institute that you will be heading, tell us more about the key idea behind this initiative.

MB: My ambition is to make AITHYRA a cool new magical place in central Europe. That's something that doesn't happen every day. With the help of AI, we would like to transform the way biological sciences are done in order to drive the biological revolution in the next decade with the goal of ultimately improving human health. We want our research to have a tangible impact on diagnosing and curing diseases, developing new drugs, understanding better how biological systems work in our body, and how they stop working when we get sick. We want to co-locate biologists and computer scientists in the same place so we learn from each other and work on the next generation of machine learning and experimental technologies together. In my view, this is a necessary condition for the next breakthrough. It's hard to tell now where these breakthroughs might happen, so at this point we are open-minded about what biological problems we want to consider. But we would like to consider biological problems from the perspective of machine learning, so we will likely favour problems in which it is possible to collect large amounts of data and where the problem itself is likely to be amenable to machine-learning solutions.

ER: In the organization of an institute like this, will you try to combine data production and model training? Do you have some ideas how to structure the institute, such that it is tailored to AI and machine learning?

MB: Absolutely so. We would like to create a robotic lab so we can test machine learning algorithms in the loop to produce and consume the data. We would also like to improve heavy simulations with the help of machine learning and use simulated data to train machine-learning systems. Everything will be released in the public domain; we would like to contribute to the community by publishing code, models, and data.

Professor Michael Bronstein

"With the help of AI, we would like to transform the way biological sciences are done in order to drive the biological revolution in the next decade with the goal of ultimately improving human health."

ER: In terms of computing scale for training, is that an issue? What access do you have to GPU power to train all these models?

MB: Some of the models are indeed very compute-intensive but in different ways. Large-scale transformers, such as those used in LLMs applied for example on genomics data, are GPU-hungry, whereas other problems such as traditional physics simulations mostly rely on CPUs. We certainly would like to be one of the better-equipped places in Europe in terms of compute power and budget significant cost for computer infrastructure.

ER: Do you plan to have strong private–academic partnerships, or how do you see that sort of connection to translational applications?

MB: The mandate of the institute is pure research. It will be an institute of the Austrian Academy of Sciences, and a significant amount of research funding, 150 million euros over a period of 12 years, will be provided by the Boehringer Ingelheim Foundation as a nonprofit institution. Having said that, I would like to create a start-up and venture capital ecosystem around it, →

Photo: OeAW

because some of the research that we'll be producing will likely be commercializable. We would like our future researchers and students to take their ideas out and make an impact in the broader industry. My aspiration would be to make it the friendliest place for start-ups in Europe to translate research discoveries into impactful solutions for society.

ER: There's often a tension between the private and the academic world. We've seen that with AlphaFold 3: it was released and published but the code was not available, and part of the community reacted strongly to this. What are your views? Should all models be immediately open source, or is there some room for keeping them proprietary for a while?

"One of the reasons why the machine-learning community has made such rapid progress is because most of the research was open sourced."

MB: One of the reasons why the machine-learning community has made such rapid progress is because most of the research was open sourced. We definitely would like to adopt the same mindset. We would also like to collaborate with industry, but I think the majority of the work we'll be doing will contribute to the academic research community.

ER: You mentioned that scale and the amount of data are a determinant criterion for many applications and models. In the academic world, do we have access to enough computing power to reach this scale?

MB: In terms of access to computing power, I wouldn't make general statements, because I think the problems in machine learning can vary a lot depending on how computer-hungry they are. For some applications like LLMs, academia is absolutely out of the race with industry. Just to give you an example, Princeton prides itself on a cluster of something like 300 GPUs, and one of the biggest clusters in the biotech industry is Recursion with more than 500. Meta alone has hundreds of thousands and will soon surpass half a million GPUs. Universities obviously cannot compete with leading big tech companies and should rather be working on other problems that might not require immense computational resources, and I believe there are plenty of such problems in biology. The more compute-hungry problems might need to be solved by collaboration with industry.

ER: As a young institute, you will have to recruit new talent. What are the skills necessary these days for young scientists to be ready for AI in biology?

MB: Compared to a few years ago, we see a growing number of computer scientists interested in biological problems. And likewise, there are many biologists interested in using machine learning in their field. It's very good that there is this percolation. In the new institute, I would expect machine learners to at least have a broad understanding of biology, maybe a deeper understanding of the specific problems they are working on, even if they are not necessarily able to produce new results in this domain on their own. In the same way, biologists should be knowledgeable about how machine learning works and should be able to use it, even if they are not necessarily able to develop new algorithms. It's important to put them together in the same building so they breathe the same air and dream the same dreams. We need to offer an attractive work environment. We would like to be the best employer fostering an inclusive and diverse culture investing and taking care of our people.

Photo: Getty Images/Westend61

ER: Your background is in mathematics or computer science?

MB: Computer science, yes.

ER: Did you find it difficult to understand biology and to think about these problems?

Michael Bronstein: I would not claim that I understand biology, but I also don't think I would call it "difficult"– "exciting" is probably a better term. My first biological machine-learning collaboration was with Bruno Correia, a structural biologist based at EPFL in Switzerland. These were very cool problems with a lot of geometry. I find that when a newbie comes to your field and starts asking silly questions, in most cases the answer is trivial, but in other cases you actually start thinking, "Why are we doing it this way and not another way?" And when you cannot explain it to yourself, you may come to the conclusion that you can do it better or completely differently. It happened to me a couple of times and it was very rewarding. These are the kinds of interactions I would like to have between biologists and computer scientists.

ER: We are all excited about AI, and it's a profound change even in how we think about biology and new experiments. But it also raises some concerns about the power of these methods, their reliability, potential misuses, and the growing autonomy of AI systems. What should the research community do to develop AI in a responsible way that keeps the trust of decision-makers and the public in general?

MB: There are many concerns about applications where AI replaces humans or AI makes decisions that impact human life, be it the recommendation algorithms on social media or AI-assisted decisions made by governments, the judicial system, or financial institutions. I think regulation is probably inevitable to ensure that such systems are used fairly and to the benefit of the society, but I'm also concerned about over-regulating the field. We don't want to choke it while it is still nascent. In the life sciences, it is possible that we are moving towards a new way of doing science with the help of AI. Probably for the first time in the history of the scientific method, humans might be augmented and perhaps, in a more remote future, even replaced by machines in the process of generating new hypotheses, which has so far been a hallmark of human →

"Probably for the first time in the history of the scientific method, the part of hypothesis generation that has been the hallmark of human ingenuity and creativity might be augmented and perhaps, in a more remote future, even done by machines."

ingenuity and creativity. This is something that we would like to look into: can we generate new theories or design new experiments with the help of AI, basically making automated scientists?

From a philosophical perspective, it raises the important question of what science will look like when it's not done by humans. There are proponents of a "post-theory science", where we have a theory that cannot be written in the form of equations that we, humans, understand. Instead, it's a black box with many parameters that are fine-tuned on the data. The theory produces predictions that can be tested experimentally. AlphaFold is a good example. It does not offer a "theory of protein folding"; rather, it produces correct predictions that are useful in many situations.

One of the concerns raised about biological AI is biosafety. My main concern is not the ability of AI to design toxic compounds, but to design harmful organisms and viruses. We have plenty of very toxic stuff already without any AI, and the challenge in drug design is how to make it selectively toxic. At this point in time, it is a possible but, in my view, a still somewhat remote scenario. However, since the field is evolving very fast, we might transition into this more harmful reality faster than we anticipate, so it's very important to be prepared for it.

ER: You mentioned the autonomy of the living organism. Now with AI, we are reaching a high level of autonomy and there is a strong motivation to push this even further to generate more data and hypotheses. Do you think we should put some cap on this autonomy? Or should we let self-reproducing robots do drug screening on our behalf?

MB: We don't really have self-reproducing AI, and in general it works differently from biological organisms. In my opinion, we should not see AI as some kind of uncontrolled force of nature. Rather, it's a technology that we design and can shape in a way that is useful for humankind. Achieving this requires scientists to do the right things, as well as political effort and regulation. You might call me a technological positivist, but I don't see the AI doomsday scenarios as unavoidable or even likely.

ER: For the future, what might be the next critical technological or theoretical advance in AI that will bring it to the next level?

MB: I would say, a big achievement in AI for science would be accelerating or replacing traditional and notoriously heavy numerical simulations like molecular dynamics or fluid dynamics with generative machine-learning models trained on experimental or simulated data. There are still many open questions, but if successful, the impact could be tremendous because such models appear across the board in computational sciences.

ER: You have worked on many different topics, and you are a serial entrepreneur. You were head of Graph Learning at Twitter. You also mentioned a couple of companies you are involved in now. You even published a paper on social communication between whales. And now you will be the head of the new institute. How do you keep up with all of this?

MB: Well, I'm lucky to have had amazing students and collaborators. Science is a collective effort and I've had the chance to work with the best people in their respective domains. I intend to keep it this way and bring the best talent under the roof of the new institute. Biology is a very broad field with a lot of exciting problems, and the AI and machine-learning research community has a special culture allowing the field to move very fast. What we are working on now didn't exist a few years ago. Even to people in the field, the capabilities we see today would have seemed like science fiction just a decade ago. We want to bring some of this culture and speed into more traditional science.

ER: Professor Bronstein, thank you for the interview.

Disclosure and competing interests statement:
The author declares no competing interests.

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

Therefore, this pdf continues with the section Results.

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

Shared first authorship is indicated with an *.

CATCHING CALCIUM TRANSPORT IN MOTION: STRUCTURES AND DYNAMICS

cf. BIF FUTURA, VOL. 33 | 1.2018

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Supervisors: Prof. Poul Nissen, Assoc. Prof. Magnus Kjaergaard

SCa^{2+}-ATPases control cytosolic calcium levels by pumping calcium ions across the cell membrane through a cycle of conformational transitions driven by ATP hydrolysis. To avoid calcium reflux, it is crucial that these pumps run predominately in a single direction, even though all partial reaction steps are in principle reversible. The main goal of my PhD project was to understand the unidirectional transport of calcium ions at a molecular level. I solved the structures of key intermediates of *Listeria monocytogenes* Ca^{2+}-ATPase 1 (LMCA1), through both cryogenic electron microscopy (cryo-EM) and X-ray crystallography. Previous work using single-molecule Förster resonance energy transfer (smFRET) showed that a transient intermediate between the inward- and outward-open states of LMCA1, the so-called [Ca]E2P state, is the final step before calcium is irreversibly released to the outside of the membrane. This state was shown to have a distinct conformation with a partially rotated actuator domain. By solving the cryo-EM structure of the [Ca]E2P state of LMCA1, I confirmed that the actuator domain is indeed partially rotated. My structure showed that the binding site of this intermediate state occludes a calcium ion, and poor electron density for key calcium-coordinating residues revealed a dynamic calcium binding site that prepares for calcium release to the outside of the membrane. The smFRET study showed that following calcium release, LMCA1 rapidly auto-dephosphorylates to prevent backflow of calcium ions. The mechanism of this rapid process can be explained by two of my crystal structures of LMCA1 stalled with metal fluorides in outward-closed conformations. The structures reveal that even before the dephosphorylation reaction is initiated, an essential dephosphorylation loop in the actuator domain is moved into position for the reaction. With these new structures of LMCA1, I have discovered crucial details of the mechanism and dynamics of the irreversibility of Ca^{2+}-ATPases.

PUBLICATION

Hansen SB, Dyla M, Neumann C, Quistgaard EMH, Andersen JL, Kjaergaard M *et al* (2021) The crystal structure of the Ca(2+)-ATPase 1 from *Listeria monocytogenes* reveals a pump primed for dephosphorylation. *J Mol Biol* **433**: 167015

KIPFERL AND RHINO READ OUT DNA SEQUENCE AND CHROMATIN STATE TO FIGHT SELFISH ELEMENTS

cf. BIF FUTURA, VOL. 33 | 1.2018

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Institute: Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna, Austria
Supervisor: Dr Julius Brennecke

Selfish, repetitive genetic elements make up large portions of eukaryotic genomes. Their activity threatens genomic stability and must be strictly controlled. In the animal germline, PIWI-interacting RNAs (piRNAs) serve as sequence-specific guides to silence selfish transposable elements (TEs) and maintain fertility. To ensure the targeting of all TEs, piRNAs are produced from TE-rich loci called piRNA clusters. In *Drosophila*, these clusters are marked by the germline-specific heterochromatin protein 1 (HP1) variant Rhino, which induces piRNA production at these sites. Like other HP1 family members, Rhino strongly binds to nucleosomes via the heterochromatic H3K9me3 histone mark. However, H3K9me3 is not exclusive to piRNA clusters, and not all heterochromatin is bound by Rhino. The aim of my PhD project was therefore to understand the molecular mechanisms underlying Rhino's highly specific localization to piRNA clusters. Using fly genetics, confocal microscopy, molecular biology, and next-generation sequencing, I found that Rhino interacts with a DNA-binding zinc finger protein, which acts as a guide for Rhino and stabilizes it at most piRNA clusters. I showed that the binding sites of this previously uncharacterized guidance factor, which I named Kipferl, are enriched with a short DNA motif. Rhino is recruited only when Kipferl binding coincides with the presence of H3K9me3. Moreover, I identified a Rhino-specific residue as being critical to the interaction between Rhino and Kipferl, which explains why Kipferl acts as a guide for Rhino but not for other HP1 proteins. Unexpectedly, I showed that without Kipferl, Rhino is not only lost from most piRNA clusters but also relocalizes to satellites, another kind of selfish, repetitive genetic element. This indicates that satellites evolved other means to recruit Rhino and likely benefit from high levels of Rhino binding. My work uncovered a big missing piece of the piRNA pathway and provided insight into the logic of HP1 protein diversification and the role of genomic conflicts in Rhino evolution.

PUBLICATIONS

Baumgartner L, Ipsaro JJ, Hohmann U, Handler D, Schleiffer A, Duchek P, Brennecke J (2024) Evolutionary adaptation of an HP1-protein chromodomain integrates chromatin and DNA sequence signals. *Elife* **13**: RP93194

Baumgartner L, Handler D, Platzer SW, Yu C, Duchek P, Brennecke J (2022) The Drosophila ZAD zinc finger protein Kipferl guides Rhino to piRNA clusters. *Elife* **11**: e80067

CRYO-ELECTRON TOMOGRAPHY REVEALS AUTOPHAGY AT HIGH RESOLUTION INSIDE THE CELL

cf. BIF FUTURA, VOL. 34 | 2.2019

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In macroautophagy, a cup-shaped double-membrane structure called the phagophore engulfs cellular waste, closes into an autophagosome, and delivers its cargo for degradation, thereby removing damaged organelles and enabling nutrient recycling. How different membrane sources contribute to building the phagophore and how phagophore shape changes during growth have been difficult to study due to the limited resolution of conventional electron microscopy. In my PhD project, I studied autophagy at high resolution directly in starving yeast and mammalian cells using correlative cryo-electron tomography (cryo-ET). In yeast, my labmate Cristina Capitanio and I found that the double membrane of autophagosomes is thinner than that of any other organelle. This suggests that phagophores grow mostly by direct channelling of lipids from the endoplasmic reticulum (ER) into the membrane; we estimate that vesicle fusion contributes only 20-40 % of the membrane. Using a method that we devised, we sorted all captured phagophores by their growth stage. This revealed their dynamic structural changes: from early to late phagophores, the double membrane becomes thinner. This leads to an increase in the rim curvature, which likely drives constriction of the rim, thus limiting the size of bulk autophagosomes. Notably, the thin double membrane seems to be conserved from yeast to mammalian cells. However, autophagosomes in starving yeast contain mostly cytosol with ribosomes, whereas most autophagosomes in mammalian cells contain at least one piece of the ER. Rather than assuming a uniform shape, the pieces of the ER inside autophagosomes have a large range of morphologies, from bent sheets to intricate tubular networks. Cryo-ET thus offers a high-resolution view of what happens during autophagy inside the cell, yielding precise measurements and revealing unexpected relationships that can be used to model and understand autophagy even better.

