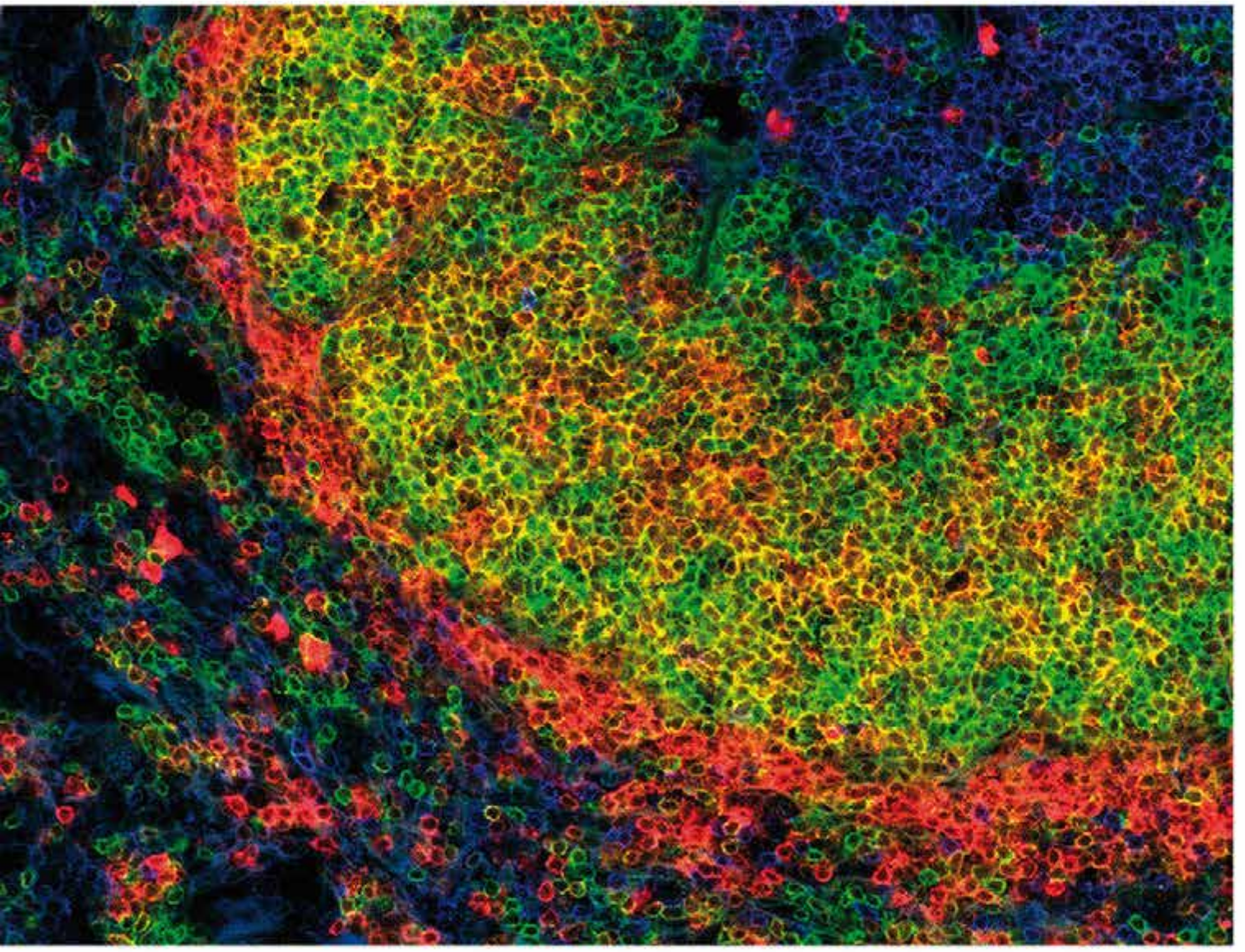
RESEARCH INSTITUTE OF MOLECULAR PATHOLOGY
VIENNA BIOCENTER

2013



Boehringer Ingelheim

IMP
Research Institute of Molecular Pathology

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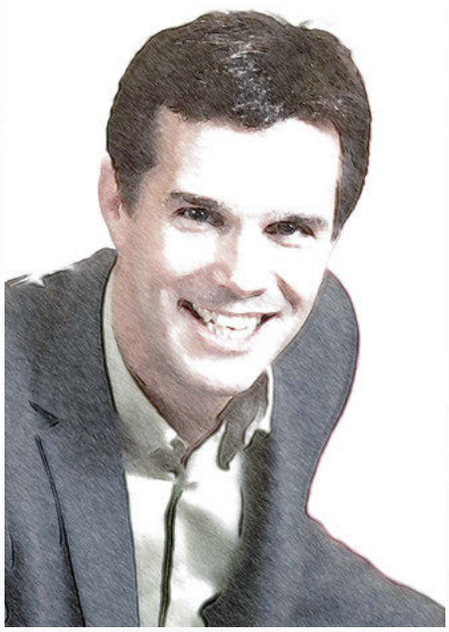


HARALD ISEMANN
Managing Director/ Finance and Administration

INTRODUCTION

2013 has been a special year for the IMP. It has been a year with many personnel changes in the faculty, including a change in directorship and, particularly exciting for us all, we have been able to develop plans for a new research building whose construction will begin at the end of next year.

Many changes that we have seen in 2013 go back to fundamental, visionary decisions that were taken when the IMP was first conceived in the mid-1980s. One of these was the design of the IMP as a 'self-renewing' institute with a career structure that fosters high turnover of scientists working at the institute. Another one was the decision that research at the IMP should only be motivated by curiosity and not applicability, despite its affiliation with Boehringer Ingelheim right from the beginning. A third was the plan to support the research groups with top quality scientific services who provide expertise in various 'high tech' areas.

This year, we have seen all of these three principles at work. There has been a high turnover at the faculty level. Most notably, Barry Dickson retired from his position as Scientific Director at the end of 2012 and moved, together with his wife Krystyna Keleman and some of their group members, to the Janelia Farm Research Campus at the Howard Hughes Medical Institute in September. Jan-Michael Peters, Deputy Director since 2011, succeeded Barry as the new Scientific Director of the IMP in July, while Meinrad Busslinger became the new Scientific Deputy Director. Barry and Krystyna have shaped science at the Vienna Biocenter Campus for many years. In particular, they established circuit neuroscience as a new research topic at the IMP and created the Vienna Drosophila RNAi library (VDRC). In the process, Barry recruited a number of outstanding neuroscientists to the IMP.

In the summer, we also saw the departure of another faculty member, Carrie Cowan. Carrie, who was group leader at the IMP for the past six years, accepted a position as Deputy Dean of the Watson School at the Cold Spring Harbor Laboratories. Also, Thomas Marlovits, a joint appointment with our collaboration partner IMBA, accepted a professorship at the University of Hamburg. Until the new facilities there are completed, Thomas will stay on as affiliated member of the IMP/IMBA faculty. We would like to thank Barry, Krystyna, Carrie and Thomas for their great contributions to the IMP and wish them all the best for their future.

In the meantime, two new group leaders started their research at the IMP at the beginning of 2013. Luisa Cochella, previously at Columbia, focuses on spatio-temporal specificity of miRNA function and its role in cell-type specification. Rushad Pavri, who joined us from Rockefeller University, studies the molecular mechanisms of antibody gene diversification, the phenomenon by which the immune system generates the vast repertoire of high-affinity antibodies that form the basis of all long-term immunity against pathogens like bacteria and viruses. Both have already proven to be wonderful additions to the IMP faculty.

The IMP's second key principle, to perform basic biomedical research which is motivated by the curiosity of world-class scientists, has once again proven to be a great success. As described in the following pages of this report, IMP scientists have made fundamental contributions to our understanding of molecular biology, immunology, neurobiology and disease modeling. They have developed ground-breaking technologies such as STARR-seq for enhancer mapping and microscopic imaging techniques that enable the simultaneous recording of many neurons at once. At the same time, other developments in 2013 have shown that basic research, despite being only driven by the motivation 'to understand how things work' can also make innovative contributions to applied science in the long run. Inspired by basic cell biological experiments carried out by former IMP Director Kim Nasmyth in budding yeast, and by the Peters research group in vertebrate cells, Boehringer Ingelheim has developed an inhibitor of Polo-like kinase 1 (PLK1), called Volasertib. In the first clinical trials for treatment of acute myeloblastic leukaemia, Volasertib performed so well that the Federal Drug Administration in the US gave it the 'Breakthrough Therapy' designation in June of this year.