PUBLICATIONS

Bieber A*, Capitanio C*, Erdmann PS, Fiedler F, Beck F, Lee CW *et al* (2022) *In situ* structural analysis reveals membrane shape transitions during autophagosome formation. *Proc Natl Acad Sci USA* **119**(39): e2209823119

Bieber A*, Capitanio C*, Wilfling F, Plitzko J, Erdmann PS (2021) Sample preparation by 3D-correlative focused ion beam milling for high-resolution cryo-electron tomography. *J Vis Exp* **176**: e62886

DESCENDING NETWORKS TRANSFORM COMMAND SIGNALS INTO POPULATION MOTOR CONTROL

cf. BIF FUTURA, VOL. 36 | 1.2021

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Supervisor: Prof. Pavan Ramdya

Animals, including humans, are capable of a remarkable variety of behaviours. To transform intentions into actions, movement instructions must pass from the brain to downstream motor circuits through descending neurons (DNs). DNs include small sets of command-like neurons that are sufficient to drive behaviours, such as walking or grooming. However, the circuit mechanisms of those command-like neurons were unclear. In my PhD project, I showed through simultaneous activation of command-like DNs and functional recording of many other DNs that command-like DNs in the fruit fly *Drosophila melanogaster* directly recruit networks of additional DNs to orchestrate complex behaviours. Specifically, I found that command-like DNs – previously thought to drive behaviours alone – in fact co-activate larger populations of DNs. In collaboration with other members of the Ramdya group, I analysed the fly connectome and found direct excitatory connections between command-like DNs and networks of interconnected DNs in the fly brain. By experimentally ablating these connections, we revealed that DNs with many downstream DNs require network recruitment to drive complete behaviours. Without strong DN–DN connectivity, they drive only simpler stereotyped movements – for example, those requiring fewer joints and less flexibility that can be repeatedly generated without much variability. Through the connectome analysis, we also showed that DN networks reside within behaviour-specific clusters that inhibit one another. These results support a mechanism for command-like descending control in which behaviours are generated through the recruitment of increasingly large DN networks that compose actions by combining multiple motor subroutines. A similar mechanism might be leveraged for descending control in other species, including mammals, and could be used to inspire the design of more flexible artificial controllers for applications in engineering and robotics.

PUBLICATIONS

Braun J*, Hurtak F*, Wang-Chen S, Ramdya P (2024) Descending networks transform command signals into population motor control. *Nature* **630**: 686–694

Hermans L*, Kaynak M*, Braun J, Rios VL, Chen C-L, Friedberg A *et al* (2022) Microengineered devices enable long-term imaging of the ventral nerve cord in behaving adult *Drosophila*. *Nat Commun* **13**(1): 5006

ALL-OPTICAL INTERROGATION OF EPISODIC MEMORY IN THE HIPPOCAMPUS

cf. BIF FUTURA, VOL. 33 | 2.2018

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Discipline: Neurobiologist, MSc
Institute: Wolfson Institute for Biomedical Research, University College London, UK
Supervisor: Prof. Michael Häusser

Episodic memory captures particular experiences in their spatial and temporal context. The hippocampus is thought to support this ability by generating neural sequences of place cells (which encode location) and time cells (which encode the timing of events) that associate events across space and time. However, experimental evidence linking such sequences to memory and behaviour is largely correlational, and the causal role of these activity patterns is not yet known. Through all-optical interrogation of neural circuits, which combines two-photon imaging with holographic two-photon optogenetics, the activity of hundreds of neurons can be simultaneously read out and manipulated at cellular resolution in mice while they solve cognitive tasks. In my PhD project, I used this approach to probe the causal role of hippocampal activity patterns in episodic memory. I contributed to providing the first direct evidence of a causal role for place cells in memory-guided spatial behaviour. Targeted activation of place cells outside their natural firing field during a virtual reality navigation task drove specific behaviours that were associated with the stimulated cells' spatial tuning. I also investigated the role of time cell sequences in associating events across time. Using two-photon imaging, I recorded such sequences during a temporal association memory task. Sequences spanned a temporal gap between stimuli more evenly in running mice, and more linear sequences resulted in faster learning of new associations between these stimuli – suggesting that the organization of sequences is important for learning. Next, I developed an all-optical strategy for activating such sequences across the temporal gap during learning. Photostimulation resulted in the formation of new firing fields in the activated neurons, thereby imprinting these neurons into the endogenous sequences. My work provides causal evidence linking place cell activity and hippocampal sequences to the neural code of episodic memory.

PUBLICATION

Robinson N, Descamps L, Russell L, Buchholz M, Antonov G, Lau J *et al* (2020) Targeted activation of hippocampal place cells drives memory-guided spatial behavior. *Cell* **183**(7): 2041–2042

NUTRIENT TRANSPORTER SCREEN IDENTIFIES METABOLIC REQUIREMENTS FOR METASTASIS

cf. BIF FUTURA, VOL. 35 | 2.2020

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Supervisor: Prof. Sarah-Maria Fendt

Cancer metastasis is a multi-step process in which cancer cells disseminate from a primary tumour and spread throughout the body to different organs. When metastasizing cancer cells arrive at secondary organs, they must adapt their metabolism to the local nutrient environment. Which nutrients are important at different sites of metastasis is not known. A better understanding of these requirements could help to prevent metastatic relapse in patients. To address this, I performed a loss-of-function CRISPR screen against solute carrier (SLC) transporters in a breast cancer mouse model to define the nutrient requirements of lung and liver metastases. I found that most of the SLC transporter dependencies of the metastases were organ-specific, meaning that cancer cells from the same tumour have different nutrient requirements based on where they colonize. One example was the mitochondrial iron importer Slc25a37 (mitoferrin-1), whose loss inhibited liver but not lung metastases in the 4T1 mouse model of metastasis. I also showed that the SLC25A37 protein was expressed more highly in liver than in lung metastases of human breast cancer patients. Using metabolomics techniques, I found that Slc25a37 expression was required in the liver to support haem synthesis in cancer cells in mouse models of metastasis. Loss of Slc25a37 inhibited haem synthesis in liver metastases but not in lung metastases. Genetically deleting haem importers in cancer cells then injecting them into mice showed that cancer cells in the lung rely on extracellular haem import and are therefore less sensitive to loss of Slc25a37. By measuring haem metabolites in metastatic tissue in mice, I found that haem in both lung and liver metastases is metabolized to bilirubin, which is necessary for metastasis survival in both organs. By comprehensively defining the nutrient transport liabilities of metastases in different tissues, I have provided evidence that these liabilities are partly related to nutrient availability in the tumour microenvironment. This work may stimulate further studies on the nuanced role of iron metabolism in cancer and the role of haem in cancer progression.

PUBLICATION

Altea-Manzano P, Cuadros A, Broadfield LA (2020) Nutrient metabolism and cancer in the *in vivo* context: a metabolic game of give and take. *EMBO Rep* **21**(10): e50635

USING GENETIC AND CHEMICAL SCREENS TO DISCOVER *HNF1A* REGULATORS IN HUMAN BETA CELLS

cf. BIF FUTURA, VOL. 35 | 1.2020

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Institute: Centre for Genomic Regulation (CRG), Barcelona, Spain
Supervisor: Prof. Jorge Ferrer

Hepatocyte nuclear factor 1A (HNF1A) is a core transcription factor in insulin-secreting beta cells within pancreatic islets. Heterozygous mutations in *HNF1A* lead to haploinsufficiency and monogenic diabetes. Moreover, loss-of-function *HNF1A* variants increase the risk of type 2 diabetes (T2D), whereas gain-of-function variants decrease it. Stimulating the expression of functional *HNF1A* alleles thus represents a promising strategy for treating T2D. However, pathways that regulate *HNF1A* expression and HNF1A-dependent functions in human beta cells are poorly understood. To explore HNF1A-dependent mechanisms, I generated a model of HNF1A-deficient diabetes using human pluripotent stem-cell-derived islets and compiled a genome-wide list of HNF1A direct targets. I showed that re-expressing *HNF1A* in HNF1A-deficient cells restores the transcription of key HNF1A direct targets and improves beta cell function. I then performed a genome-wide CRISPR activation screen in human beta cells and identified 285 activators and 77 repressors of *HNF1A*. By integrating this data with a drug repurposing screen, I found common genes and pathways regulating *HNF1A*, including calcium channel activity and signalling through prostanoid or neurotransmitter receptors. The regulatory effects of selected pathways were supported by analysis of gene co-expression in primary human islet cells and genetic analysis, which showed that variants increasing the expression of *HNF1A* activator genes in pancreatic islets decrease T2D susceptibility. In addition to this resource of genes and small molecules that regulate *HNF1A* expression, I generated a model of *HNF1A* haploinsufficiency to test their potential to restore HNF1A-dependent transcription and enhance beta cell function. My work thus elucidates gene regulatory networks that are critical for human beta cell function and provides a disease-relevant model to further develop disease-modifying therapies for diabetes.

PUBLICATION

Atla G, Bonàs-Guarch S, Cuenca-Ardura M, Beucher A, Crouch DJM, Garcia-Hurtado J *et al* (2022) Genetic regulation of RNA splicing in human pancreatic islets. *Genome Biol* **23**(1): 196

MOLECULAR MECHANISMS OF CYTOKINE CLEAVAGE BY INFLAMMATORY CASPASES

cf. BIF FUTURA, VOL. 35 | 2.2020

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Discipline: Biochemist, MSc
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Supervisor: Prof. Jonathan Kagan

The ability to induce inflammation is critical to defend against infection. Pro-inflammatory interleukin (IL)-1 family cytokines are synthesized as inactive pro-forms that must be cleaved by inflammatory caspases to become active. Only caspase-1 was thought to efficiently cleave IL-1 cytokines. However, species of the order Carnivora (which includes dogs and cats) lack caspase-1 but have a hybrid caspase-1/4 that resembles human caspase-4. In my PhD project, I showed that canine caspase-1/4 performs all caspase-1 functions, including cleaving IL-1 cytokines. Functional analyses revealed that a conserved patch of positively charged amino acids near the active site of carnivoran caspase-1/4 and caspase-1 is crucial for cleavage. This enabled me to redesign caspase-4 to cleave the IL-1 cytokine IL-1β. In cells, redesigned caspase-4 and carnivoran caspase-1/4 operated as a single-protein signalling pathway that is activated in response to bacterial infection, leading to cleavage and release of IL-1β independently of caspase-1. I explored whether similar pathways exist in humans and found that human caspase-4 can process the IL-1 cytokine IL-18. Bacterial infection induces caspase-4-dependent IL-18 cleavage and release from human macrophages, independent of caspase-1. In collaboration with the Wu lab at Harvard, I used cryo-electron microscopy to solve the first structures of caspases bound to a cytokine. They revealed that caspase-4 and caspase-1 engage pro-IL-18 through a unique mechanism involving two distinct interfaces. Uncleaved pro-IL-18 has extensive structural differences from mature IL-18; cleavage of the pro-form induces conformational changes that allow the mature cytokine to engage the IL-18 receptor on target cells. My work revealed insights into caspase-mediated IL-1 cytokine maturation and signalling that provide molecular targets for the design of drugs against inflammatory diseases.

PUBLICATIONS

Dong Y*, Bonin JP*, Devant P*, Liang Z*, Sever AIM, Mintseris J *et al* (2024) Structural transitions enable interleukin-18 maturation and signaling. *Immunity* **57**: 1533–1548.e10

Devant P*, Dong Y*, Mintseris J, Ma W, Gygi SP, Wu H *et al* (2023) Structural insights into cytokine cleavage by inflammatory caspase-4. *Nature* **624**: 451–459

Devant P, Cao A, Kagan JC (2021) Evolution-inspired redesign of the LPS receptor caspase-4 into an interleukin-1β–converting enzyme. *Sci Immunol* **6**: eabh3567

A SEARCH FOR FUNCTIONAL CONNECTIVITY RULES IN THE THALAMUS AND HIPPOCAMPUS

cf. BIF FUTURA, VOL. 33 | 2.2018

MARTIN FERNHOLZ
Discipline: Neuroscientist, MSc
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Supervisor: Prof. Tobias Bonhoeffer

The brain follows largely unknown wiring rules to establish its functional connectivity. In my PhD project, I investigated these rules in two circuits. First, members of the Bonhoeffer group and I investigated how visual information from two eyes is merged at the first synapse after the retina, the dorsolateral geniculate nucleus (dLGN). Using a novel optogenetic approach, we discovered that although more than 60 % of dLGN neurons are structurally connected to both eyes, more than 95 % of the functional input comes from one eye. This functional monocularity is exacerbated by differences in the expression of two subtypes of glutamate receptors in dLGN neurons at these synapses. We concluded that functional binocular convergence is limited in this part of the visual pathway; instead, fine-scale input selection and refinement result in a "winner takes all" wiring rule. Second, I investigated the functional connectivity rules of dendritic spines – small protrusions that form the postsynaptic part of most excitatory synapses of the brain. New spines form after periods of strong neuronal activity or during learning. However, the identity of the presynaptic partners with which spines form functional synapses remains elusive. Resolving and quantifying dendritic spines with high throughput is challenging. I improved the throughput of an existing *in vitro* assay by establishing volumetric two-photon imaging and by developing DeepD3 – an open deep-learning framework for detecting dendritic spines and dendrites. DeepD3 enables fast and reproducible quantification, a time-intensive and error-prone process that is typically done manually. With these improvements, I performed pharmacological and computational experiments aimed at isolating events of synaptic transmission in spines. Unfortunately, neither experimental approach proved effective. Nevertheless, my work paves the way for future efforts to investigate the connectivity rules of dendritic spines with the required throughput.

PUBLICATIONS

Fernholz MHP, Guggiana Nilo DA, Bonhoeffer T, Kist AM (2024) DeepD3, an open framework for automated quantification of dendritic spines. *PLOS Comput Biol* **20**(2): e1011774

Bauer J*, Weiler S*, Fernholz MHP*, Laubender D, Scheuss V, Hübener M, Bonhoeffer T, Rose T (2021) Limited functional convergence of eye-specific inputs in the retinogeniculate pathway of the mouse. *Neuron* **109**(15): 2457–2468

SPECIES-SPECIFIC SPLICING OF *CAMK2B*, A KEY REGULATOR OF SYNAPTIC PLASTICITY

cf. BIF FUTURA, VOL. 33 | 2.2018

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Supervisors: Prof. Markus Wahl, Prof. Florian Heyd

Advances in RNA sequencing have revealed the significant role of alternative splicing in transcriptome diversity, especially in higher-order organisms. Several studies have shown that alternative splicing is controlled in a species-specific manner, but its regulation and functionality remain enigmatic. In my PhD project, I focused on the species-specific splicing of the calcium/calmodulin-dependent protein kinase IIβ (CaMKIIβ), a key regulator of synaptic plasticity. Having identified several primate-specific splice variants during my MSc project, I aimed to decipher the exact regulatory mechanism and its functional effects at the protein, cellular, and organismal levels in my PhD project. Through a combination of minigene splicing assays and RNA sequencing analysis, I identified a *cis*-regulatory splicing element in *CAMK2B* and showed that its sequence differs between primates and other mammals. During primate evolution, the branch point sequence was weakened by mutation and thus renders its downstream exon as an alternative exon. Weakened canonical splice elements appear to globally render constitutive exons as alternative exons, thus providing a paradigm for the *cis*-directed species-specific regulation of alternative splicing. On a functional level, I identified differences in the enzymatic activity and the altered substrate spectra of various CaMKIIβ isoforms. I used CRISPR-Cas9 to introduce the weaker primate branch point into the mouse genome, resulting in human-like *CAMK2B* splicing in the brains of mutant mice. I also observed strong impairment of long-term potentiation in CA3-CA1 synapses of these mice, thus connecting branch point-controlled, species-specific alternative splicing with a fundamental function in learning and memory.