The scientific excellence of the IMP has also been reflected in several other ways this year, for example by the great success that IMP scientists have had in attracting highly competitive grants, and by the honour of hosting some of the world's most famous scientists. We are particularly proud that another three IMP group leaders, Luisa Cochella, Johannes Zuber and Dave Keays, were awarded ERC Starting grants in 2013, and that Dave Keays also obtained the START Prize of the Austrian Science Fund. These achievements were celebrated together with the other Vienna Biocenter prize winners in a remarkable ERC "Eighties Revival Convention". The event gave us the delightful opportunity to listen to the only band on this planet consisting exclusively of ERC awardees. Two of our illustrious seminar speakers worth special mentioning were Nobel laureates John Gurdon and David Baltimore, who told the audience in the fully-packed IMP lecture hall about their exciting scientific work from the 1950s through to the present day.

The third principle, to support research groups with state-of-the-art technologies via service groups, has been further applied in 2013. Several new groups have started their operation as part of the Campus Science Support Facilities (CSF). Advanced Microscopy, Biocomputing and a Preclinical Imaging Facility have begun to provide services to the research groups on Campus this year. The key instrument of the Preclinical Imaging Facility is a 15.2 Tesla Ultrahigh Field Magnetic Resonance Tomograph, which is only available at one other facility worldwide. Together with the well-established IMP core facilities, we continue our strategy to have our infrastructure constantly upgraded to the latest technologies available for cutting-edge research.

What will the future bring? First and foremost, we are about to recruit several new faculty members to the IMP. Secondly, we want to strengthen academic training at the IMP, so that students and postdocs will be educated in a broad variety of topics, ranging from technical skills to soft skills. We are therefore very happy that Inês Crisóstomo has taken up the position of Coordinator of Scientific Training earlier this year. Inês has begun implementing her numerous ideas on reorganizing and improving the VBC PhD program. For example, from the beginning of next year, all new PhD students will attend a three-week introductory course to get familiar with the great variety of research and core facilities on Campus.

When founding director Max Birnstiel opened the IMP on 26 May 1988, not even the most visionary supporters of this project could have predicted how much of an impact the IMP would have on biomedical research. By enabling fundamental discoveries in biology, by training young scientists from all over the world, and by forming the nucleus of the Vienna Biocenter Campus, the IMP has, together with other research institutions such as IST-Austria and CeMM, turned Vienna into an international center of research in the life sciences.

We are particularly pleased that we have been able to start the planning for a new IMP building on the Vienna Biocenter Campus, which is scheduled for completion in the fall of 2016. The draft plans of the new IMP building provide promise of a wonderful environment that nurtures the highest of scientific creativity.

While the IMP is planning the next phase of its evolution, the remarkable development of the Vienna Biocenter Campus over the past 25 years has not only been recognized by biologists but also by historians. Maria Wirth, from the Institute of 'Zeitgeschichte' in Vienna, presented her book on the history of the Vienna Biocenter Campus this year, and quite appropriately, on the occasion of Max Birnstiel's 80th birthday.

We can all be proud to be part of this development and to be given the opportunity to 'write' history together, by making discoveries at the IMP that change our concept of how the world works. Therefore, we would like to thank all IMP employees, past and present, for their dedication and commitment in making the IMP such a unique place for scientific discovery. Finally, we would like to express our gratefulness to our shareholder Boehringer Ingelheim for the continuous and generous support of the research at the IMP.

Jan-Michael Peters and Harald Isemann

WATCHING WORMS THINK

In view of the advances originating from interdisciplinary research, it is quite surprising to note that work exceeding the traditional boundaries of a subject was held in rather low esteem until very recently. Biophysics, for instance, was frequently seen as a last refuge for students who were deemed unfit to work in physics or even biology. Perhaps this prejudice was the factor that motivated Robert Prevedel not to study biophysics, but to embark on his PhD work in Anton Zeilinger's group at the University of Vienna. However, his interest in biology did not abate during this time and also not during his post-doctoral work on imaging methods in Canada. Hence he chose to come to Vienna and work in the group of Alipasha Vaziri, who has a shared appointment at IMP and the University of Vienna.

Alipasha is a physicist with a background in quantum optics, interested in problems at the interface between physics and biology. He is investigating whether quantum effects play a role in biological systems, as well as trying to develop new tools to study biological processes at higher speeds and with greater resolution. After joining Alipasha's group, Robert elected to develop new microscopic tools for imaging brain activity at an unprecedented rate. As he disarmingly admits, "When we started we didn't have a concrete neuroscientific question in mind, but were confident that our tools would open up a range of new possibilities. We expected other groups to be interested as well." As experiments in biological sciences are often limited by what is technically possible, his expectations did not seem unreasonable – and were soon fulfilled.

Physics is not the primary focus at IMP. Hence it was natural for Alipasha's neighbors to think about entirely different problems. Despite the fact that communication between the scientists might be difficult – biology and quantum physics hardly share a common language – Alipasha regularly discussed his ideas with Manuel Zimmer, who was in the office next door. Manuel is a neurobiologist interested in the function of the nervous system in the worm *Caenorhabditis elegans*. His group had recently been augmented by the arrival of Tina Schrödel, a PhD student from Erlangen.

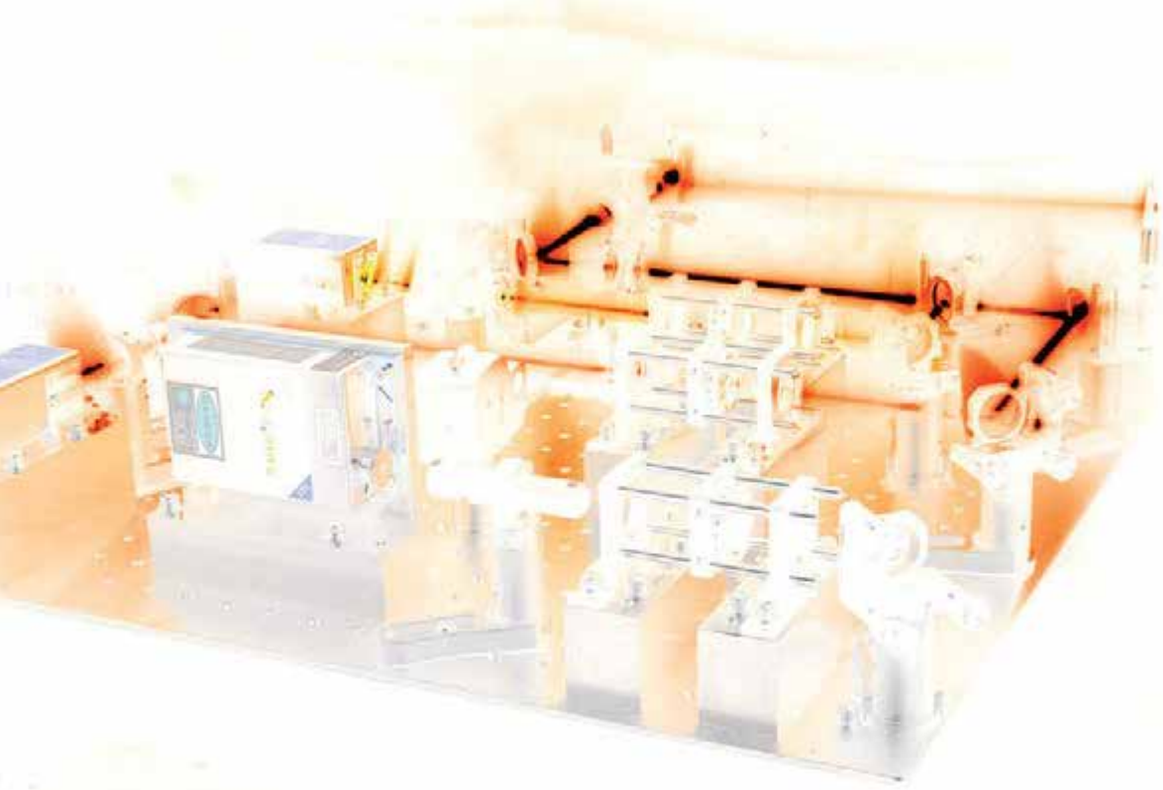
Tina had always been fascinated by the brain, particularly its ability to base decisions on information it receives and processes. She was realistic enough to recognize that the human brain is far too complicated to investigate. To make progress in this sector, it would be necessary to work with much simpler model organisms. The worm seemed an ideal choice of system: like humans, worms use information about their surroundings to decide on the best course of action. In the simple example Tina likes to cite, a worm is able to detect the level of oxygen in the surrounding soil (a low oxygen concentration indicates the presence of bacteria, on which the worm feeds), but its response to the stimulus is variable. If it is hungry it moves towards the potential meal; if it is not hungry it does not. In other words, there is a connection between sensory neurons (the input) and motor neurons (the output), and it is somehow modulated by information about the animal's nutritional state.