PUBLICATIONS

Lučić I, Jiang PL, Franz A, Bursztyn Y, Liu F, Plested AJR (2023) Controlling the interaction between CaMKII and Calmodulin with a photocrosslinking unnatural amino acid. *Protein Sci* **32**(11): e4798

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ENCODED SYNTHESIS AND EVOLUTION OF CLINICALLY APPROVED 2'-MODIFIED RIBONUCLEIC ACIDS

cf. BIF FUTURA, VOL. 34 | 2.2019

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Supervisor: Dr Philipp Holliger

Xeno-nucleic acids (XNAs) are unnatural nucleic acids with altered sugar, phosphodiester backbone, or nucleobase components. Nucleic acid chemistries with 2'-modifications, such as 2'-*O*-methyl-RNA (2'OMe-RNA) and 2'-*O*-2-methoxyethyl-RNA (MOE-RNA), have shown promise in clinical applications due to properties such as high antisense-binding affinity and increased biostability by virtue of high nuclease resistance. However, conventional phosphoramidite synthesis of these 2'-modified nucleic acids is limited in the lengths it can produce, prevents parallel exploration of different sequences and modifications, and precludes evolution. In my PhD project, I developed an encoded enzymatic synthesis approach using polymerase engineering to enable the efficient synthesis of 2'OMe-RNA and MOE-RNA. This approach opens up possibilities for the evolution and selection of 2'-modified protein-binding aptamers with therapeutic potential. I used structure-guided semi-rational engineering on a thermophilic archaeal polymerase to identify and mutate a two-residue nascent-strand steric gate near its active site. This modification alleviated steric clashes within the polymerase, enabling processive synthesis of 2'-modified nucleic acids, including mixed-chemistry 2'OMe-/MOE-RNA aptamers, and unlocking their evolution. Furthermore, I established a reverse transcriptase-free selection procedure for selecting MOE-RNA aptamers, enabling me to re-select a 2'OMe-RNA vascular endothelial growth factor (VEGF) aptamer in the MOE-RNA chemistry. I also established procedures for the *de novo* selection of Tau-binding RNA, 2'OMe-RNA, and MOE-RNA aptamers. This involved four rounds of selection of random libraries to reduce the sequence diversity enough to allow deep sequencing and massively parallel screening for antigen binding using an Illumina HiSeq instrument. By advancing these aptamer selections further, my work lays the groundwork for the development of the first MOE-RNA aptamer. It also demonstrates the potential of encoded synthesis of 2'-modified nucleic acids in creating biostable aptamers using nucleic acid chemistries that are approved for human use.

PUBLICATION

Freund N*, Taylor AI*, Arangundy-Franklin S, Subramanian N, Peak-Chew S-Y, Whitaker AM *et al* (2023) A two-residue nascent strand steric gate controls synthesis of 2'-*O*-methyl- and 2'-*O*-(2-methoxyethyl)-RNA. *Nat Chem* **15**: 91–100

INVESTIGATION OF DNA METHYLATION TURNOVER IN PLURIPOTENCY AND EARLY DIFFERENTIATION

cf. BIF FUTURA, VOL. 36 | 1.2021

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Supervisor: Prof. Alexander Meissner

Throughout embryonic development, cells undergo a series of lineage decisions that are orchestrated by genetic and epigenetic mechanisms, including DNA methylation. In human pluripotent stem cells, thousands of highly methylated regions are targeted by DNA demethylases. Their local demethylation activity is counteracted by the activity of *de novo* DNA methyltransferases, resulting in a balance referred to as DNA methylation turnover. The function and mechanism of this phenomenon, which was only discovered a few years ago, are not yet understood. In my PhD project, I investigated the emergence and regulation of DNA methylation turnover during human pluripotency and early differentiation using a combination of experimental and analytical approaches. Using publicly available whole-genome DNA methylation data, I revealed that this dynamic mechanism occurs substantially at genomic regions undergoing demethylation during *in vitro* differentiation of human pluripotent stem cells into the three germ layers, but it is also active at genomic loci linked to mature lineage decisions. By revealing local differentiation-related changes in the activity of DNA demethylases, I discovered for the first time that *de novo* DNA methylation turnover is established in transient progenitor populations. This suggests an extended regulatory role for DNA methylation turnover beyond pluripotency. Furthermore, my analysis confirms that DNA methylation turnover is highly specific to certain retrotransposons, whose silencing is frequently associated with DNA methylation. In particular, of all the families of retrotransposon elements that I analysed, two evolutionarily young subfamilies of endogenous retroviruses 1 (ERV1) and K (ERVK9) were most prominently targeted by the DNA methylation turnover in human pluripotent stem cells. As these subfamilies were previously shown to be bound by pluripotency factors, including the transcription factor NANOG, my results provide a possible mechanism underlying the DNA methylation turnover during pluripotency. By exploring the target-specific regulation of DNA methylation turnover, my work emphasizes its potential role in human cell differentiation during embryonic development.

PUBLICATIONS

The results of this project have not yet been published.

FROM SOAP TO GLUE: THE SURFACE OF CHROMOSOMES IS A PARADOX DURING MITOSIS

cf. BIF FUTURA, VOL. 34 | 2.2019

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Supervisor: Dr Sara Cuylen-Häring

Every time a cell divides, it faces the challenge of accurately transmitting its chromosomes to its daughter cells. To achieve this goal, cells compact and individualize their chromosomes during mitotic entry to facilitate the attachment of spindle microtubules to their kinetochores. During exit from mitosis, however, chromosomes cluster in a spindle-independent manner. Chromosome individualization and clustering are regulated by the surfactant-like protein Ki-67, which localizes to the surface of mitotic chromosomes. How Ki-67 radically modifies its properties to switch from a chromosome repellent to an attractant during anaphase remained unknown. In my PhD project, I used quantitative live-cell microscopy and protein engineering experiments to show how Ki-67 can be converted from an electrostatically repulsive barrier into an electrostatically driven adhesive. My results support a model in which, during early mitosis, phosphorylated Ki-67 protrudes from the chromosome surface towards the cytoplasm, thereby ensuring the dispersion of individual chromosomes by a surfactant-like mechanism. Upon mitotic exit, dephosphorylation of Ki-67 causes its collapse and activates a highly conserved positively charged patch within its amino terminus. This abrupt change in the properties of Ki-67 triggers the formation of an RNA-containing liquid phase on the surface of chromosomes, which generates the adhesive force that clusters chromosomes and excludes cytosolic material prior to nuclear reformation. Thus, the results of my work revealed how phase separation is coordinated with lipid membrane confinement to re-establish nuclear-cytoplasmic compartmentalization.

PUBLICATIONS

Hernandez-Armendariz A, Sorichetti V, Hayashi Y, Koskova Z, Brunner A, Ellenberg J *et al* (2024) A liquid-like coat mediates chromosome clustering during mitotic exit. *Mol Cell* **84**(17): 3254–3270.e9

Sridharan S, Hernandez-Armendariz A, Kurzawa N, Potel CM, Memon D, Beltrao P *et al* (2022) Systematic discovery of biomolecular condensate-specific protein phosphorylation. *Nat Chem Biol* **18**(10): 1104–1114

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

EXAMINING CO-TRANSLATIONAL ASSEMBLY BY SYNTHETIC RNA TETHERING

cf. BIF FUTURA, VOL. 35 | 1.2021

TOBIAS HOCHSTÖGER
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Supervisor: Dr Jeffrey A. Chao

While many protein complexes can be assembled *in vitro*, the correct assembly of protein complexes in living cells is more challenging. This is due in part to the abundance of potential non-specific interactors that can drive the formation of deleterious protein aggregates. One strategy to avoid non-specific interactions, termed co-translational assembly, can occur for heteromeric protein complexes when the nascent chains attached to ribosomes interact directly with their binding partner, circumventing diffusion-based searching. Co-translational assembly can take place when a nascent chain is bound by an already folded free protein or when two nascent chains come into contact due to colocalization of the encoding mRNAs. During my PhD, I investigated co-translational assembly using a single-molecule imaging approach to visualize reporter mRNAs and their translation in human cells. Using split enzyme reporters of varying affinity and two orthogonal intracellular nanobodies against distinct epitope arrays, I engineered an approach to image the translation of both nascent polypeptides simultaneously. This approach revealed that mRNAs encoding a synthetic heterodimer can be colocalized simply due to the high affinity of the interacting nascent chains, whereas such interactions were absent for low-affinity interactors. Furthermore, I developed a synthetic mRNA tether, analogous to a multivalent mRNA binding protein, that enabled me to colocalize mRNAs through the binding of orthogonal coat proteins to stem-loop structures in the mRNA 3'-untranslated regions. Characterization of the mRNA tether revealed a robust degree of colocalization at 1:1 stoichiometry, no inhibition of translation, and – surprisingly – no overall changes in the levels of protein complex assembly as measured by bulk enzymatic assays. This finding suggests that mRNA colocalization may be beneficial only for protein subunits that are prone to misfolding and need to be co-translationally assembled. My work advances our understanding of the mechanisms that promote co-translational assembly, which are particularly relevant to protein misfolding diseases, and provide a framework to further interrogate the functional relevance of co-translational nascent chain interactions.

PUBLICATIONS

The results of this project have not yet been published.

RECORDING NEURAL ACTIVITY VIA SPLIT SELF-LABELLING PROTEIN TAGS

cf. BIF FUTURA, VOL. 33 | 2.2018

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Institute: Max Planck Institute for Medical Research, Heidelberg, Germany
Supervisor: Prof. Kai Johnsson

Understanding brain function involves visualizing neurons in action within neural circuitry. As calcium ions (Ca^{2+}) are correlated with neuronal activity, biosensors have been developed to record Ca^{2+} concentration changes. However, existing tools often rely on indirect methods, such as reporter gene systems or CRISPR-Cas-based tools, which have low spatiotemporal resolution or indirect coupling between the signal and readout. Optical and optogenetic approaches have better temporal resolution but may lack continuous and multiplexed recording capabilities. In my PhD project, I aimed to overcome these limitations by developing a Ca^{2+} recorder using the self-labelling protein HaloTag. With a split-HaloTag variant consisting of a large inactive protein fragment that can be reversibly complemented by a small decapeptide connected to Ca^{2+}-responsive domains, I created a Ca^{2+}-dependent protein labelling technique called Caprola. Caprola irreversibly labels cells only in the presence of both elevated Ca^{2+} and an applied fluorophore substrate. The recording period is only determined by substrate addition and wash-out. I explored the versatility of Caprola by functionally characterizing it in immortalized cell lines and primary rat neurons together with Ca^{2+} mobilizing agents or electrical stimulation. Moreover, I achieved up to three sequential recordings, allowing temporal distinction of Ca^{2+} activity periods. The persistent fluorescence enabled me to sort glioblastoma cells based on Ca^{2+} levels, followed by transcriptomic analysis. Collaborating with the German Cancer Research Center, I identified genes that were enriched in high-Ca^{2+} cells and that correlate with tumour connectivity and poor patient survival. Lastly, I used Caprola in zebrafish larvae during visual stimulation to show that the technique can directly record neuronal activity at cellular resolution. Caprola complements existing methods and could enhance our understanding of the cellular states driving biological function.

PUBLICATIONS

Huppertz M-C*, Wilhelm J*, Grenier V, Schneider MW, Falt T, Porzberg N *et al* (2024) Recording physiological history of cells with chemical labeling. *Science* **383**: 890–897

Hai L, Hoffmann D, Wagener R, Hausmann D, Azorin D, Xie R *et al* (2024) A clinically applicable connectivity signature for glioblastoma includes the tumor network driver CHI3L1. *Nat Commun* **15**: 968

mRNA DEGRADATION REGULATES GENE EXPRESSION VIA TRANSCRIPTIONAL ADAPTATION

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Supervisor: Prof. Didier Stainier

Almost every class of molecules, from proteins to non-coding RNAs, has a role in gene expression. Somewhat unexpectedly, mRNA degradation fragments, which at first glance appear to be a waste product of cytoplasmic mRNA processing, can also take part in tuning transcriptional output. This process, termed transcriptional adaptation (TA), occurs when mRNA-destabilizing mutations lead to the transcriptional upregulation of so-called adapting genes. The precise mechanism of TA is the subject of ongoing research. The goal of my PhD project was to create an inducible TA system so that the main trigger of TA and the process itself could be studied in a tightly controlled manner. I engineered mammalian cells to overexpress inducible, nonsense-mediated decay-prone transgenes and modified mammalian cells to express Cas13d, an mRNA-targeting CRISPR family effector. Using my inducible system, I showed that TA can be triggered on demand, thus enabling the study of both direct and indirect TA gene targets from the initial stages of TA. Time-course Cas13d experiments, in which the upregulation of adapting genes was tracked after mRNA degradation was initiated, revealed that genes with higher sequence similarity to the perturbed genes are upregulated more rapidly than those with lower similarity. The results of these experiments also suggest that epigenetic properties of the TA response vary depending on the trigger: Cas13d-mediated degradation of wild-type mRNAs led to immediate transcriptional enhancement that was independent of epigenetic changes, whereas Cas9 mutants presented significant and measurable alterations in chromatin accessibility. My research provides valuable knowledge and tools that advance the understanding of TA and gene expression regulation in response to physiological and supraphysiological mRNA degradation.

PUBLICATIONS

Jiang Z*, El-Brolosy MA*, Serobyan V*, Welker JM, Retzer N, Dooley CM *et al* (2022) Parental mutations influence wild-type offspring via transcriptional adaptation. *Sci Adv* **8**(47): eabj2029

Jakutis G, Stainier DYR (2021) Genotype-phenotype relationships in the context of transcriptional adaptation and genetic robustness. *Annu Rev Genet* **55**: 71–91

MAMMALIAN OOCYTES STORE PROTEINS ON CYTOPLASMIC LATTICES

cf. BIF FUTURA, VOL. 34 | 2.2019

IDA MARIE ASTAD JENTOFT
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Institute: Max Planck Institute for Multidisciplinary Sciences (MPI-NAT), Göttingen, Germany
Supervisor: Dr Melina Schuh

The oocyte is the largest cell in the biologically female body. Its large cytoplasm stores maternally deposited factors such as mRNA and proteins to support the first stages of embryonic development. These factors are responsible for driving the transition from egg to embryo. Although our understanding of maternal mRNA storage is increasing, much less is known about how oocytes store proteins for early embryogenesis. The oocyte cytoplasm is filled with enigmatic proteinaceous structures called cytoplasmic lattices (CPLs). CPLs were discovered more than 60 years ago, but their composition and role in egg and embryonic development remain elusive. It has been shown that peptidylarginine deiminase 6 (PADI6) and proteins of the subcortical maternal complex (SCMC) are important for CPL formation and that loss of CPLs prevents embryonic development beyond the two-cell stage. However, the link between PADI6, the SCMC and the CPLs was unclear. In my PhD project, I combined live-cell imaging and pan-expansion microscopy (pan-ExM) and found that CPLs are made up of PADI6 and SCMC proteins. I expressed fluorescently tagged reporter constructs of these proteins in growing oocytes and found that PADI6 and the SCMC proteins form short fibres that crowd the entire oocyte cytoplasm. Pan-ExM also allowed me, for the first time, to visualize CPLs with light microscopy and to confirm the co-localization of CPLs with PADI6 and the SCMC proteins. Next, I used comparative mass spectrometry to investigate why loss of CPLs in the oocyte leads to early embryonic arrest. I found that several proteins were depleted in the oocyte in the absence of CPLs. Importantly, these proteins were associated with several essential cellular processes in the early embryo. Using pan-ExM, I found that many of the depleted proteins localize to CPLs in wild-type oocytes. My work thus describes a novel maternal protein storage compartment in mammalian oocytes. By producing and stockpiling essential proteins on CPLs during oocyte growth, the egg can meet the immediate needs of the early embryo. This study provides insights into poorly understood yet fundamental aspects of oocyte biology as well as causes of female infertility.

PUBLICATION

Jentoft IMA, Bäuerlein FJB, Welp LM, Cooper BH, Petrovic A, So C *et al* (2023) Mammalian oocytes store proteins for the early embryo on cytoplasmic lattices. *Cell* **186**(24): 5308–5327

IDENTIFYING A SOCIAL MOTION DETECTOR IN THE VISUAL SYSTEM OF DEVELOPING ZEBRAFISH

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Supervisor: Prof. Herwig Baier

Social affiliation is a basic social behaviour emerging from individual-level behavioural rules, such as long-distance attraction and short-distance repulsion. It also underpins the collective dynamics seen, for example, in fish swarms and bird flocks. Little is known about the neural mechanisms underlying these behaviours. In my PhD project, I used biological motion, a potent visual trigger for affiliation in developing zebrafish, to investigate their visual system. Since social affiliation behaviour emerges in zebrafish only after several weeks of age, I adapted an imaging procedure for larval zebrafish so that volumetric two-photon imaging could be used on juvenile animals. This allowed me to record – for the first time – large-scale activity of thousands of neurons simultaneously. By applying anatomical alignment and neural time series analyses, I discovered a previously unknown cluster of neurons in the dorsal thalamus that strongly and selectively responded to the burst-and-glide motion frequencies that juvenile zebrafish display at this developmental stage. I mapped the anatomical cluster density to an existing electron-microscopic whole-brain reconstruction, which allowed me to identify the synaptic inputs and outputs of this cluster. This revealed a neural pathway from the eye via this cluster to multiple brain regions, including evolutionarily conserved areas for social behaviour in the hypothalamus. Finally, by removing the thalamic neuronal cluster via either targeted high-intensity two-photon laser pulses or a chemogenetic agent in transgenic animals, I showed that the animals kept a minimum average distance to virtual conspecifics but had strongly reduced attraction to social stimuli. This finding demonstrates that the dorsal thalamus cluster is essential for the attraction part of the attraction-repulsion interplay in social affiliation behaviour of juvenile zebrafish. Multiple studies have since reported a homologous cluster in the rodent thalamus with a similar gene expression profile for the processing of auditory and somatosensory social cues, indicating that the discovered dorsal thalamus cluster is an evolutionarily conserved brain area for processing sensory social cues.

PUBLICATION

Kappel JM, Förster D, Slangewal K, Shainer I, Svara F, Donovan JC *et al* (2022) Visual recognition of social signals by a tectothalamic neural circuit. *Nature* **608**: 146–152

STRUCTURAL INSIGHTS INTO TRIM21 UBIQUITINATION MECHANISMS

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Discipline: Molecular Biotechnologist, MSc
Institute: MRC Laboratory of Molecular Biology, Cambridge, UK
Supervisors: Dr David Neuhaus, Dr Leo C. James

Tripartite motif-containing protein 21 (TRIM21), a RING E3 ligase, detects antibody-coated viruses in the cytosol, leading to viral degradation and stimulation of proinflammatory pathways. TRIM21 also has significant translational potential in targeted protein degradation – which is harnessed in methods such as Trim-Away. The aim of my PhD project was to unravel TRIM ubiquitination mechanisms from structural, biochemical, and cellular perspectives. E3 ligases act by engaging ubiquitin-charged E2 enzymes (E2~Ub). I determined the crystal structure of TRIM21 in complex with a stable Ub-charged E2 enzyme, ubiquitin-conjugating enzyme E2-N (Ube2N). Combined with biochemical and cellular findings, the structure unveiled TRIM21's specific selection for Ube2N among many E2 enzymes using a mechanism conserved in many E3 ligases. Next, I captured ubiquitinated TRIM21, Ube2N~Ub, and ubiquitin-conjugating enzyme E2 variant 2 (Ube2V2) in the act of ubiquitination in a crystal structure, allowing me to reveal the structural basis of K63-Ub chain elongation. In this process, multiple TRIM21 ligases use a trans mechanism to elongate ubiquitin chains on other TRIM21 ligases. Once TRIM21 is carrying a chain of four Ubs, it switches to a faster cis mechanism. Finally, I discovered that TRIM21 ubiquitinates itself, the antibody, and the substrate. TRIM21 could also ubiquitinate and degrade lysine-less and N-acetylated substrates, suggesting non-canonical ubiquitination. These findings have profound implications for understanding the mechanisms of RING ligases, both within and beyond the TRIM family, and for developing innovative TRIM21 applications.

PUBLICATIONS

Kiss L, Rhinesmith T, Luptak J, Dickson CF, Weidenhausen J, Smyly S *et al* (2023) Trim-Away ubiquitinates and degrades lysine-less and N-terminally acetylated substrates. *Nat Commun* **14**: 2160

Kiss L, James LC (2022) The molecular mechanisms that drive intracellular neutralization by the antibody-receptor and RING E3 ligase TRIM21. *Semin Cell Dev Biol* **126**: 99–107

Kiss L, Clift D, Renner N, Neuhaus D, James LC (2021) RING domains act as both substrate and enzyme in a catalytic arrangement to drive self-anchored ubiquitination. *Nat Commun* **12**: 1220

Kiss L*, Zeng J*, Dickson CF, Mallery DL, Yang JC, McLaughlin SH *et al* (2019) A trionic anchor mechanism drives Ube2N-specific recruitment and K63-chain ubiquitination in TRIM ligases. *Nat Commun* **10**: 4502

ORGANIZATION OF SISTER CHROMATIDS IN REPLICATED CHROMOSOMES

cf. BIF FUTURA, VOL. 34 | 2.2019

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After DNA replication, each chromosome contains two sister chromatids. Their spatial organization, which contributes to fundamental processes such as DNA damage repair and chromosome segregation, is established by protein complexes called cohesins. One such complex, cohesive cohesin, entraps two sister chromatids and ensures their stable cohesion. Little is known about how cohesive cohesin is loaded and positioned along the genome or how this contributes to the 3D organization of replicated chromosomes. In my PhD project, I used sister-chromatid-sensitive Hi-C (scsHi-C) to study sister chromatid contacts across the genome in human cells. With this technology, nascent DNA strands are labelled during replication and then computationally detected in sequencing reads. The directionality of the labelled reads allows the Hi-C contacts to be split into intra-chromatid or inter-chromatid interactions, thereby enabling me to explore the spatial organization of sister chromatids. By analysing the intra-chromatid genome-wide scsHi-C maps of human immortalized HeLa cells, I discovered a significant misalignment between sister chromatids occurring in the same direction genome-wide. This misalignment was most pronounced at the localization sites of cohesive cohesin. Using perturbation experiments, I demonstrated that this misalignment depends on cohesive cohesins but is largely independent of cohesin-mediated DNA looping, CCCTC-binding factors that block cohesin movement, and transcription. By investigating sister chromatid conformation during cell cycle progression, I found that the misalignment is established early during DNA replication, supporting a model where cohesion establishment at the replication fork promotes a global register shift. I speculate that this shift might arise from the intrinsic asymmetry of DNA replication, in which different machineries synthesize leading and lagging DNA strands. My work offers insights into the spatial organization of sister chromatids and cohesive cohesin biology. It also serves as a valuable resource for modelling the structure and mechanisms underlying the establishment of the 3D organization of replicated chromosomes in human cells.

PUBLICATION

Ochs F, Green C, Szczurek AT, Pytowski L, Kolesnikova S, Brown J *et al* (2024) Sister chromatid cohesion is mediated by individual cohesin complexes. *Science* **383**(6687): 1122–1130

SKIN-DEPENDENT DEVELOPMENT OF MURINE MECHANOSENSORY NEURONS

cf. BIF FUTURA, VOL. 34 | 2.2019

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Discipline: Developmental Neurobiologist, MD
Institute: Department of Neurobiology, Howard Hughes Medical Institute, Harvard Medical School, Boston, MA, USA
Supervisor: Dr David Ginty

Tactile sensation is mediated by low-threshold mechanoreceptors (LTMRs). At the ends of these sensory neurons are specialized structures called end organs. In hairless (glabrous) skin, end organs called Meissner corpuscles are nested inside the dermis and detect the lightest forces on the skin. In hairy skin, hair follicle-associated end organs wrap around hair follicles and form lanceolate (or lance-shaped) endings, responding to puffs of air and to directional movement. These end organs can be labelled using the same genetic methods and are found in rodent and mammalian species, but they have distinct morphological properties depending on the skin type they innervate. In my PhD project, I studied how LTMR end-organ diversity is achieved in different skin types. First, by tracking sensory neuron endings in the skin of genetically labelled mice across development with single-neuron resolution, I found that structural specialization of LTMRs in hairy skin and glabrous skin arises at nearly identical times during development. I identified neurons whose axons branch along the border of glabrous and hairy skin, forming Meissner corpuscles in the former and lanceolate endings in the latter. These findings raise the possibility that developing LTMRs, which are morphologically similar until early postnatal timepoints, can form either type of end organ depending on the skin type they innervate. Using immunochemistry and RNAscope assays on mutant mice with ectopic glabrous skin on their dorsal paws, I found that the LTMRs innervating those regions formed end organs in accordance with the altered skin type. Lastly, I deleted bone morphogenetic protein receptor type Ia (*Bmpr1a*) in sensory neurons in mice, the ligands of which are highly enriched in glabrous skin but not in hairy skin. This led to strikingly aberrant Meissner corpuscles but did not affect end organs in hairy skin. By showing that embryonic LTMRs are unspecified and rely on signals from their target tissues to flexibly acquire skin-type-specific structural properties, my work aids our understanding of how sensory neurons of touch develop and how their dysfunction can lead to neuropathies and pain.

PUBLICATION

Koutsioumpa C, Santiago C, Jacobs K, Lehnert BP, Barrera V, Hutchinson J *et al* (2023) Skin-dependent development of murine mechanosensory neurons. *Dev Cell* **58**: 2032–2047

TRANSCRIPTIONAL CONTROL OF BASAL-LIKE PANCREATIC CANCER

cf. BIF FUTURA, VOL. 34 | 2.2019

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Discipline: Molecular Biologist, MD
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Supervisor: Prof. Christopher Vakoc

Aggressive cancers are characterized by a 'loosened' cellular identity, whereby the orderly differentiation and gene expression profiles of tumour cells are disrupted. Basal-like carcinomas, which can arise in many different organs, are tumours that acquire a morphology and a gene expression pattern that are usually restricted to cells in the skin and other surface tissues. One prominent example is pancreatic cancer, in which basal-like differentiation carries a dire prognosis. While the clinical relevance of this aberrant cancer cellular state has been established, its underlying molecular mechanisms remain largely unknown. The goal of my PhD project was to define the key genetic factors controlling the basal-like state of pancreatic ductal adenocarcinoma. To interrogate the role of individual genes in maintaining basal-like differentiation, I performed flow cytometry-based genetic screens in cellular models of human basal-like pancreatic cancer. This unbiased method nominated MED12, a large component of the general transcription activator complex Mediator, as a critical factor maintaining the basal state. Through follow-up epigenomic and biochemical experiments, I found a direct protein-protein interaction between MED12 and the transcription factor p63, a master regulator of basal-like carcinomas. I discovered that MED12 acts as a molecular bridge between p63 and the rest of the Mediator complex, which in turn sustains p63-dependent transcriptional activity. Lastly, I demonstrated both *in vitro* and *in vivo* that MED12 is selectively required for the survival of basal-like pancreatic cancer cells. My results define the gene regulatory landscape of the basal state of pancreatic cancer and unveil a novel target for therapies to treat this deadly disease. My findings also highlight the power of high-throughput genetic approaches in revealing elusive protein-protein interactions that control cellular identity. Future work will aim to validate the p63-MED12 complex as a potential therapeutic target in aggressive basal-like carcinomas by devising ways to perturb this interaction.

PUBLICATIONS

The results of this project have not yet been published.

THE ROLE OF STEM CELL SIGNALLING IN PITUITARY DEVELOPMENT AND TUMORIGENESIS

cf. BIF FUTURA, VOL. 33 | 1.2018

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Institute: Great Ormond Street Institute of Child Health (GOS ICH), University College London (UCL), UK
Supervisor: Prof. Juan Pedro Martinez-Barbera

Cellular senescence is a metabolically active state of cell-cycle arrest that can occur following a persistent DNA-damage response. Senescent cells and their associated pro-inflammatory secretions, which are known as the senescence-associated secretory phenotype (SASP), have been implicated in cancer. Senescent cells are also associated with adamantinomatous craniopharyngioma (ACP), a paediatric pituitary tumour. In my PhD project, I investigated the role of senescent cells and the SASP in ACP tumorigenesis *in vivo*. To characterize the contribution of SASP factors, I used a transgenic mouse line, $R26^{lsl\text{-}mBRF1}$, which expresses a mutant, constitutively active negative regulator of cytokine signalling, BRF1. This protein targets the mRNA of cytokines, decreasing their secretion. By crossing $R26^{lsl\text{-}mBRF1}$ mice with established mouse models of ACP tumorigenesis, I showed that reduction of SASP factors by BRF1 resulted in a significant decrease in tumour burden and increased survival, highlighting the detrimental role of SASP in ACP. Furthermore, I generated a novel transgenic mouse model, $p21^{FDR}$, that allowed me to isolate, genetically trace, and pharmacogenetically ablate *in vivo* cells expressing p21, a marker of cellular senescence. I validated this new genetic tool in the context of ACP and showed that senescent cells can be ablated successfully. The Martinez-Barbera lab is now using the $p21^{FDR}$ mouse model to study the dynamics of senescent cells in ACP tumorigenesis. While studying SASP, I discovered that BRF1 is a marker of $SOX2^+$ stem cells in human and mouse pituitaries. Although a population of these cells resides in the pituitary gland, their role is elusive. I showed that expression of the constitutively active BRF1 in pituitary progenitor cells resulted in severe hypopituitarism due to a failure of lineage-specified cells to terminally differentiate. Hormone production in the pituitaries of $R26^{lsl\text{-}mBRF1}$ mice, however, was rescued *ex vivo* through co-culture with wild-type pituitaries and *in vivo* with chimeric pituitaries, suggesting a paracrine mechanism. My research highlights a critical role for senescent cells in promoting tumorigenesis and provides evidence for a new paracrine mechanism by which $SOX2^+$ stem cells direct cell differentiation during pituitary development.