C. elegans has a little more than 300 neurons and a total of about 8,000 synaptic connections, making the system several orders of magnitude simpler than the human nervous system. Current efforts in human brain research are being devoted to establishing the human "connectome", a map of how individual neurons in the brain are connected to one another. However, as Tina points out, "The worm connectome was worked out 25 years ago, but we still don't know how the worm's brain works." Robert explains, "Joining the dots between the neurons can never provide the full picture. It only describes the system's constraints – what it can and cannot do – but gives no information on the dynamics of what actually happens."

Thus, we still have a lot to learn about the relatively simple brain of the worm, especially with regard to the dynamics of the system, i.e. what happens when the worm "thinks". Tina and Robert decided to tackle this challenge. When neurons are active they release calcium ions, which can be detected by a fluorescent sensor known as GCaMP. Previous work in other groups had disclosed two or three neurons at a time, but it was conceivable that Robert's advancements in imaging technology might permit many more neurons to be examined simultaneously. Tina and Robert were hoping to visualize about 8-10 neurons at a time, which would have signified a major step forward compared to what had been previously possible. Robert had been working with a two-photon technique based on extremely short laser pulses that can be "sculpted" to focus on an area of about 60 microns in diameter while retaining good axial resolution. He and Alipasha had also developed a set-up for rapid detection of photons, enabling them to avoid the customary trade-off between speed and sensitivity.

Tina's and Robert's initial experiments were thwarted by the dense packing of neurons in the brain. As Tina recalls, "The neurons turned out to be so close together that we were unable to distinguish them on 3D images – all we could see were clouds of neurons. But then we came up with the idea of attaching a nuclear localization signal to our calcium reporter so we could focus on calcium in the nuclei. This trick gave us much sharper images, on which we were able to identify the individual neurons."

The results far exceeded the researchers' dreams: they could almost simultaneously observe the activity of about 100 neurons, corresponding to about 70% of the neurons in the worm's brain. The imaging set-up could take five pictures of the brain's volume per second, providing a level of temporal resolution that is easily sufficient to study the "thought" processes in the animal, which are known to have a time scale of a



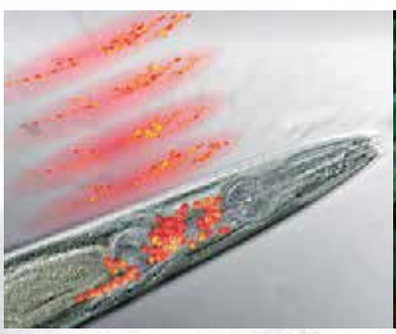
Robert Prevedel

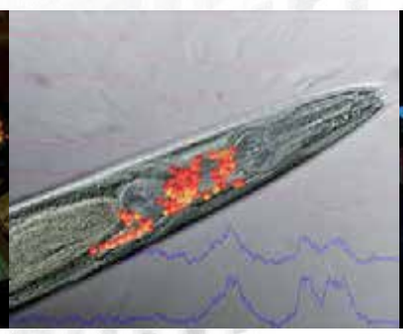
few seconds. The new system was fast enough to visualize what the neurons do in response to the presence of oxygen. Tina and Robert found that about a half of the total neurons were active in the basal condition (at atmospheric oxygen levels, i.e. 21%), while neurons of the so-called BAG and URX groups were consistently activated when the oxygen concentration was shifted between 21% and 4%. The findings were published in October in *Nature Methods* (2013, Vol. 10, 1013–1020).

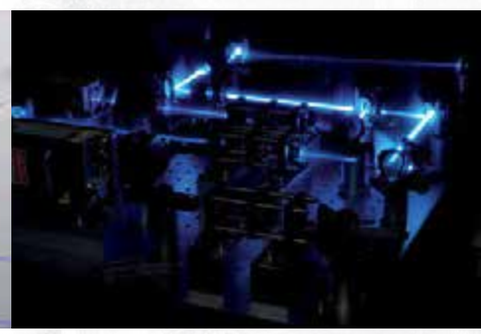
The importance of the work goes far beyond the immediate identification of neurons that help the worm to decide how to respond to oxygen. The technique has the potential to revolutionize our understanding of how brains assimilate and process information, and thus direct behavior. The new set-up can be extended, thanks to the vast array of genetic tools available in model organisms. Individual neurotransmitters can be eliminated and individual neurons triggered (using optogenetics, a further exciting development), which means that responses to a wide range of different inputs can be investigated.
Alipasha and Manuel intend to continue their collaboration and have specific projects in mind. Robert wishes to develop the method further. For technical reasons the worms are currently confined within a microfluidic chip and are unable to move. It is thus not possible to correlate the activity of motor neurons to the animal's behavior. Any possible feedback loops in the decision-making process are inactivated. Robert is contemplating the incorporation of a tracking system to overcome these limitations and thus investigate freely moving worms. If he is successful we will have all the tools we need to observe the worm's brain in action.

Of course, it should be relatively straightforward to adapt the technique for use in other systems. Technical considerations currently make it difficult to image regions larger than 200 x 200 x 100 microns, but these dimensions are sufficient to handle the majority of the fly brain or parts of the brain of the zebra fish, such as the olfactory bulb, in which input and output neurons are fairly close together. Unfortunately this is not the case in larger brains, such as those of mammals. In this setting the method cannot yield comparable comprehensive information about these systems. It is simply not possible to image the entire brain of a mouse at once. Tina is undaunted by the difficulty of working with mammalian brains. "I'd be happy if we could manage to understand the worm's brain while I'm still alive," she comments, adding "although many features of the worm may well turn out to be conserved in mammals – this is one of the reasons why we study model organisms."

Robert's hope when he came to Vienna was that he would be able to apply his knowledge of physical principles to the study of biological systems. This has clearly been fulfilled and, as Robert modestly admits, "I never thought I'd be able to contribute to a field that is so different from anything I've studied." The success of IMP's interdisciplinary approach shows, once again, that advances may stem from bringing different fields together, and clearly puts IMP at the forefront of worldwide efforts in understanding brain function.







THE VIRTUE OF PERSISTENCE - THE STARR-SEQ STORY

Research in biological sciences is clearly driven to a large extent by technology. Without X ray microscopy and NMR spectroscopy we would have a very limited understanding of molecular structure, while recent advances in sequencing technology have revolutionized genetics. Even simple techniques (such as those for the separation, visualization and large-scale preparation of proteins and nucleic acids) are indispensable in the field. But how many of us stop to think about the effort involved in the development of each of these now standard methods?

Methodological development is intrinsically beset by problems. Not only is it necessary to venture into the unknown, it can be difficult or nearly impossible to obtain third-party funding (national funding agencies have a worrying obsession with hypothesis-driven research and are extremely reluctant to support the development of techniques). If the project fails and the method does not work, the results cannot be published anywhere. As a consequence, most people are reluctant to devote their time or risk their careers on developing methods that are actually needed to advance the field. Last but not least, it is obviously difficult to encourage a prospective PhD student to gamble his or her future on work of this kind.

One person who is well aware of how difficult it is to establish a new method is Cosmas Arnold. Cosmas came to IMP from Regensburg almost five years ago, to work in Alex Stark's group. As he recalls, "I had a firm offer from FMI in Basel and was quite sure I would go there, but I met Alex and he was really convincing." He set himself the ultimate challenge of understanding the function of the regulatory non-coding DNA in the genome.

Thanks to recent advances in sequencing methodology, we have a good understanding of the genes encoded in the DNA of a wide variety of organisms. However, we have made much less progress in understanding how the expression of these genes is regulated in a tissue- and stage-specific manner. It has been believed for a long time that control is exerted via a range of enhancer sequences and non-coding regions of DNA bound by transcription factors that activate the individual genes. The theory is based on the assumption that various cells, and cells in different stages of development, utilize distinct enhancers. Unfortunately, there are simply too few enhancer sequences known to enable specific patterns to be detected and gene activity to be predicted.

The field was calling for a method to identify all enhancers that function in a given cell. This is what Cosmas and Alex decided to develop. Their initial approach was to clone random pieces of the *Drosophila* genome into a reporter plasmid carrying the gene for green fluorescent protein (GFP), and use fluorescence-activated cell sorting (FACS) to identify cells in which the GFP gene was activated. The plan was to use PCR to extract the DNA containing the enhancers. Unfortunately, the fluorescent cells had been transfected by not just one but thousands of different plasmids, and it was impossible to determine which of them carried an enhancer sequence. In fact, the fragments extracted from GFP-positive cells were no different from those in GFP negative cells. The solution sounds trivial in hindsight, but it was about a year before the researchers came up with the idea of cloning pieces of genomic DNA (possibly containing an enhancer sequence) behind the transcriptional start site of the reporter gene. Now any enhancer would effectively transcribe itself, and the level of RNA produced would provide a quantitative measure of the enhancer's activity.