PUBLICATION

Haston S, Gonzalez-Gualda E, Morsli S, Ge J, Reen V, Calderwood A *et al* (2023) Clearance of senescent macrophages ameliorates tumorigenesis in KRAS-driven lung cancer. *Cancer Cell* **41**: 1242–1260. e6. doi:10.1016/j.ccell.2023.05.004.

USE OF SPIKE-INS IN RNA-SEQ TO STUDY SINGLE-CELL DRUG RESPONSES IN PANCREATIC ISLETS

cf. BIF FUTURA, VOL. 33 | 1.2018

BRENDA MARQUINA SANCHEZ
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Institute: CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria
Supervisor: Dr Stefan Kubicek

The glucagon-secreting alpha cells and the insulin-secreting beta cells of pancreatic islets are closely related developmentally and undergo transdifferentiation through a few reported genetic modifications or drug treatments. In my PhD project, I used single-cell RNA sequencing to dissect the cell-type-specific effects on pancreatic islets of treatment with two drugs that alter cellular identity: artemether and an inhibitor of forkhead box O (FOXO). I showed that during sample preparation, cell lysis can lead cell-free RNA to be co-encapsulated with other cells and constitute up to 20% of the single-cell sequencing reads. This is a common issue in islet single-cell sequencing and can be easily identified, because non-endocrine cells express hormones. To overcome this issue, I added mouse spike-in cells to the human cells during sample preparation and vice versa. Using a bioinformatic pipeline, I could then identify, characterize, and remove the contamination. This pipeline is a useful tool to ensure clean islet transcriptomes for studying drug effects. I showed that FOXO inhibition induced de-differentiation of alpha and beta cells in mouse and human samples, which was characterized by a reduction in glucagon and insulin, respectively, as well as in key transcription factors and functional genes. In alpha cells, artemether caused the fraction of alpha cells that also express insulin to triple in the human sample and double in the mouse. In both species, this subpopulation of alpha cells was also characterized by a general loss of alpha-cell identity and an induction of beta-cell identity, as shown by the increase in the expression of genes involved in beta-cell development, insulin secretion, and insulin signalling. In beta cells, artemether led to de-differentiation and insulin repression in mouse samples but not in human samples. Deciphering this difference requires further study. This new method for quantitative, error-correcting single-cell RNA-sequencing data normalization using spike-in reference cells helps to resolve the complex cell-specific effects of pharmacological perturbations with high resolution and accuracy.

PUBLICATION

Marquina-Sanchez B*, Fortelny N*, Farlik M*, Vieira A, Collombat P, Bock C *et al* (2020) Single-cell RNA-seq with spike-in cells enables accurate quantification of cell-specific drug effects in pancreatic islets. *Genome Biol* **21**: 106

DECIPHERING CD8⁺ T CELL-MEDIATED IMMUNITY AGAINST SARS-COV-2

cf. BIF FUTURA, VOL. 35 | 1.2020

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Supervisor: Prof. Dirk H. Busch

Despite the pivotal role of CD8⁺ T cells in combating viral infections, their characterization in the immune response to severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) has been limited. My PhD project focused on a comprehensive examination of SARS-CoV-2-specific CD8⁺ T cells in patients who had recovered from a mild course of COVID-19. Members of the Busch lab and I developed a proprietary pool of putative immunogenic peptides and used peptides specific to 12 types of human leukocyte antigens to identify SARS-CoV-2-specific CD8⁺ T cells in 91% of patients. We even observed these cells two years after infection. To decipher the transcriptomic profiles and T-cell receptor (TCR) sequences of the cells, we used single-cell RNA sequencing. The SARS-CoV-2-specific TCR repertoire displayed significant diversity across patients and epitopes supporting the generation of a polyfunctional T-cell response upon infection. To characterize the specificity and functionality of TCRs, I reassembled TCR sequences *in silico* and integrated them into peripheral blood mononuclear cells from healthy donors using CRISPR-Cas9-mediated orthotopic TCR replacement. My detailed assessments, including measurements of TCR affinity, functional avidity, cellular avidity, and killing assays, revealed that the reassembled TCRs were highly functional and cytotoxic. By demonstrating that SARS-CoV-2-specific CD8⁺ T-cell responses are durable, diverse, and functionally robust, I have laid the foundation for TCR characterization to advance viral T-cell therapy.

PUBLICATIONS

Carr A*, Mateyka LM*, Scheu S, Bici A, Paijmans J, Reijmers RM *et al* (2024) Advances in preclinical TCR characterization: Leveraging cell avidity to identify functional TCRs. *Biol Chem* **405**(7–8): 517–529

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Wagner KI*, Mateyka LM*, Jarosch S*, Grass V, Weber S, Schober K *et al* (2022) Recruitment of highly cytotoxic CD8⁺ T cell receptors in mild SARS-CoV-2 infection. *Cell Rep* **38**(2): 110214

3D MOTION AND TORQUE GENERATION OF CROSS-LINKING KINESIN MOTORS

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Institute: TUD Dresden University of Technology, Dresden, Germany
Supervisor: Prof. Stefan Diez

The cell uses the spindle apparatus to faithfully segregate chromosomes. In this process, the spindle fibres, made of microtubules (MTs), are organized by the motor protein kinesin-5 and its antagonist kinesin-14. The two-dimensional sliding of MTs by motor proteins has been well studied, but less is known about their three-dimensional (3D) motion. In my PhD project, I further developed a 3D sliding assay in which a long MT is suspended between two pedestals, making it freely accessible. I added human kinesin-5 or kinesin-14 and short MTs and used fluorescent microscopy to analyse their sliding along the long MT. Due to their sideways motion, both motor proteins drove the helical motion of the short MTs around the long MT, but only when the short MTs were anti-parallel (with polarity in opposite direction) with the long MT. Parallel short MTs did not slide, but kinesin-5 caused them to move sideways around the long MT. The 3D sliding assay also revealed the distance between the short and long MTs (motor extension), which decreased with increasing sliding velocity, indicating a correlation between motor conformation and activity. When kinesin-5 and kinesin-14 were present at an equimolar ratio, their activities were balanced, with neither net forward nor sideways motion. To measure the force in the sideways direction, or torque, I developed a MT coiling assay in which one end of a long MT was immobilized and the other end was pushed and rotated by kinesin-5 or kinesin-14. I showed that the MT buckled, coiled, and wound into a helix, demonstrating that the motors generate torque. One of my collaborators used computational simulations to quantify the torques of motor proteins for the first time. My work provides a detailed picture of the function of mitotic motors, which could allow for flexible filament organization and torque regulation in the mitotic spindle.

PUBLICATIONS

Meißner L, Niese L, Diez S (2024) Helical motion and torque generation by microtubule motors. *Curr Opin Cell Biol* **88**: 102367

Meißner L, Niese L, Schüring I, Mitra A, Diez S (2024) Human kinesin-5 KIF11 drives the helical motion of anti-parallel and parallel microtubules around each other. *EMBO J* **43**: 1244–1256

Mitra A, Meißner L, Gandhimathi R, Renger R, Ruhnow F, Diez S (2020) Kinesin-14 motors drive a right-handed helical motion of antiparallel microtubules around each other. *Nat Commun* **11**: 2565

INHIBITION OF THE PROTON-ACTIVATED CHLORIDE CHANNEL BY PIP_2 LIPID

cf. BIF FUTURA, VOL. 36 | 1.2021

LJUBICA MIHALJEVIĆ
Discipline: Biochemist, BSc
Institute: Johns Hopkins School of Medicine, Baltimore, MD, USA
Supervisor: Dr Zhaozhu Qiu

The proton-activated chloride (PAC) channel is a ubiquitously expressed pH-sensing ion channel that regulates endosomal acidification and macropinosome shrinkage by releasing chloride from the organelle lumens. The PAC channel is also trafficked to the cell surface, where it is activated under pathological conditions related to acidosis, such as stroke, and contributes to acid-induced cell death. Although its structure and function are well characterized, its regulation remains largely unexplored. The most common and best-studied lipid regulator of ion channel function is phosphatidylinositol (4,5)-bisphosphate (PIP_2), a negatively charged phospholipid found predominantly in the inner leaflet of the plasma membrane. At least ten ion channel families depend on PIP_2 for their activity, but only one chloride channel has been reported to be inhibited by PIP_2. The goal of my PhD project was to determine whether PIP_2 could regulate the PAC channel. Using a whole-cell patch clamp technique with HEK293 cells endogenously expressing the PAC channel, I showed that the addition of PIP_2 decreased chloride currents in real time. To reveal the mechanism underlying PIP_2 inhibition, my collaborators and I solved the cryo-electron microscopy structure of the PAC channel bound to diC8-PIP_2. Unexpectedly, this structure revealed that PIP_2 binds to the PAC channel in the outer membrane leaflet. I also showed that the presence of the PIP_2-binding site depends on the PAC channel adopting a desensitized conformation, which itself depends on pH; the percentage of PAC inhibition by PIP_2 decreased with increasing pH. Furthermore, I found that a higher number of phosphates and a longer acyl chain increase the inhibitory potency of phosphatidylinositols. This indicates that the negative charge on the inositol head and acyl chain insertion into the membrane synergistically contribute to the binding and stabilization of the desensitized state of the PAC channel. The results of my work could be exploited for the design of PAC inhibitors and provide a useful tool for the future design of potential therapeutics for acidosis-related diseases involving the PAC channel.

PUBLICATION

Mihaljević L*, Ruan Z*, Osei-Owusu J, Lü W, Qiu Z (2023) Inhibition of proton-activated chloride (PAC) channel by PIP_2. *Elife* **12**: e83935

SINGLE-MOLECULE STUDIES OF THE EUKARYOTIC REPLICATIVE HELICASE CDC45/MCM2-7/GINS

cf. BIF FUTURA, VOL. 35 | 2.2020

DANIEL RAMÍREZ MONTERO
Discipline: Biophysicist, MSc
Institute: Delft University of Technology (TU Delft), The Netherlands
Supervisor: Prof. Nynke H. Dekker

Cells need to make an exact copy of their genome before cell division to ensure that each daughter cell obtains one full copy. This process is carried out by the replisome, which is driven by the replicative helicase Cdc4/Mcm2-7/GINS (CMG). Fully understanding CMG is thus crucial to grasping how the replisome achieves this remarkable task. However, the assembly and activation of CMG require a minimal set of 36 proteins, making it one of the most complex systems ever studied at the single-molecule level. In my PhD project, I developed a powerful hybrid assay combining cutting-edge biochemistry with single-molecule microscopy. This assay allowed me to directly observe and quantify the motion of CMG at the single-molecule level for the first time, using correlative optical tweezers and confocal microscopy. Working with other members of the Dekker group, I observed that CMG exhibits two distinct types of motion along DNA: unidirectional translocation and diffusion. Furthermore, we showed that ATP binding halts CMG diffusion, facilitating the initial melting of DNA required to initiate DNA replication. Our findings revealed an unexpected role for motion during CMG activation and provided mechanistic insights into the most important step in replication initiation. In addition to being used to investigate DNA replication, this assay can easily be adapted to study other similarly complex biochemical reactions at the single-molecule level, allowing the field of single-molecule biophysics to access a new level of biological complexity.

PUBLICATIONS

Ramírez Montero D, Sánchez H, van Veen E, van Laar T, Solano B, Diffley JFX, *et al* (2024) Hybrid ensemble and single-molecule assay to image the motion of fully reconstituted CMG. *J Vis Exp* **209**: e67076

Liu Z*, van Veen E*, Sanchez H, Solano B, Palmero Moya FJ, McCluskey KA *et al* (2024) A biophysics toolbox for reliable data acquisition and processing in integrated force-confocal fluorescence microscopy. *ACS Photonics* **11**(4): 1592–1603

Ramírez Montero D, Liu Z, Dekker NH (2024) *De novo* fabrication of custom-sequence plasmids for the synthesis of long DNA constructs with extrahelical features. *Biophys J* **123**(1): 31–41

Ramírez Montero D, Sanchez H, van Veen E, van Laar T, Solano B, Diffley JFX, *et al* (2023) Nucleotide binding halts diffusion of the eukaryotic replicative helicase during activation. *Nat Commun* **14**: 2082

SYSTEMATICALLY IDENTIFYING AND FUNCTIONALLY CHARACTERIZING TRANSCRIPTIONAL REGULATORS

cf. BIF FUTURA, VOL. 34 | 2.2019

FILIP NEMCKO
Discipline: Molecular Biologist, MSc
Institute: Research Institute of Molecular Pathology (IMP), Vienna, Austria
Supervisor: Prof. Alexander Stark

Mammalian proteomes are remarkably complex, comprising over 20,000 proteins that orchestrate diverse biological processes, including gene transcription. More than 2,400 of these proteins are considered putative transcriptional regulators, but their specific contributions to transcription remain largely unknown. This knowledge gap can be attributed to the limitations of current annotation methods and to the fact that a substantial proportion of the proteome remains poorly characterized, leaving much of the protein function landscape undiscovered. To address this challenge, I developed ORFtag, a versatile method for massively parallel tagging and probing protein functions on a proteome-wide scale. ORFtag uses retroviruses bearing a promoter, peptide tag, and splice donor site. Upon large-scale transduction of cultured cells, ORFtag cassettes randomly integrate into the genome and drive the transcription of nearby endogenous gene loci by splicing of the functional tag to splice-acceptor sites downstream of the integration site, creating near-N-terminal fusion proteins. ORFtag can be used to generate fusions of endogenous genes with a wide range of functional tags, facilitating proteome-wide functional screening. I systematically fused proteins to the DNA-binding domain of the bacterial tetracycline repressor (TetR), enabling their recruitment to TetO binding sites located upstream of an integrated green fluorescent protein (GFP) reporter, and screened for transcriptional regulators. My screens across mouse and human cell lines revealed both established and novel regulators, including very long proteins not accessible by other methods. In addition, our collaborators used ORFtag to find post-transcriptional regulators – highlighting the potential of this new method to uncover proteins involved in a variety of biological processes.

PUBLICATIONS

Nemčko F*, Himmelsbach M*, Loubiere V, Yelagandula R, Pagani M, Fasching N *et al* (2024) Proteome-scale tagging and functional screening in mammalian cells by ORFtag. *Nat Methods* **21**: 1668–1673

Klaus L, de Almeida BP, Vlasova A, Nemčko F, Schleiffer A, Bergauer K *et al* (2023) Systematic identification and characterization of repressive domains in Drosophila transcription factors. *EMBO J* **42**: e112100

Nemčko F, Stark A (2022) Proteome-scale identification of transcriptional activators in human cells. *Mol Cell* **82**(3): 497-499

STUDYING DNA REPLISOME COMPONENTS IN SISTER CHROMATID COHESION

cf. BIF FUTURA, VOL. 34 | 2.2019

SUDIKCHYA SHRESTHA
Discipline: Molecular Geneticist, BSc
Institute: The Francis Crick Institute, London, UK
Supervisor: Prof. Frank Uhlmann

DNA replication and chromosome segregation are coordinated through sister chromatid cohesion, where replicated chromosomes are held together until their separation during anaphase. In sister chromatid cohesion, a multi-subunit protein complex called cohesin entraps DNA within its ring conformation. How sister chromatid cohesion is established is not well understood, but it has been suggested that it involves a successful encounter between cohesin and the replisome. In my PhD project, I investigated the role of three replication fork components – topoisomerase 1 interacting factor 1 (Tof1), chromosome segregation in meiosis protein 3 (Csm3), and mediator of replication checkpoint 1 (Mrc1) – in cohesion establishment. The Tof1-Csm3 complex and Mrc1 have roles during DNA replication, including replication checkpoint signalling, securing replication fork speed, and recruiting topoisomerase I and the histone chaperone FACT (facilitates chromatin transactions). By modulating each of these functions independently, I showed that none of them explains the contribution of Tof1-Csm3 and Mrc1 to cohesion establishment. Instead, using purified components, I revealed direct and multipronged protein interactions of Tof1-Csm3 and Mrc1 with cohesin. These findings suggest that a series of physical interactions between replication fork components and cohesin could facilitate the establishment of sister chromatid cohesion during DNA replication. Structural studies often show cohesin adopting a folded conformation; if cohesion establishment involves the replisome passing through the cohesin ring, this could potentially be enabled by Tof1-Csm3 and Mrc1 facilitating the unravelling of the cohesin ring structure. Alternatively, Tof1-Csm3 and Mrc1 may tether cohesin between the points at which the complex unloads ahead of the replication fork and reloads behind the replication fork. Biochemical reconstitution of cohesion establishment through bulk assays or single-molecule studies will be instrumental for a complete molecular understanding of how cohesin complexes are established during DNA replication.

PUBLICATION

Shrestha S, Minamino M, Chen ZA, Bouchoux C, Rappsilber J, Uhlmann F (2023) Replisome-cohesin interactions provided by the Tof1-Csm3 and Mrc1 cohesion establishment factors. *Chromosoma* **132**: 117–135

MECHANISTIC INSIGHTS INTO THE DNA DOUBLE-STRAND BREAK REPAIR FACTOR MRN

cf. BIF FUTURA, VOL. 35 | 2.2020

ERIK VAN DE LOGT
Discipline: Biochemist, MSc
Institute: Gene Center Munich, Ludwig-Maximilians University of Munich, Germany
Supervisor: Prof. Karl-Peter Hopfner

DNA double-strand breaks (DSBs) can lead to large chromosome aberrations, cell death, and tumorigenesis. Two main pathways have emerged to repair these deleterious DNA lesions: non-homologous end joining and homologous recombination (HR). Error-free HR requires extensive resection of the broken DNA strands, leaving a single-stranded DNA overhang that is used to search for the complementary sequence on the sister chromatid, followed by template-dependent repair. The MRN protein complex – comprising the manganese-dependent nuclease Mre11, the ATPase Rad50, and the eukaryote-specific regulatory subunit Nbs1 – acts early in HR to recognize and tether DNA ends and initiate resection. Using cryo-electron microscopy, cross-linking mass spectrometry, and biochemical experiments, I solved the first full-length structure of MRN and thereby demonstrated how this complex integrates its scaffolding and enzymatic functions. I showed that a eukaryote-specific extension of Mre11 allows MRN to scan along the genome in an ATP-independent manner. Upon DNA-end recognition by MRN, the coiled-coils of Rad50 act as a chemo-mechanical element, clamping the DNA between them. MRN then undergoes major conformational rearrangements to cleave the DNA endonucleolytically before the break. During this process, the zinc-hook domains of Rad50's long coiled-coils enable two MRN complexes to interact, thus facilitating DNA tethering over long distances. I found that disturbing this interaction severely impairs DSB repair via not only HR but also non-homologous end joining. This finding points towards a broader function of the MRN complex. My results explain MRN's mode of action in DNA tethering and processing, a mechanism central to HR and at the forefront of meiotic defects and cancer development.

PUBLICATIONS

Rotheneder M*, Stakyte K*, van de Logt E*, Bartho JD*, Lammens K, Fan Y *et al* (2023) Cryo-EM structure of the Mre11-Rad50-Nbs1 complex reveals the molecular mechanism of scaffolding functions. *Mol Cell* **83**: 167–185

Gut F*, Kashammer L*, Lammens K, Bartho JD, Boggusch AM, van de Logt E *et al* (2022) Structural mechanism of endonucleolytic processing of blocked DNA ends and hairpins by Mre11-Rad50. *Mol Cell* **82**: 3513–3522

Stakyte K*, Rotheneder M*, Lammens K*, Bartho JD*, Gradler U, Fuchss T *et al* (2021) Molecular basis of human ATM kinase inhibition. *Nat Struct Mol Biol* **28**: 789–798

CHARACTERIZATION OF REGULATORS OF CPG ISLAND ACTIVITY IN MOUSE STEM CELLS

cf. BIF FUTURA, VOL. 34 | 2.2019

MARLENA JUDITH WISSER
Discipline: Biochemist, MSc
Institute: Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland
Supervisor: Prof. Dirk Schübeler

CpG island (CGI) promoters control the expression of over two-thirds of mammalian genes. How a high frequency of unmethylated CpGs – the hallmark of CGI promoters – contributes to promoter activity is not fully understood. In mouse embryonic stem cells (mESCs), high CpG density is thought to enhance promoter activity independently of transcription factors that bind complex DNA motifs. In my PhD project, I explored whether proteins that bind unmethylated CpGs facilitate this CpG density-dependent effect. To identify candidates in mESCs, I used oligo-affinity purification, quantitative mass spectrometry and promoters with varied CpG densities to identify proteins that preferentially bind the CpG-rich promoters. For selected targets (zinc finger and BTB domain-containing protein 2, ZBTB2; sterile alpha motif domain-containing 1, SAMD1; lysine methyltransferase 2B, KMT2B; and lysine demethylase 2B, KDM2B), I validated my findings genome-wide in mESCs using published chromatin immunoprecipitation data sets. Analysis of DNA motifs by me and a bioinformatician in the Schübeler group suggests that sequence information beyond CpGs can be important for binding, potentially explaining differential binding. By genetically deleting the selected targets in mESCs and measuring RNA levels, I found that their transcriptional effects at bound, CpG-rich sites were small. On average, transcriptional activity was slightly higher for KMT2B and ZBTB2 and slightly lower for KDM2B and SAMD1 at these sites than at CpG-poor sites. I confirmed my findings for ZBTB2 and SAMD1 using sensitized luciferase reporters stably expressed from a defined locus in mESCs. My work advances our knowledge of the CGI proteome in mESCs and confirms that proteins binding low-complexity DNA motifs contribute to CpG density-specific activity. Understanding how CpG density contributes to CGI promoter activity is crucial for predicting CGI promoter regulation and gene expression in development and disease.

PUBLICATIONS

Welte T, Goulois A, Stadler MB, Hess D, Soneson C, Neagu A *et al* (2023) Convergence of multiple RNA-silencing pathways on GW182/TNRC6. *Mol Cell* **83**(14): 2478–2492.e8

Schübeler D, Grand R, Luebke M (2021) *Use of a combination of an orphan motif and CpG density to control expression of a heterologous transgene.* European Patent Office, EP4256058

PHOSPHATASES AFFECT THE ORDER OF CDK SUBSTRATE PHOSPHORYLATION DURING THE CELL CYCLE

cf. BIF FUTURA, VOL. 35 | 2.2020

THERESA ZEISNER
Discipline: Biochemist, MSci
Institute: Cell Cycle Laboratory, The Francis Crick Institute, London, UK
Supervisor: Sir Paul Nurse

Ordered cell cycle progression is essential for the faithful propagation of the eukaryotic genome. It is regulated via the reversible phosphorylation of hundreds of proteins through the interplay of cyclin-dependent kinases (CDKs) and phosphatases. Phosphoproteomic studies have revealed CDK substrates and their phosphorylation timing during the cell cycle. However, phosphatases have historically been disregarded as unspecific housekeeping enzymes. Thus, our understanding of the role of phosphatases in ordering CDK substrate phosphorylation timing or which CDK substrates are targeted by which phosphatases is incomplete. Using a phosphoproteomics approach in fission yeast, I identified which phosphatases target which CDK substrates *in vivo* by comparing the dephosphorylation kinetics of CDK substrates in the presence and absence of each of four phosphatases: protein phosphatase 2A with a B55 regulatory subunit (PP2A-B55), PP2A-B56, protein phosphatase 1 (PP1), and cell division cycle 14 (CDC14). I found that most of the sites on CDK substrates were dephosphorylated by a specific phosphatase, with only a few jointly targeted by multiple phosphatases. The phosphatases differ in their preference for the phosphorylated residue, the surrounding amino acids, and short linear interaction motifs. To determine whether these differences affect the ordering of CDK substrate phosphorylation, I used phosphoproteomic time-course data to show that CDK substrates phosphorylated by PP2A-B55 and PP1 are, on average, phosphorylated later during the cell cycle than those targeted by CDC14 or PP2A-B56. This suggests that PP2A-B55 and PP1 may restrict some sites on CDK substrates from being phosphorylated early during the cell cycle, thereby imposing order on the timing of CDK substrate phosphorylation. I used a single-cell phosphosensor to show that, consistent with this finding, phosphorylation of a PP2A-B55 substrate happens earlier in the absence of PP2A-B55. Together, these data show that there is a division of labour between cell cycle phosphatases, which each target distinct sites on CDK substrates. Overall, my work highlights the importance of phosphatases in regulating the timing of CDK substrate phosphorylation during the cell cycle.

PUBLICATION

Basu S, Patterson JO, Zeisner TU, Nurse P (2022) A CDK activity buffer ensures mitotic completion. *J Cell Sci* **135**: jcs259626

MECHANISM OF CYCLIN E-INDUCED WHOLE-GENOME DUPLICATION

cf. BIF FUTURA, VOL. 34 | 2.2019

JINGKUN ZENG
Discipline: Cell Biologist, BSc
Institute: The Francis Crick Institute, London, UK
Supervisor: Dr John Diffley

Most human cancer cells are aneuploid. Extensive aneuploidies can be caused by whole-genome duplication (WGD), in which a cell's chromosome number is doubled. WGD is preceded by genetic alterations, but how these alterations lead to WGD is largely unclear. Although mutations in p53, a tumour suppressor that maintains genome integrity, are associated with WGD, this process can also occur in p53 wild-type tumours. In a p53 wild-type background, amplification of the cyclin E gene is associated with WGD. In my PhD project, I investigated the role of cyclin E in WGD. Using cell models that permit the inducible overexpression of cyclin E, I showed that the whole genome is duplicated in cells overexpressing cyclin E. Using an optimized live-cell imaging technique called FUCCI (fluorescent, ubiquitination-based cell cycle indicator), I performed detailed cell-cycle analysis. I discovered that cyclin E-overexpressing cells undergo WGD by bypassing mitosis, meaning the cells enter G1 after an extended G2 arrest without an intervening mitosis. The G2 arrest indicates that the G2 checkpoint is activated as a result of the replicative stress generated by cyclin E overexpression. I showed that replicative stress in general can cause mitotic bypass, as inhibiting DNA replication with aphidicolin also leads to mitotic bypass. Unexpectedly, I found that p53 is required for replicative stress-induced mitotic bypass; knocking out p53 abolished mitotic bypass almost completely, and cells entered mitotic catastrophe instead. I showed that p21, a downstream target of p53, mediates mitotic bypass by inhibiting cyclin-dependent kinases. This in turn leads to activation of the anaphase promoting complex/cyclosome (APC/C), an E3 ubiquitin ligase, which degrades the G2 marker cyclin B, effectively causing cells to enter G1 with double the DNA content. I call this new G1 phase endoreduplication cycle G1 (EC-G1). I found that most aphidicolin-induced EC-G1 cells are in a senescent-like state, but cyclin E overexpression can drive these cells into S phase, thereby allowing whole-genome duplication. My work provides new insights into the chromosomal abnormalities that are common in cancer cells, aiding our understanding of cancer evolution.

PUBLICATION

Zeng J*, Hills S*, Ozono E, Diffley J (2023) Cyclin E-induced replicative stress drives p53-dependent whole-genome duplication. *Cell* **186**: 528–542.e514

MD FELLOWSHIPS 2023

With its MD fellowships, the Boehringer Ingelheim Fonds helps outstanding medical students to pursue an ambitious experimental project in basic biomedical research. Candidates study in Germany and change their workplace (institution and city) for at least ten months to join an internationally renowned laboratory. Here, we present the 17 fellows who were granted an MD fellowship in 2023.

KONRAD BRAMBACH
Molecular characterization of an attention-like state in Drosophila

BENJAMIN DUISBERG
Identifying novel transcriptional modulators of autophagy in neurodegenerative diseases

RAHEL FAUTH
Investigating riboflavin kinase as a metabolic vulnerability in acute myeloid leukaemia

RIEKE-MARIE HACKBARTH
Epistatic effects of TREM2 and AXL on microglial transcription and cognitive improvement in AD mice

ROBERT HAVKIN
How rapamycin-insensitive mechanisms affect TSC2-deficient iPSC-derived neurons

MERLIN HEISER
Investigating the effects of sleep fragmentation in mice after heart attack

NOEL-ADRIAN HOLLOSI
How p53, vascularization, and the tumour immune microenvironment interact in liver cancer

LAURA CHRISTIN KIDA
Lineage tracing of natural killer cell evolution via mitochondrial single-cell sequencing

DU HANH NGUYEN
Extracellular vesicles and purinergic signalling in intestinal immune regulation

JANA NGUYENOVA
Effects of extended liver venous deprivation on liver regeneration in a cirrhosis background

DAVID REEG
A $CD8^+$ T cell-based approach to analyse the pathogenesis underlying post-acute sequelae of COVID-19

MARIE REHM
Defining the mechanisms of cell type-specific metabolic control of neuronal activity in the brain

ANNA MARIA REINEHR
Characterizing the impact of two candidate RNA-binding proteins on breast cancer metastasis

MARLENE SCHEFFOLD
Testing small molecule modulators of ATG9A trafficking in Ap4b1 knockout mice

TIL STEINICKE
DNA methylation-based classification of acute leukaemia

ANNA SOPHIE TIEFENBACHER
The intracellular molecular mechanism behind elevated Aβ during HSV1 infection

ENRICA WEDIG
Liver microenvironment during tissue injury and regeneration

MOLECULAR CHARACTERIZATION OF AN ATTENTION-LIKE STATE IN DROSOPHILA

KONRAD BRAMBACH

Duration: 09/23–08/24

Project at: The Crickmore Lab, Harvard University, Boston, MA, USA

Supervisor: Dr Konstantinos Dimitriadis

Home University: Ludwig-Maximilians University Munich (LMU)

IDENTIFYING NOVEL TRANSCRIPTIONAL MODULATORS OF AUTOPHAGY IN NEURODEGENERATIVE DISEASES

BENJAMIN DUISBERG

Duration: 03/23–03/24

Project at: University of Cambridge, UK

Supervisor: Prof. David C. Rubinsztein

Home University: Technical University of Munich

INVESTIGATING RIBOFLAVIN KINASE AS A METABOLIC VULNERABILITY IN ACUTE MYELOID LEUKAEMIA

RAHEL FAUTH

Duration: 10/23–08/24

Project at: Harvard Medical School, Dana-Farber Cancer Institute, Boston, MA, USA

Supervisor: Prof. Kimberly Stegmaier

Home University: Heidelberg University Hospital

EPISTATIC EFFECTS OF TREM2 AND AXL ON MICROGLIAL TRANSCRIPTION AND COGNITIVE IMPROVEMENT IN AD MICE

RIEKE-MARIE HACKBARTH

Duration: 03/23–02/24

Project at: Yale University, New Haven, CT, USA

Supervisor: Prof. Carla V. Rothlin and Prof. Sourav Ghosh

Home University: University Medical Center Hamburg-Eppendorf (UKE)