Instead of sequencing the plasmid DNA, the researchers would isolate RNA from the transfected cells. Of course, it was necessary to remove the plasmid DNA. This turned out to be far more tricky than expected because the plasmids were extremely stable. The results were still too "noisy" to permit enhancers to be detected. The researchers tried several cloning strategies, various PCR conditions, and even different sources of polymerase to make the method work, but were repeatedly thwarted by the noise in the system. Although he can now afford to relax, Cosmas clearly remembers his mood at the time. "It was extremely frustrating. I didn't even have a negative result to show for my efforts. When my second 'Friday seminar' came up, all I could say was that the method still didn't work."

Despite these setbacks, Cosmas and Alex remained convinced of the approach. As Cosmas says, "It seemed simply unbelievable that it would not work." As they were both aware of the fact that Cosmas had to complete his PhD on time, they agreed to give the experiment "one final shot" before cutting their losses and finding an entirely different project for him. By this time they had inserted an intron into the reporter plasmid and were using a two-stage PCR involving a primer over the splice junction to improve the signal-to-noise ratio. They were also working with primers that had been specially engineered to protect them from degradation by the natural exonuclease activity of the polymerase (which came from a small company in South Africa – the scientists tried ten different enzymes before finding one that amplified all DNA in the genome in an unbiased fashion). For the final attempt, Cosmas tested the method on a library cloned from a 17-kb fragment of the fly genome which was known to carry an enhancer that was active in the cells. His memory of this key experiment is still vivid. "The previous six months had been really tough, but this time we finally saw a signal. This was the proof of principle we were waiting for. All we had to do was scale the technique up so that we could use it on the entire genome."

Luckily, scaling up via 160 kb to the entire *Drosophila* genome worked without too many problems. Cosmas and Alex had their hands on a

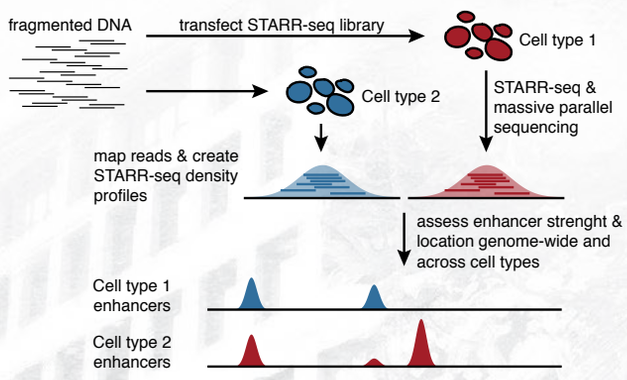
GFP expression in the somitic tissue of the Drosophila ovary driven by an enhancer identified by STARR-seq in ovarian somatic cells (OSC)

Cosmas Arnold

method which would detect all enhancers that worked in cell types and under conditions they could specify. The scientists presented the new technique, which they named STARR-seq (self-transcribing active regulatory region sequencing), at meetings in Heidelberg and Cold Spring Harbor, and formally published it in March 2013 in *Science* (Vol. 339, 1074-1077). The response was overwhelming. The paper was featured as a "research highlight" in *Nature Methods*, and was commented in *Nature Reviews*, *Nature Structural & Molecular Biology, Nature Biotechnology* and even the German-language *Laborjournal*. As the commentaries in these journals showed, more than ten years had elapsed in waiting for a functional test of enhancers that could be used on a genome-wide scale.

The Science paper essentially describes the method and shows that it can identify functional enhancer sequences in two different cell types. After nearly three years of development, Cosmas and Alex had a tool to address biological questions that had previously been out of reach. Not only are they able to define common features of enhancers that function in individual cell types or in particular stages of development (possibly even teaching computers to recognize such elements), they can examine the enhancers of genes that are activated in response to particular signals (such as after hormonal stimulation), and are able to investigate the conservation of enhancer sequences in evolution. The possibilities are limited only by the imagination of the people doing the work. As Cosmas says, "When we announced our results it was like a mini-earthquake in the field. We have since sent our plasmid out to a host of other groups. Most of the people in Alex's lab are now using the technique. We have managed to scale it up further and can use it to identify enhancers in mammalian genomes – including the human genome, which is 20 times bigger than that of the fly."

An immediate upshot of his success is that Cosmas was able to finalize his PhD. Not surprisingly, his thesis examiners were highly impressed by his persistence. Research absolutely requires developmental work of the type that Cosmas undertook with determination and patience, but the community does not usually acknowledge the efforts that go into methodological advances. Cosmas himself insists on acknowledging the part played by his colleagues, especially Martina Rath and the IMP/IMBA/CSF core facilities, in helping him through the difficult times, when even he started to doubt that they would ever get the method to work. With the advantage of hindsight it was clearly all worthwhile – and IMP has produced not only a method but also a paper that is sure to become a citation classic.



Workflow of STARR-seq - Identification of cell type -specific enhancers

MEINRAD BUSSLINGER

Stem cell commitment in hematopoiesis

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Daniela Kostanova Poliakova / Research Assistant
Karina Schindler / Research Assistant
Erika Kiligan / Research Technician

[1] until April
[2] until August
[3] until September
[4] since October

Acquired immunity to foreign pathogens depends on the differentiation of B and T lymphocytes from hematopoietic stem cells, which is controlled by a multitude of transcription factors. We are interested in understanding the process by which transcription factors regulate the commitment of early hematopoietic progenitors to lymphoid lineages and control their subsequent differentiation to mature B and T cells. We investigate the underlying transcriptional control mechanisms by using mouse transgenic, cell biological, and genome-wide molecular approaches.

B cell development

A fundamental question in hematopoiesis is how stem cells and early progenitors become committed to a single developmental pathway, and then differentiate into mature cell types of the selected lineage. The entry of lymphoid progenitors into the B cell lineage depends on several transcription factors, including E2A, EBF1, and Pax5. E2A and EBF1 function as B cell specification factors while Pax5 functions as the B cell commitment factor that restricts the developmental potential of hematopoietic progenitor cells to the B cell pathway. We have recently shown that the transcription factor Ikaros controls signaling of the pre-B cell receptor by activating genes coding for signal transducers and repressing genes involved in the down-regulation of pre-BCR signaling. As all four transcription factors are also expressed in mature B cells, we currently study their molecular functions in controlling B cell immune responses to foreign pathogens (Figure 1). Whereas the identity of mature B cells strictly depends on Pax5, the transcriptional regulator Blimp1 controls terminal differentiation to plasma cells. By analyzing the molecular mechanism of Blimp1 action, we recently showed that Blimp1 shuts down the B cell gene expression program by directly repressing B-cell-specific genes, while promoting plasma cell differentiation by activating genes involved in the unfolded protein response and in immunoglobulin secretion.

T cell development

Signaling through the Notch1 receptor is essential for initiating the development of T cells in the thymus. Besides, early T cell specification depends on the transcriptional regulator GATA3. We are currently elucidating the molecular functions of these two transcription factors in early T lymphopoiesis by conditional mutagenesis, gene expression profiling, and ChIP sequencing.

Transcriptional networks

Global genomic approaches are ideal for elucidating the transcriptional network controlling the development of B and T cells. To achieve this aim, we define the regulatory landscape of pro-T, pro-B, mature B, and plasma cells by genome-wide mapping of DNase I hypersensitive sites, transcription start sites and chromatin modifications, in order to delineate active enhancers and promoters. By ChIP sequencing we identify the binding sites of various transcription factors at these regulatory elements. Conditional mutagenesis combined with mRNA sequencing is used to study the dependence of target gene expression on the different transcription factors. These genome-wide approaches have already provided important insights into the transcriptional network controlling early and late B cell development.