HOW RAPAMYCIN-INSENSITIVE MECHANISMS AFFECT TSC2-DEFICIENT IPSC-DERIVED NEURONS

ROBERT HAVKIN

Duration: 12/23–11/24

Project at: Harvard Medical School, Boston Children's Hospital, MA, USA

Supervisor: Prof. Mustafa Sahin

Home University: Charité – Universitätsmedizin Berlin

INVESTIGATING THE EFFECTS OF SLEEP FRAGMENTATION IN MICE AFTER HEART ATTACK

MERLIN HEISER

Duration: 10/23–09/24

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

Supervisor: Prof. Arash Haghikia

Home University: Charité – Universitätsmedizin Berlin

HOW P53, VASCULARIZATION, AND THE TUMOUR IMMUNE MICROENVIRONMENT INTERACT IN LIVER CANCER

NOEL-ADRIAN HOLLOSI

Duration: 09/23–09/24

Project at: Harvard Medical School, Boston, MA, USA

Supervisor: Prof. Dan G. Duda

Home University: Münster University Hospital

LINEAGE TRACING OF NATURAL KILLER CELL EVOLUTION VIA MITOCHONDRIAL SINGLE-CELL SEQUENCING

LAURA CHRISTIN KIDA

Duration: 12/23–04/25

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

Supervisor: Prof. Caleb Lareau

Home University: Charité – Universitätsmedizin Berlin

EXTRACELLULAR VESICLES AND PURINERGIC SIGNALLING IN INTESTINAL IMMUNE REGULATION

DU HANH NGUYEN

Duration: 11/23–09/24

Project at: Harvard University Boston, MA, USA

Supervisor: Prof. Simon C. Robson, Prof. Maria Serena Longhi

Home University: University Medical Center Hamburg-Eppendorf (UKE)

EFFECTS OF EXTENDED LIVER VENOUS DEPRIVATION ON LIVER REGENERATION IN A CIRRHOSIS BACKGROUND

JANA NGUYENOVA

Duration: 10/23–10/24

Project at: Yale University, New Haven, CT, USA

Supervisor: Prof. Julius Chapiro

Home University: Charité – Universitätsmedizin Berlin

A CD8⁺ T CELL-BASED APPROACH TO ANALYSE THE PATHOGENESIS UNDERLYING POST-ACUTE SEQUELAE OF COVID-19

DAVID REEG

Duration: 10/23–10/24

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

Supervisor: Prof. E. John Wherry

Home University: University of Freiburg

DEFINING THE MECHANISMS OF CELL TYPE-SPECIFIC METABOLIC CONTROL OF NEURONAL ACTIVITY IN THE BRAIN

MARIE REHM

Duration: 06/23–05/24

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

Supervisor: Prof. Christopher Dulla

Home University: Charité – Universitätsmedizin Berlin

CHARACTERIZING THE IMPACT OF TWO CANDIDATE RNA-BINDING PROTEINS ON BREAST CANCER METASTASIS

ANNA MARIA REINEHR

Duration: 08/23–01/25

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

Supervisor: Prof. Sohail Tavazoie

Home University: Münster University Hospital

TESTING SMALL MOLECULE MODULATORS OF ATG9A TRAFFICKING IN AP4B1 KNOCKOUT MICE

MARLENE SCHEFFOLD

Duration: 03/23–02/24

Project at: Harvard Medical School, Boston, MA, USA

Supervisor: Dr Darius Ebrahimi-Fakhari

Home University: Max Planck Institute for Medical Research, Heidelberg

DNA METHYLATION-BASED CLASSIFICATION OF ACUTE LEUKAEMIA

TIL STEINICKE

Duration: 04/23–09/24

Project at: Harvard Medical School, Boston, MA, USA

Supervisor: Prof. Volker Hovestadt

Home University: University of Münster

THE INTRACELLULAR MOLECULAR MECHANISM BEHIND ELEVATED Aβ DURING HSV1 INFECTION

ANNA SOPHIE TIEFENBACHER

Duration: 09/23–08/24

Project at: Harvard Medical School, Boston, MA, USA

Supervisor: Dr William Eimer

Home University: Heidelberg University

LIVER MICROENVIRONMENT DURING TISSUE INJURY AND REGENERATION

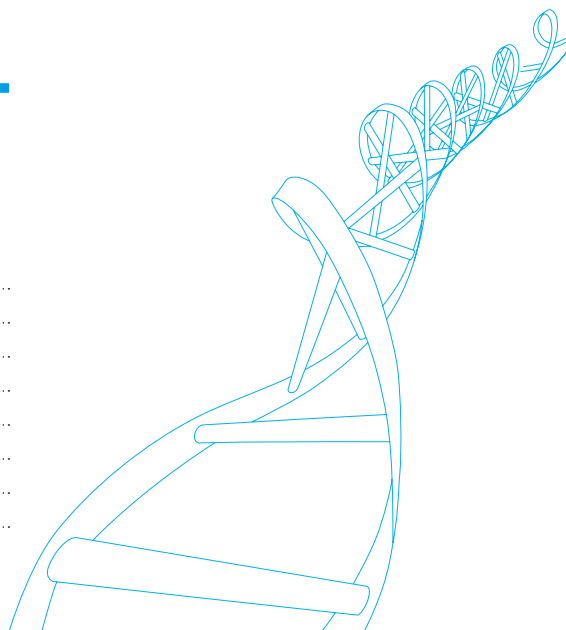
ENRICA WEDIG

Duration: 03/23–03/24

Project at: Harvard Medical School, Boston, MA, USA

Supervisor: Prof. Wolfram Goessling

Home University: University Hospital of Munich (LMU)



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.

PAPERS IN THE SPOTLIGHT

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

MISSING STORAGE LATTICE IN EGGS CAN CAUSE INFERTILITY

One of the first concerns of new mothers is to feed their offspring. New research shows that they begin doing so with their eggs.

As they grow, kids need a lot of food. The same applies to developing embryos directly after fertilization. Oocytes produce many proteins for later use by the embryo. But how do they store them safely and prevent degradation and early activation? It was long known, for example, that birds use their egg yolk as storage, but now we have learned how mammals solve this essential problem. For about 60 years, we knew mammalian egg cells possess cytoplasmic lattices. Ida Jentoft in the lab of Melina Schuh at the MPI for Multidisciplinary Sciences in Göttingen, Germany, and her collaborators have unveiled the structure of these mysterious structures pervading the cell in unprecedented detail and determined their role as a safe storage for proteins needed by the embryo.

Ida imaged oocytes of mice and the resulting embryos using expansion microscopy, which can physically enlarge a cell while keeping intact the location of its structures relative to each other. Her super-resolution images showed that the lattices were made up of bundles of elliptical filaments, stacked in a way to maximize surface area – ideal for storage. And indeed, the lattices were loaded with proteins. Ida also identified the building blocks of the lattices: PADI6 (peptidylarginine deiminase 6) and the proteins of the SCMC (subcortical maternal complex). Both were known to be involved in the lattices and in fertility, but details were lacking.

Ida also engineered mouse oocytes without PADI6 and SCMC. The resulting oocytes lacked lattices and had much lower stores of maternal proteins. The embryos stopped dividing or were heavily impaired. The scientists therefore concluded that loading proteins onto the lattices protects them from the roving recycling squad of the cell until the embryo starts developing. The authors confirmed that these findings also apply to humans, enabling us to better understand certain forms of infertility. For instance, couples with mutations in PADI6 and SCMC will most likely not benefit from *in vitro* fertilization and may be spared the ordeal.

REFERENCE

Jentoft IMA, Bäuerlein FJB, Welp LM, Cooper BH, Petrocic A, So C *et al* (2023) Mammalian oocytes store proteins for the early embryo on cytoplasmic lattices. *Cell* **186**: 5308–5327, https://doi.org/10.1016/j.cell.2023.10.003

Ida Jentoft, fellowship: 2019–2022

Photos: iStock/Yummy pic (top); private (Jentoft)

YOUR FIRST YEARS, NOT FORGOTTEN, JUST OUT OF REACH

Having no memories of early childhood is normal for humans and many mammals. During her PhD on infantile amnesia in Tomás Ryan's lab at Trinity College Dublin, Sarah Power found that early memories are present but cannot be activated by adults – up to now. For humans and mice, it was known that an intense immune response during pregnancy (maternal immune activation, or MIA) can lead to autism spectrum disorder (ASD). Sarah worked with mice in which MIA leads to male offspring with ASD-like behaviour. Unexpectedly, she found that such MIA males show no infantile amnesia.

She used new optogenetic methods to enable other mice to also recall early memories. The ensemble of nerve cells active when a memory is formed is considered the engram of this memory. It forms a connected network spread out over the brain. Cells located in the dentate gyrus (DG) of the hippocampus have a special role: they connect memories to a certain place, time, or context, making it possible to separate memories. During recall, the network of cells is reactivated: we remember.

Sarah tagged the cells of engrams formed while infant mice learned fear, navigation, and recognition tasks. In the adult mice, she then activated only those cells of a tagged engram located in the DG. As other engram cells also became active, Sarah concluded that the engrams formed in infancy are intact in the brain of adult mice and can be artificially activated.

On the cellular level, she found that compared with the control group MIA males had more engram cells in the DG with more and larger dendritic spines. The MIA males were also better at activating just the cells belonging to the relevant engram. She also demonstrated that cytokine IL17a activity is key to prevent infantile amnesia in these mice. Together, her results show that infantile amnesia is an inherent part of natural embryonic brain development and has links to ASD and the immune system. She now also works with children to better understand memory and its counterpart, forgetting, which are topics highly relevant to both health and education.

REFERENCE

Power SD, Stewart E, Zielke LG, Byrne EP, Douglas A, Ortega-de San Luis C *et al* (2023) Immune activation state modulates infant engram expression across development. *Sci Adv* **9**: 45, eadg9921, DOI: 10.1126/sciadv.adg9921

Sarah Power, fellowship: 2018–2021

You probably won't be able to remember events from your early years, no matter how intensely you experienced them.

Photos: Stocksy/Hernandez & Sorokina (bottom); private (Power)

PERSPECTIVES

FROM HIGH-RISK, HIGH-GAIN RESEARCH PROJECTS TO HEAD OF CORE RESEARCH FACILITIES

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

INTERVIEW WITH TINA DAVITER

Tina Daviter worked in Marina Rodnina's lab at the University of Witten/Herdecke, Germany, and earned her PhD in physical biochemistry there in 2003. She then moved to the UK, where from 2003 to 2005 she was a postdoc in Venki Ramakrishnan's lab at the MRC Laboratory of Molecular Biology in Cambridge before joining Gabriel Waksman's lab at Birkbeck, University of London. In both labs, she contributed her biophysical perspective to structural biology. At the Ramakrishnan lab, she was involved in work leading to the 2009 Nobel Prize. In January 2007, Tina became manager of the newly established Biophysics Centre at Birkbeck, supporting researchers and handling diverse responsibilities, from teaching students to strategic investment. As a founding member of a European biophysics facilities network, she also bolstered core facility exchange. In 2020, Tina was named the first head of Core Research Facilities at the Institute of Cancer Research (ICR), where she is now more deeply involved in strategy implementation, governance, and career support, but misses bench work.

What fascinates you about working in a core facility?

Being involved in all the different projects and seeing the whole breadth of research going on. Matching the needs of the users with the available equipment and teasing the best out of it.

How would you define your role as head of Core Research Facilities?

I make sure that my facility managers have everything they need: equipment, space, funding, people, training, even a career path. I also ensure that their operations are integrated into the wider institutional administration. Sometimes that places me between a rock and a hard place. All sides want to make research happen, even finance, but finding funds is hard. Consequently, a large part of my work is to build relationships, create opportunities, and influence decision making at all levels.

What facilities do you manage?

Light microscopy and flow cytometry facilities, both on two sites, a genomics one and two new ones, a pathology and an antibody facility. Team size varies between one and eight. In any given year, we collaborate with about 125 groups and deliver on average 325 training sessions totalling about 1,000 hours.

Are you still hands on?

No, and I do miss the feeling of a pipette. But I love the proximity to research and my background is essential.

Name one little-known fact about core facilities.

How much like running a small business it is: balancing user fees to recover cost against affordability for scientists, monetarizing our equipment through additional applications and advertising to new, also commercial, users, and finding sponsors for a proof of principle study.

Are core facilities more than a service to science?

I think core facility staff add a lot of value to research. They are key enablers as technical experts. For some microscopes it takes a year to learn how to drive them. But core facility staff provide much more. As science gets more technology-driven, they become essential collaborators. They consult on which equipment or approach to use to answer a research question, advise on sample preparation and how to acquire high-quality publishable data, set standards, and ensure research integrity. I would say this takes about 50% of their time.

Do core facilities offer a distinct career path?

An emerging one, yes. I – and others in similar positions – are trying to find ways to foster this by developing a parallel path to an academic career, ideally with interchange at all levels and visibility of our unique roles and responsibilities. Staff join at any level, as an apprentice or former group leader. Facility jobs are often permanent positions offering more security.

What would you like to tell users about core facilities?

Make the most of the expertise and knowledge of the staff to get excellent data and results, but also acknowledge this cooperation. Because we are a part of science, and we love making your experiments work.

Photo: private

PROFILES

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

Edward Lemke has been elected **fellow of the Biophysical Society** in the US in honour of his outstanding scientific excellence in regard to the function of intrinsically disordered proteins and his contributions to furthering the field of biophysics. Founded in 1958, the society currently consists of over 7,000 members in academia, government, and industry around the world.

PROFESSOR HAUKE HILLEN
Institute: University Medical Center Göttingen, Germany
Fellowship: 2014–2016

LUKAS MAGER
Institute: University Hospital of Tübingen, Germany
Fellowship: 2012–2014

MAGDALENE SCHLESIGER
Institute: Heidelberg University Hospital, Germany
Fellowship: 2011–2014

In September 2023, three BIF fellows received an **ERC Starting Grant**. They are among the approx. 15 % of successful applicants to receive roughly 1.5 million euros each. **Hauke Hillen**'s project is "MitoRNA: Structural Studies of the Human Mitochondrial RNA Life Cycle", **Lukas Mager**'s is called "SOAR: Systematic Triangulation of Pathobiont–Host Interactions", and **Magdalene Schlesiger**'s is called "Drugs and Memory: How Do Drug-Associated Contexts Drive Behaviour? The Role of Entorhinal Circuitry in Addiction".

ASSISTANT PROFESSOR MICHAEL-JOHN DOLAN
Institute: Trinity College Dublin, Ireland
Fellowship: 2012–2014

REBECCA JORDAN
Institute: The University of Edinburgh, UK
Fellowship: 2014–2016

ATTYA OMER
Institute: San Raffaele Telethon Institute for Gene Therapy, Milano, Italy
Fellowship: 2015–2017

PROFESSOR COLINDA SCHEELE
Institute: , VIB KU Leuven Center for Cancer Biology, Leuven, Belgium
Fellowship: 2015–2017

In fall 2024, another four BIF fellows were awarded an **ERC Starting Grant.** The selected projects are **Rebecca Jordan**'s "Mechanisms of Cortical Predictive Learning", **Attya Omer**'s "Harnessing Hematopoietic Stem Cell Breakthroughs to Pioneer Advances in Transplantation Therapies", **Colinda Scheele**'s "Probing the Malignant Potential of Mutant Clones in Healthy Mammary Tissue by Successive Mutagenesis", and **Michael Dolan**'s "Micro-Dissect: Dissection of Microglial State Biology in Brain Repair". Michael has also been appointed assistant professor in Trinity's School of Genetics and Microbiology at the University of Dublin.