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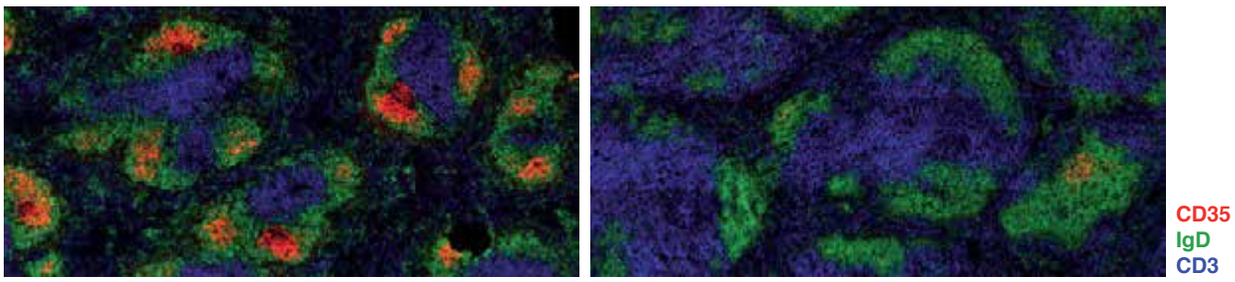

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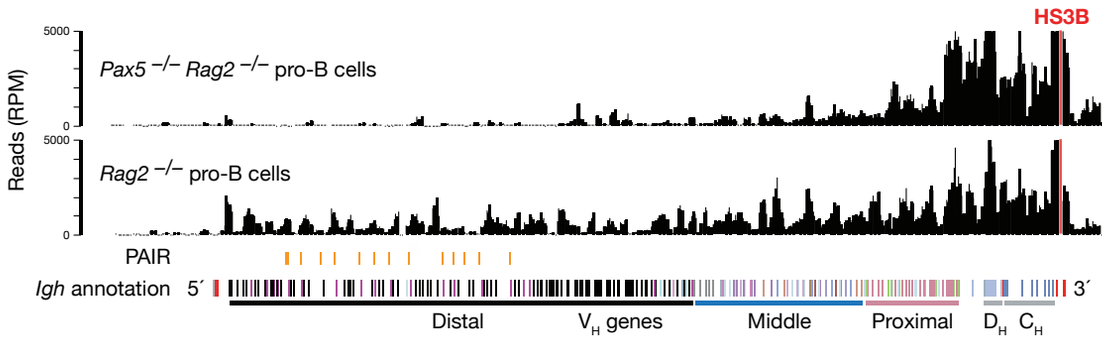

Figure 1: ***Critical role of Pax5 in B cell immunity.*** *The Cd23-Cre line deletes the floxed (fl) Pax5 allele in mature B cells of control Cd23-Cre Pax5$^{fl/+}$ and experimental Cd23-Cre Pax5$^{fl/-}$ mice, which were immunized with the antigen NP-KLH. Immunostaining of spleen sections from control mice identified CD35^{+} follicular dendritic cells (red), mature IgD^{+} B cells (green), and CD3^{+} T cells (blue). However, in the absence of Pax5, germinal centers with follicular dendritic cells were not formed in experimental mice.*

Figure 2: ***Pax5-dependent long-range interactions across the Igh locus in committed pro-B cells.*** *Uncommitted Pax5$^{-/-}$ Rag2$^{-/-}$ and committed Rag2$^{-/-}$ pro-B cells were analyzed by 4C-sequencing using a viewpoint (HS3B in red) at the 3′ end of the Igh locus. The 4C-seq reads were plotted as reads per million mapped sequence reads (RPMs). The distinct V_H gene families (different colors) in the distal, middle and proximal V_H gene regions are shown together with the D_H and C_H elements in the 3′ proximal Igh domain.*

Spatial regulation of V(D)J recombination

The development of B cells and αβ T cells depends on the functional rearrangement of *Igh* and *Igk* or *Tcrb* and *Tcra* loci, respectively. All four loci are large in size (0.7 to 3 megabases), have a complex structure, and undergo reversible contraction by looping in rearranging lymphocytes. Locus contraction is thus a general mechanism that juxtaposes distantly located V genes of the large V gene cluster next to D or J segments in the 3′ proximal domain, which facilitates V-(D)J recombination. We previously demonstrated the essential role of Pax5 in the control of *Igh* locus contraction, and identified Pax5-activated intergenic repeats (PAIRs) in the distal V_H gene cluster as potential regulatory elements involved in this process. By performing high-resolution mapping of long-range interactions, we recently showed that local interaction domains establish the three-dimensional structure of the extended *Igh* locus in lymphoid progenitors (Figure 2). In committed pro-B cells, these local domains engage in long-range interactions across the *Igh* locus (Figure 2), which depend on the regulators Pax5, YY1 and CTCF. The large V_H gene cluster thereby undergoes flexible long-range interactions with the more rigidly structured 3′ proximal domain, which ensures similar participation of all V_H genes in V_H-DJ_H recombination to generate a diverse antibody repertoire.

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TIM CLAUSEN

Molecular mechanisms of protein quality control

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All cells have evolved a sophisticated protein quality control system by which molecular chaperones and proteases monitor the functionality of each protein, thus reducing the amount of misfolded molecules that may undergo dangerous interactions. Our group performs a structure-function analysis of several eukaryotic and prokaryotic quality control factors with the purpose of disclosing novel strategies to combat protein-folding diseases and bacterial pathogenicity.

Proteolytic cascades mediating cell-cell signaling

Cellular differentiation is a process commonly associated with multi-cellular organisms and their development. However, differentiation into specialized cell types is also employed by a range of bacteria. One classic example is the endospore-forming bacterium *Bacillus subtilis* that can differentiate into dormant and stress-resistant spores to survive harsh environmental conditions. Spore formation in *Bacillus subtilis* relies on a regulated intramembrane proteolysis (RIP) pathway that synchronizes mother-cell and forespore development. To address the molecular basis of this SpoIV transmembrane signaling, we performed a structure-function analysis of the activating protease CtpB. Crystal structures reflecting distinct functional states show that CtpB constitutes a ring-like protein scaffold penetrated by two narrow tunnels. Access to the proteolytic sites sequestered within these tunnels is controlled by PDZ domains that rearrange upon substrate binding. Accordingly, CtpB resembles a minimal version of a self-compartmentalizing protease regulated by a unique allosteric mechanism. Moreover, biochemical analysis of the PDZ-gated channel combined with sporulation assays reveals that activation of the SpoIV RIP pathway is induced by the concerted activity of CtpB and a second signaling protease, SpoIVB. This proteolytic mechanism is of broad relevance for cell-cell communication, illustrating how distinct signaling pathways can be integrated into a single RIP module. Depending on the origin of the signaling proteases, such a mechanism could coordinate either different transcriptional programs within a single cell or, when the signaling proteases are secreted from two neighboring cells, link the RIP signal cascade to the cell-cell communication that coordinates developmental programs, among other aspects.

Phospho-arginine (pArg), a novel protein modification connected with bacterial stress response

The reversible phosphorylation of proteins plays a critical role in regulating almost any cellular process. Although most studies analyzing protein phosphorylation have been focused on pSer, pThr and pTyr, phosphorylation of His, Arg and Lys may constitute an equally important modification that appears to be underrepresented in the current literature. In 2008 we discovered that McsB of *B. subtilis* is a protein arginine kinase that phosphorylates and inhibits the stress-response repressor CtsR. Last year we completed studies that led to the identification of a highly specific arginine phosphatase (YwlE) counteracting McsB. Structural and biochemical data of YwlE-substrate complexes disclosed a surprisingly simple polarity filter that is installed to distinguish pArg from other phospho-residues. A single active site residue, a threonine, is used to identify the phosphorylated guanidinium

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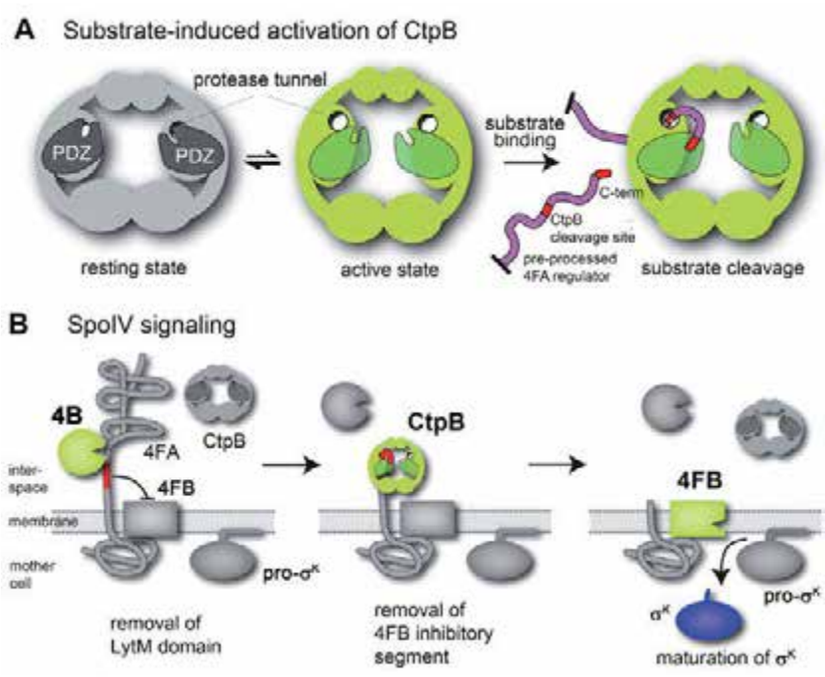

Figure 1: *Model for cooperative cleavage of 4FA by the signaling proteases 4B and CtpB. (A) Cartoon illustrating the allosteric regulation of CtpB. (B) The SpoIV proteolytic cascade transmits the sporulation signal from forespore to mother cell. Our data indicate that the three participating proteases 4B, CtpB, and 4FB act sequentially in the RIP pathway (the respective active forms are shown in green).*

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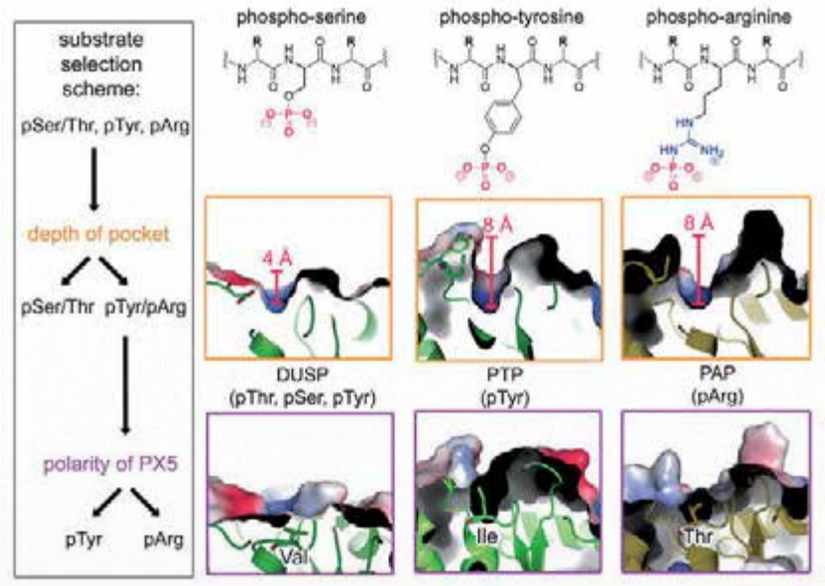

Figure 2: *Determinants of substrate specificity within the LMW-PTP family. Substrate selectivity against different phosphorylated residues is achieved by a two-step mechanism. First, phosphatases have evolved binding pockets of characteristic depth that reflect the size of their cognate substrates. Second, PAPs appear to have installed a polarity filter, Thr11, to detect the presence of the hydrophilic guanidinium group of the pArg substrate.*

group and select pArg against pTyr, pSer and pThr. Based on these findings, we discovered a pArg phosphatase in Drosophila, but could not assess the biological relevance of pArg in eukaryotes. On the contrary, we obtained preliminary evidence that in bacteria, pArg phosphorylation may serve as a degradation signal targeting substrates to the ClpCP protease. To address this point we plan to perform a detailed structural characterization of the McsB arginine kinase and its partner protein McsA. A crystal structure of the McsB kinase would be interesting for several reasons: a) may delineate the molecular basis of how McsB marks specific stress-response factors for degradation, b) it may provide a detailed insight into an unprecedented protein phosphorylation mechanism, and c) it may facilitate the search for potential protein arginine kinases in eukaryotic cells.

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LUISA COCHELLA

Spatio-temporal specificity of miRNA function and its role in cell-type specification

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One of the main goals of developmental biology is to understand how the different cell types that constitute a multicellular organism are specified during its development. We are currently exploring the contribution of miRNA-mediated gene expression regulation to the diversification of the genetic programs that control cell differentiation. We are interested in how miRNAs themselves are regulated to achieve highly specific expression patterns in order to generate this diversity of cell types, as well as how miRNAs were integrated into gene regulatory networks during evolution.

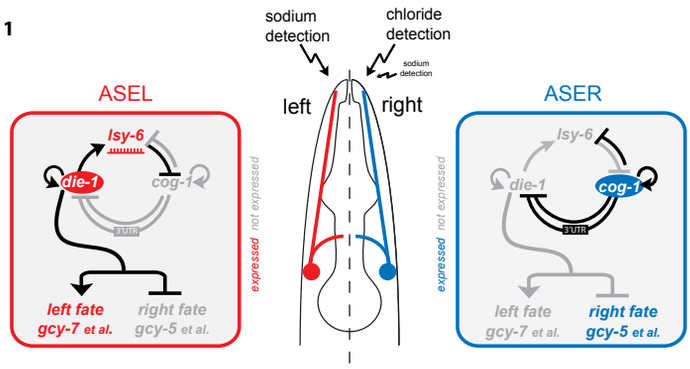
Description of research activities

MicroRNAs play integral regulatory roles in the genetic networks underlying the development and physiology of multicellular organisms. In particular, a number of reasons make miRNAs ideally suited to diversify genetic programs and contribute to the variety of cell types that constitute an adult organism: **a**) The dramatic expansion in the number of miRNAs during evolution, coinciding with the onset of multicellularity, has made them numerous and diverse in their targeting capacity, **b**) They display complex and dynamic spatio-temporal expression patterns, **c**) Regulatory interactions involving miRNAs can arise more easily during evolution than those involving protein factors due to fewer sequence constraints, and therefore miRNAs can modify preexisting genetic networks to produce stable and heritable phenotypes.

Of special interest is the role of miRNAs in the specification of the vast diversity of cell types found in the nervous system - an organ system with an unparalleled cellular complexity, where miRNAs are particularly enriched. In fact, in a few cases miRNAs were reported to increase neuronal diversity during development. For example, in *C. elegans, lsy-6* diversifies the ASE-class neurons to generate two subclasses with different sensory capacities (Figure 1). Our previous work has shown how - in order to distinguish these two neuronal classes - *lsy-6* is exclusively expressed in one but not the other neuron (Figure 2). To determine the contribution of miRNAs in generating cell-type diversity, we are continuing to use the nematode *C. elegans* as a model system. *C. elegans* has a relatively simple nervous system, yet with a large number of well characterized neuronal classes (at least 118). While there are obvious differences between such a simple nervous system and the more complex mammalian nervous system, the underlying genetic networks controlling cell-type specification are remarkably conserved. In addition, *C. elegans* is a powerful genetic model organism and an optimal system to study multiple aspects of gene expression regulation in an entire living organism, yet with single-cell resolution.

As a first step towards determining the contribution of miRNAs to cellular diversity, we are trying to understand how miRNAs are themselves expressed in a cell-type and stage-specific manner. This is crucial to place them in the context of the genetic networks they are a part of, and thus fully grasp their contribution to gene expression regulation.

miRNA biogenesis is controlled at the transcriptional as well as post-transcriptional level. We previously explored the transcriptional regulation of the miRNA *lsy-6*, and uncovered a novel two-step mechanism for transcriptional activation that results in the exquisitely specific expression of this miRNA (Figure 2 – Cochella and Hobert. *Cell*, 2012).



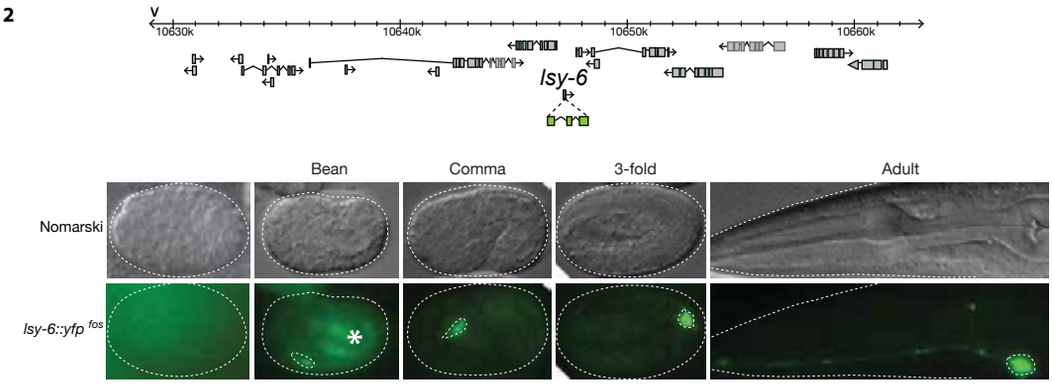


Figure 1: ***Gene regulatory network controlling ASE neuron asymmetry.*** *The ASE neurons are a pair of gustatory neurons in the head of C. elegans. While bilaterally symmetric in many ways, these neurons display a functional asymmetry in the environmental cues they sense. The miRNA lsy-6 functions in the left ASE neuron to repress its target - the transcription factor cog-1 - and results in the adoption of the left specific fate, which involves the expression of left-specific chemoreceptors. Lack of lsy-6 in the right ASE neuron results in a different set of chemoreceptors being expressed. Adapted from Didiano, et al. RNA, 2010.*

Figure 2: ***Expression pattern of the lsy-6 miRNA.*** *We generated a fluorescent reporter by introducing yfp into the lsy-6 locus contained in a large genomic clone. Transgenic worms carrying this reporter show that the miRNA lsy-6 is expressed in a single cell throughout the life of the worm. This cell is the ASEL neuron, and lsy-6 is present in ASEL from its birth until adulthood.*

While this shows that transcriptional regulation plays a crucial role in establishing cell- and stage-specific expression, the role of post-transcriptional regulation is essentially unexplored. Using forward genetic screens and a directed candidate approach, we will identify post-transcriptional regulators of miRNA biogenesis and function and determine their role in development and physiology.