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

In October 2023, **Barbara Treutlein** was awarded an **ERC Synergy Grant** for the project "AxoBrain: Mapping the Axolotl Brain and Its Regeneration". Her co-grantees are Elly Tanaka and Kevin Briggmann. In the words of ERC President Maria Leptin, "The selected projects are shining examples of audacious scientific thinking." Synergy Grants are meant for projects deemed too complex for a single group and therefore fund groups of outstanding researchers to pool their skills. At about the same time, Barbara was also co-awarded the **Jubilee Prize** worth 250,000 Swiss francs by the **Max Cloëtta Foundation** for outstanding achievements and active research in the field of biomedicine.

PROFESSOR CHRISTOPH THAISS
Institute: University of Pennsylvania, USA
Fellowship: 2013–2015

Christoph Thaiss has been named **Core Investigator at the Arc Institute.** Its programmes support ambitious and collaborative research across disciplines. Arc gives scientists no-strings-attached, multi-year funding and invests in developing experimental and computational technologies. This allows Arc researchers to pursue curiosity-driven research into their boldest ideas at a level often not possible in traditional academic settings. Headquartered in Palo Alto, California, Arc is a nonprofit organization founded on the belief that many important scientific programmes can be enabled by new organizational models. Arc operates in partnership with Stanford University, UCSF, and UC Berkeley.

Photos: Lemke: IMB; Hillen: MPI_MN; Mager: C_Uniklinik Tübingen; Schlesiger: private; Dolan: P Sharp_SharPPI; Jordan: D.Robertson; Omer: private; Scheele: private; Treutlein: ETH Zurich; Thaiss: private

PROFILES

IDA JENTOFT
Institute: Research Institute of Molecular Pathology (IMP), Vienna, Austria
Fellowship: 2019–2022

JOHANNES KAPPEL
Institute: Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland
Fellowship: 2019–2022

ARIEN SCHIEPERS
Institute: Memorial Sloan Kettering Cancer Center, New York, USA
Fellowship: 2018–2020

STANISLAU YATSKEVICH
Institute: Genentech, Inc., South San Francisco, USA
Fellowship: 2019–2022

Our younger fellows have also been very successful and here we provide two examples of their awards. A total of 103 institutions from across the globe nominated their star PhD for the **International Birnstiel Award 2023**. The prize is now in its fifth year and has become one of the most competitive awards of its kind. Three of the six prestigious awards for doctoral studies in the molecular life sciences went to BIF fellows: **Ida Jentoft, Arien Schiepers**, and **Stanislau Yatskevich**. **Ida** was also one of two fellows honoured with the **2024 Otto Hahn Medal** of the Max Planck Society. **Johannes Kappel** was the second. In addition, **Ida** was selected for the Otto Hahn Award, which is given to select medal winners and provides for a long-term research residency abroad, followed by leadership of a research group on the scientist's own research topic at one of the Max Planck Institutes. The award is intended to pave the way for a long-term scientific career in Germany. Johannes was also awarded an **EMBO Postdoctoral Fellowship** for his project "Reconstructing the Wiring Logic of a Spatial Cognitive Map", which has taken him from the MPI for Biological Intelligence in Munich to the FMI in Bern, Switzerland.

DAVID HASELBACH
Institute: Research Institute of Molecular Pathology (IMP), Vienna, Austria
Fellowship: 2011–2013

LEIF LUDWIG
Institute: Charité Univesitätsmedizin Berlin and Max-Delbrück-Center Berlin, Germany
MD fellowship: 2011–2012

PROFESSOR MIRIAM STÖBER
Institute: University of Geneva, Switzerland
Fellowship: 2009–2011

The end of 2023 also saw the selection of the three **EMBO** Young Investigators **David Haselbach**, **Leif Ludwig**, and **Miriam Stöber**. The programme supports young group leaders in setting up their laboratories. Awardees receive financial support for networking for four years and benefit from training opportunities, support for their lab members, and mentoring. They also become part of the more than 700-strong network of EMBO Young Investigators, Installation Grantees, and Global Investigators.

PROFESSOR MARIANNE BOES
Institute: University Medical Centre Utrecht, Utrecht, the Netherlands
Fellowship: 1998–2000

PROFESSOR PASCAL FALTER-BRAUN
Institute: Helmholtz Munich, Neuherberg, Germany
Fellowship: 1996–1999

Marianne Boes is the lead scientist for the research consortium CLARITY (Causative Link between respirAtory syncytial viRus and chronic lung diseases: Identifying Targets for therapY), which has been funded by the **European Commission with a grant of 7 million euros**. **Pascal Falter-Braun** from Helmholtz Munich is also part of the research team.

PROFESSOR HERWIG BAIER
Institute: MPI for Biological Intelligence, Martinsried, Germany
Fellowship: 1991–1994

PROFESSOR RAINER FRIEDRICH
Institute: Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland
Fellowship: 1995–1997

Fresh off the press: an **ERC Synergy Grant** has gone to our fellows **Herwig Baier** and **Rainer Friedrich** for their joint project "Neuronal Implementation of Cognitive Maps for Navigation". Further partners are Jennifer Li and Drew Robson. The 10 million euro grant will fund a six-year study on how zebrafish represent their environment internally – a project that could reshape our understanding of intelligence.

Photos: Jentoft: S. Pfoertner, MPI-MS; Kappel: private; Schiepers: private; Yatskevich: private; Haselbach: IMP; Ludwig: private; Stoeber: private; Boes: private; Falter-Braun: private; Baier: private; Friedrich: private

PROFILES

PROFESSOR RALPH BOCK
Institute: MPI of Molecular Plant Physiology, Golm, Germany
Fellowship: 1993–1996

PROFESSOR JAN ELLENBERG
Institute: Karolinska Institutet, Stockholm, Sweden
Fellowship: 1995–1998

In 2024, two additional **ERC Advanced Grants** were awarded to our fellows, one for **Ralph Bock's** project "PlaMitEng: Plant Mitochondrial Genome Engineering: Technology Development and Application to Study Fundamental Aspects of Mitochondrial Gene Expression", and the other for Jan Ellenberg's "MITOFOLD: Revealing the Structure and Mechanism of Mitotic Chromosome Folding Inside the Cell". This is Jan's second Advanced Grant. He has also been appointed the new director of the SciLife Lab and professor at Karolinska Institutet as well as affiliated professor at Stockholm University and KTH Royal Institute of Technology.

PROFESSOR VOLKER BORMUTH
Institute: Sorbonne Université, Paris, France
Fellowship: 2005–2008

PROFESSOR MICHAEL KRIEG
Institute: The Institute of Photonic Sciences, Castelldefels, Barcelona, Spain
Fellowship: 2006–2008

Two fellows received **ERC Proof of Concept Grants**: **Volker Bormuth** and **Michael Krieg**. Volker's project is called "MIOBAS: Method for Integrated All-Optical Biological Analysis at Scale" and Michael's is "ES LowLiteScope: A Light-Efficient Microscope for Fast Volumetric Imaging of Photon Starved Samples".

PROFESSOR FLORIAN SCHMIDT
Institute: University Hospital Bonn, Germany
Fellowship: 2008–2010

At the end of 2023, **Florian Schmidt** was granted an **ERC Consolidator Grant** for his project: "DEFLAMMATION: Negative Regulation of Inflammatory Responses Revealed with Camelid Nanobodies". He was also appointed Professor for Immunology of Infections at the University Hospital in Bonn, Germany. His group studies molecular mechanisms of innate immune and inflammatory responses and does comparative studies with multiple viruses using customized nanobodies.

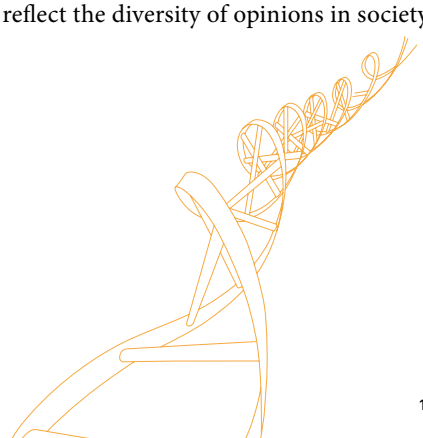
PROFESSOR MICHAEL RAPÉ
Institute: University of California, Berkeley, USA
Fellowship: 2001–2002

PROFESSOR EMRE YAKSI
Institute: Kavli Institute for Systems Neuroscience, Trondheim, Norway
Fellowship: 2004–2006

In its 60th anniversary year, **EMBO** elected 100 new **members** and 20 associate members to join its community. **Emre Yaksi** is now a member. His group studies sensory computations in the vertebrate forebrain using zebra fish. **Michael Rapé** is based in California and was already elected an associate member in 2023 for his work on protein degradation in development and disease. The now more than 2,100 members (among them more than 30 BIF fellows) guide the execution of the EMBO programmes and activities, for example by evaluating funding applications, serving on the EMBO Council and committees, and contributing to initiatives such as training, policy, outreach, and mentorship.

PROFESSOR ALDO FAISAL
Institute: Imperial College London, UK, University of Bayreuth, Germany
Fellowship: 2000–2003

In October 2024, **Aldo Faisal** was named a **member of the German Ethics Council** by the president of the German Parliament for his expertise in AI, neuroscience, and digital health. The members of the council serve in an honorary capacity for a period of four years and can be reappointed no more than once. They hold scientific, medical, theological, philosophical, ethical, social, economic, and legal expertise. In addition to scientists from the above-mentioned fields, the council also consists of members who are particularly familiar with ethical issues in the life sciences and reflect the diversity of opinions in society.



Photos: Bock: MPI-MP; Ellenberg: Karolinksa Institut; Bormuth: private; Krieg: ICFO; Schmidt: UKB_Winkler; Rapé: private; Yaksi: G Mogen Kavli Institute for Systems Neuroscience; Faisal: private

A BIF FELLOW'S GUIDE TO ...

TÜBINGEN

Travelling is fun – especially if you get insider tips from locals! In each edition of FUTURA, one fellow shows you around their city. In this edition your guide is Andrey Zyryanov, who reports from Tübingen, Germany.

FACTS & FIGURES

Country: Germany
Population: about 92,000
Area: about 108 km²
Students: about 28,500
Famous for one of the world's oldest universities, the discovery of "nuclein", now known as the DNA, and beautifull St. George's Collegiate Church.
Website: www.tuebingen.de

WHERE TO STAY

Hotel Krone: conveniently located at the city centre.
Hotel Domizil: a cosy boutique hotel located next to the Neckar River.
Hotel am Schloss: housed in an old building next to the castle with a magnificent view of the city.

NIGHTLIFE

Schwarzes Schaf: a lively night club playing various music styles.
Blauer Salon: an atmospheric bar in a squat with cheap drinks and a 15th-century gravestone welcoming you at the entrance.
Pausenhof: a small cosy bar in the southern part of the city.

RESTAURANTS

Gasthaus Bären: visit this restaurant if you want to try out classical Swabian cuisine.
Liquid Kelter: go to this bar whenever you fancy a cocktail!
An An: a great selection of Vietnamese dishes.
Freistil: come here to enjoy a large variety of beers with a view of the Neckar River.

ACTIVITIES

Winter: thermal baths with a view of the Swabian Alb in Bad Urach, chocolate and Christmas markets in the old town of Tübingen.
Spring: visit the Botanical Garden of the University of Tübingen.
Summer: swim and play sports at the Freibad Tübingen.
Fall: classical music concerts at St. George's Collegiate Church.

BEST SIGHTS

St. George's Collegiate Church 1 **:** a beautiful late Gothic church – the central landmark of Tübingen.
Kloster und Schloss Bebenhausen 2 **:** a peaceful monastery complex on the outskirts of Tübingen established in the late 12th century.
Paleontological Collection of the University of Tübingen 3 **:** unique and spectacular skeletons and fossils of pre-historic animals and plants.

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

Andrey Zyranov is 25 years old and comes from Russia. He is studying at the University of Tübingen and his supervisors are Dr Yulia Oganian and Olga Dragoy, PhD.

Photos: Barbara Honner (top left and top right); private (top centre and bottom)

PROFILES

PROFESSOR SARAH TEICHMANN
Institute: University of Cambridge, United Kingdom
Fellowship: 1997–1999

PROFESSOR GURUPRASAD MEDIGESHI
Institute: IISER Indian Institute of Science Education and Research Tirupati, India
Fellowship: 2001–2003

ASSISTANT PROFESSOR MATTEO VILLA
Institute: Medical University Graz, Vienna
Fellowship: 2011–2014

We also have a number of new professors and appointments among our fellows (if you are not named here, please get in touch, as we may not have gotten the good news yet!): **Sarah Teichmann** has been appointed Professor for Stem Cell Medicine at the University of Cambridge. She moved her group to the Cambridge Stem Cell Institute at the beginning of 2024 and is excited as the new position will open up new opportunities for her Human Cell Atlas discoveries as a reference for patient disease samples and cell and tissue engineering. **Guruprasad Medigeshi** has been appointed Professor of Biology at the Indian Institute of Science Education and Research Tirupati in India. His research is devoted mainly to host–pathogen interactions in human viral infections with a particular focus on flaviviruses, e.g. the dengue virus and respiratory viruses such as respiratory syncytial virus. **Matteo Villa** is now Assistant Professor of Experimental Rheumatology and Translational Immunology at Medical University Graz, Austria.

BIF'S SISTER FOUNDATIONS

60TH ANNIVERSARY OF THE HEINRICH WIELAND PRIZE

On 24 October, the Boehringer Ingelheim Foundation, BIF's sister foundation, awarded Benjamin F Cravatt, a professor at Scripps Research Institute, La Jolla, USA, the 2024 Heinrich Wieland Prize. He was selected for developing a new method to identify disease-causing proteins and drugs targeting those proteins.

Proteins are the building blocks of life, enabling and regulating the body's chemical reactions. Cravatt aims to understand how certain proteins contribute to disease and improve treatments. To achieve this, he invented a technology that measures the activity of all proteins within a cell – around 100,000 in humans. This groundbreaking method, called activity-based protein profiling (ABPP), is widely used globally and has already led to the development of new drugs. In honour of the 60th anniversary of the award, the prize money was raised to 250,000 euros and the accompanying symposium was extended to a full day. It included a poster session and a wide range of topics covered by senior and junior scientists.

Photos: Teichmann: private; Medigeshi: private; Villa: Marcus-Rockoff

UPCOMING EVENTS

21–26 FEB 2025
Communication training, Lautrach, Germany
Communication seminar for non-German-speaking PhD and MD fellowship holders working in Europe. The meeting will take place in Lautrach, Germany. Participants will have the opportunity to work on their writing and presentation skills with various coaches, as well as to learn more about designing graphs and figures. Further details will be sent with the invitation.

12–16 MAR 2025
130th International Titisee Conference, Titisee, Germany
The 130th ITC, titled "Stress Signalling in Development and Disease", will be chaired by Michael Rapé (Berkeley, CA, USA) and Brenda A. Schulman (Martinsried, Germany) and will highlight the interdisciplinary science that is driving the discovery, mechanistic dissection, and therapeutic targeting of stress response pathways.

ITC participation is by invitation only.

21–22 MAR 2025
Meeting of BIF's Board of Trustees
The trustees decide on the allocation of fellowships, review the proposals for the International Titisee Conferences, and handle all matters of fundamental importance for the foundation.

8–12 MAY 2025
North America meeting, Woods Hole, USA
Seminar for alumni and current PhD and MD fellows working in North America. Participants will present their scientific results. The programme is complemented by keynote presentations and talks on career opportunities and other topics. It includes tours of the scenic surroundings in Cape Cod.

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

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