A further advantage of working with *C. elegans* is that the genomes of a number of related nematode species have now been fully sequenced, and these species are being extensively characterized at various levels. This provides us with the possibility to compare gene regulatory networks that have acquired a miRNA component specifically in some of these species but not in others. In particular, the miRNA *lsy-6* which diversifies the ASE-class of neurons (Figure 1), is lacking in the nematode *Pristionchus pacificus*, although the rest of the gene regulatory network appears to be in place. Using comparative genetic approaches, we can determine the effect of adding miRNA to a pre-existing gene regulatory network.

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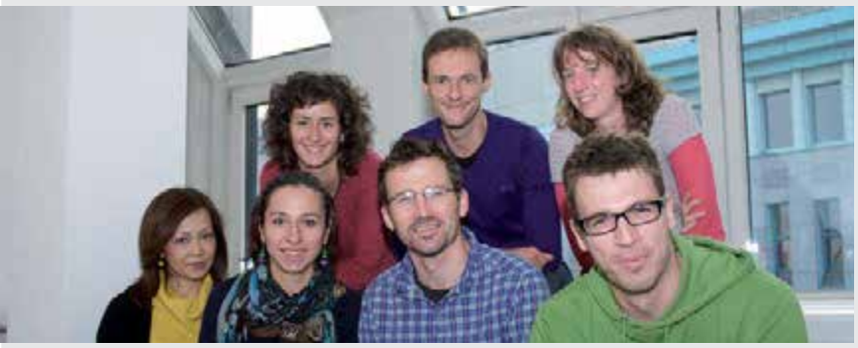

CARRIE COWAN

Symmetry breaking during cell polarization

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In June 2013, Carrie Cowan left the IMP to take up a position as Associate Dean of the Watson School of Biological Sciences at Cold Spring Harbor Laboratory.

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Cell polarity allows for spatial specializations within a cell, such as directed transport, migration, or growth. Cell polarity also controls asymmetric cell division, a dominant mechanism for dictating cell fate changes during development. The diverse roles of this fundamental organizational concept mean that understanding cell polarity is essential to understanding both normal development and diseases. We are investigating how cell polarity is established.

We are using one-cell *C. elegans* embryos as a model system to determine how cell polarity is established. One-cell *C. elegans* embryos polarize in response to a signal from the sperm-provided centrosomes. Centrosomes induce a local structural reorganization of the acto-myosin network: whereas most of the cortex undergoes stochastic contractions, the area nearest the centrosomes becomes non-contractile. This local change in cortex activity marks the functional symmetry-breaking event that allows polarization. Once cortical symmetry has been broken, mutual antagonism between antagonistic polarity proteins - the anterior and posterior PAR proteins - facilitates self-organizing polarization. PAR polarity controls cell fate determinant segregation and asymmetric cell division.

Using a combination of forward and reverse genetics, in vivo biochemistry, high-resolution time-lapse microscopy, ultrastructural reconstruction, automated quantitative analysis, and mechanical manipulations, we are investigating the following questions:

How do centrosomes communicate with the cortex?

After the sperm centrosomes are delivered to the egg during fertilization, they wander randomly in the cytoplasm for approximately thirty minutes. A dense network of cytoplasmic microtubules prevents centrosomes from moving too far away from the cortex. Upon a cell cycle signal, centrosomes are activated and signal to the cortex to change acto-myosin contractility. Centrosomes can initiate polarity from any position within the embryo, but the efficiency of polarization increases when centrosomes are close to the cortex. We are investigating the mechanisms and functions of centrosome positioning to determine how accurate information is supplied to the cortex during symmetry breaking.
In a genetic screen for centrosomal molecules that may mediate signaling from centrosomes to the cortex, we identified the Aurora family kinase AIR-1. Embryos depleted of AIR-1 often fail to break cortical symmetry in response to the centrosomes and instead undergo spontaneous polarization. AIR-1 depleted embryos often have multiple polarity axes, leading to mis-segregation of cell fate determinants during cell division. AIR-1 is required for centrosome growth but this function is distinct from AIR-1's roles in polarity establishment. We are trying to understand how AIR-1 both positively and negatively regulates cortical symmetry breaking to ensure that a single polarity axis is formed.

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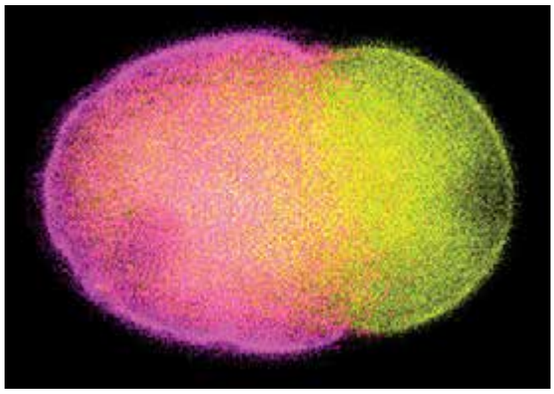

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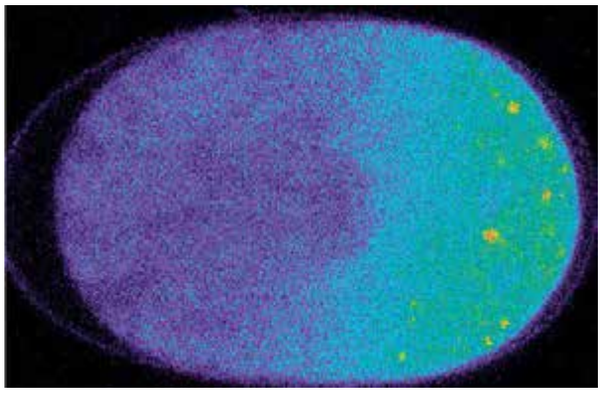

Figure 1: *Centrosome position (line) relative to the cortex (dots) during polarity establishment. The color scale indicates elapsed time (blue: -500 s, red: 300 s).*

Figure 2: *Establishment of a stable axis of PAR polarity. Anterior (PAR-6, pink) and posterior (PAR-2, yellow) cortical polarity domains.*

Figure 3: *Asymmetric distribution of the germline fate determinant PIE-1 (purple: low intensity; red: high intensity) at the beginning of cell division.*

What regulates PAR polarity in response to cortical symmetry breaking?

After the initial symmetry-breaking event in the cortex, mutually exclusive PAR protein domains drive the establishment of a stable cell polarity axis. The balance between the amounts of anterior and posterior PAR domain components appears essential for normal polarization. Controlling PAR protein amounts - both absolute and at the cortex - is an important regulatory point. Total PAR protein levels appear to depend on processing of relevant mRNAs, while the proportion of cortical PAR proteins appears to be influenced by intracellular trafficking. We are looking at the molecular mechanisms by which these pathways control cortical PAR protein localization.

How does cortical polarity control cytoplasmic asymmetry?

The establishment of polarity at the cortex provides spatial information to polarize the entire cell, ultimately allowing asymmetric changes in gene expression and cell fate. The cytoplasmic fate determinant PIE-1 is restricted to the posterior half of one-cell embryos and thus is inherited only by cells in the germline lineage. PIE-1 forms a concentration gradient in response to two distinct activities that change the apparent diffusion of PIE-1 in the cytoplasm: in the anterior, MEX-5 increases PIE-1 mobility, and in the posterior, MEX-1 decreases PIE-1 mobility. MEX-1 and MEX-5 in turn affect each other. We are using mathematical models and biochemistry to understand the parameters that are important for PIE-1 mobility.

BARRY DICKSON

Neural circuits

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[6] until July

We use molecular genetic techniques to study the function of neural circuits in Drosophila. Our goal is to understand how information processing in defined neural circuits generates complex animal behaviours. As a model system, we focus on the fly's mating behaviours. These behaviours are robust, adaptive, and particularly amenable to genetic analysis.

A major goal for neuroscience is to understand how information processing in neural circuits guides animal behaviour. At any given moment, the brain receives a rich set of sensory inputs, from both external and internal sources. This information must be integrated, interpreted in the light of knowledge gained through prior experience, and a specific behavioural action selected. These processes of sensory integration, learning and memory, decision making, and action selection are the essence of animal cognition and behaviour. Our goal is to provide a biophysical explanation for these processes.

As a model, we have chosen to study the sex life of the fruit fly *Drosophila melanogaster*. During mating, flies make decisions that are critical for their reproductive success, and hence their evolutionary fitness. Upon encountering another fly, a male decides whether or not to court, based on the current sensory input and his past experience. If he does court, the female then decides whether to accept or reject him, based on her perception of his quality as a potential mate, and her own sexual maturity and mating status. These are complex decisions made by complex brains, but working with flies has the great advantage that genetic tools can be used to identify and manipulate the relevant neurons and circuits in the brain. With these tools, it should be possible to establish causal relationships between genes, cellular biochemistry, circuit function, and animal behaviour.

The male brain

Remarkably, the different behaviours of male and female flies can largely be explained by the sex-specific splicing of a single gene – *fruitless* (*fru*). If females are forced to express male-specific fru^M transcripts, they behave like males. Conversely, males that lack fru^M behave like females.

fru is expressed in some 2000 neurons, distributed in clusters throughout the nervous system (Fig. 1). The activity of these neurons is essential for courtship behaviour. We have recently developed genetic tools that provide specific access to distinct subsets of *fru* neurons, so that we can selectively express visible markers in these cells to trace out their anatomy and connectivity, or various proteins that measure or modulate neuronal activity and allow us to study circuit function (Fig. 2). Using this genetic toolkit, we have constructed a cellular resolution wiring diagram of the entire *fru* circuit (Fig. 3) Now, we can begin to examine what each of type of *fru* neuron contributes to courtship, the nature of the signals it processes, and how and to what extent sexually dimorphic processing leads to the distinct behaviours of males and females. We have started this analysis with an analysis of the circuitry for courtship song [1], the pheromone processing pathways that feed into these song circuits [2], and the circuitry allows the male with to learn which type of female is most likely to be wooed by his song [3].

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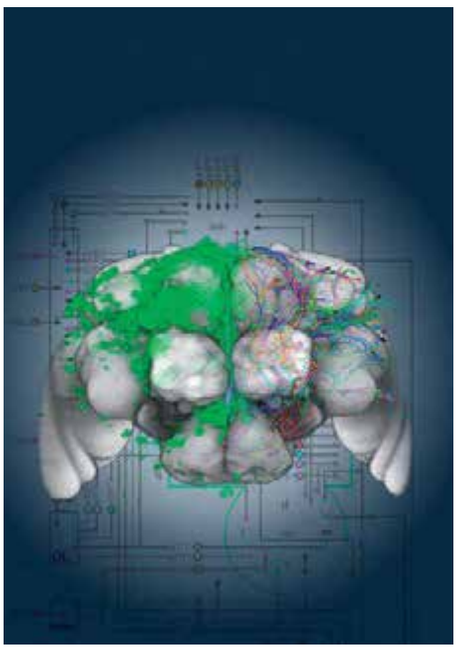

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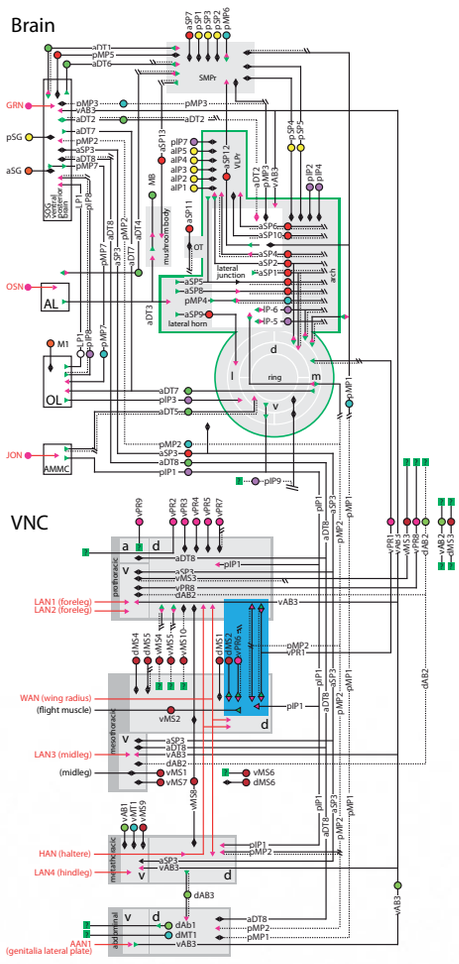

Figure 1: ***fru⁺ neurons in the CNS, shown in green.*** *Synapses are stained in magenta. This is a confocal image of the brain and ventral nerve cord of a fru^{GAL4} UAS-GFP male.*

Figure 2: ***Genetic dissection of fru⁺ neurons in the central brain.*** *The image shows processes of fru neurons in the male brain, visualized with GFP (green, left), and their digitial reconstruction into a 3D cellular resolution atlas (right).*

Figure 3: ***The fru circuit.***

The female brain

We are also currently examining the sensory pathways that process and integrate the two male signals that influence the female's mating decision: his courtship song and his pheromone profile. A third important factor in the female's decision is her own mating status. Females that have recently mated are usually reluctant to do so again. This is due to a small peptide, called the sex peptide (SP), that is present in the first male's seminal fluid. We have recently identified a molecular receptor for SP, a G-protein coupled receptor we call SPR (sex peptide receptor), and shown that it acts in a small subset of *fru⁺* sensory neurons that innervate the reproductive tract and project axons into the central nervous system. Our ongoing efforts are aimed at understanding what SP does to these neurons, and to characterise the neural pathways in the brain that further process this signal – ultimately integrating it with the song and pheromone signals to guide the female's mating decision.

WULF HAUBENSAK

Circuit mechanics of emotions in the limbic system

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Survival critically depends on recognizing what is important and initiating appropriate behavioral responses - a process modulated by emotions. Fear, for instance, associates stimuli with threats and evokes defensive behaviors, whereas reward-related emotions induce the opposite. Emotions are a central part of our mental self and linked to a variety of psychological conditions. But how are they wired in the brain? In order to investigate their underlying neural basis, we use molecular, pharmacogenetic, and optogenetic methods (Luo et al., 2008) to map neural circuits for emotional behaviors in mice. Combining these manipulations with electrophysiological methods, we explore how these circuits control emotional states and, in turn, how genes and psychoactive drugs modulate circuit activity, emotional states, and behavior.

Numerous studies have established the limbic system as the central hub in emotion processing (LeDoux, 2000). It integrates sensory information, encodes emotional states, and instructs other brain centers to regulate physiology and behavior. However, resolving how emotions emerge from the several distinct and highly interconnected neuronal populations in limbic networks remains a major challenge. To gain a foothold into the complexity of emotions on one hand and limbic circuitry on the other, we set out to study - in exemplary fashion - basic emotions such as fear and reward in selected limbic hubs.

Fear and reward in limbic circuits

We are currently developing circuit genetic tools for efficiently identifying emotional hot spots and resolving their internal architecture and functional interactions.

In a first foray, we have employed pharmacogenetics, optogenetics and viral tracing, and discovered a local inhibitory network consisting of two antagonistic neural populations in the lateral central amygdala (CEl) portion of the extended amygdala (EA) that gates amygdala output from CEm (Haubensak *et al.*, 2010). Architecture and neural modeling suggest that this network operates like a bistable switch (Figure 1). Optogenetic experiments mapped its open state to high fear/low reward states and its closed state to low fear/high reward states, indicating that, at the level of CE, opposing emotional and behavioral states are encoded in alternate and mutually exclusive neural network states. We believe that this design will enable a person to efficiently integrate emotional stimuli in order to select the appropriate behavioral response, especially when facing fear and reward simultaneously. Neural tracing experiments revealed that CEl circuitry is closely interconnected with the Bed nucleus of the stria terminalis (BNST). Preliminary evidence suggests that this projection links neural activity in CEl to BNST, and modulates autonomous responses. Thus, emotional states in CEl assemble a behavioral state from neural activity in BNST and CEm, mediating physiological and motor components of the emotional response, respectively. What, in turn, controls neural activity in CEl? CTB retrograde tracing revealed strong cortical and subcortical inputs to CEl. Modeling and optogenetic epistasis experiments suggest that CEl integrates top-down control signals and interoceptive information from these sources.

One hallmark of emotions is that they are adaptive responses to stimuli that have acquired a meaning - best illustrated by Pavlovian fear and reward conditioning. Interestingly, CEl circuitry itself seems to learn and store Pavlovian associations. Here we aim to dissect the circuit mechanisms by which CEl circuitry shapes its responses during emotional learning. Neural tracing experiments revealed strong reciprocal connectivity with inputs relaying teaching and prediction error signals of classical learning circuits. Interestingly, site perturbation of these interactions prevented fear learning, demonstrating that the shaping of CEl circuit dynamics is crucial for emotional memory.

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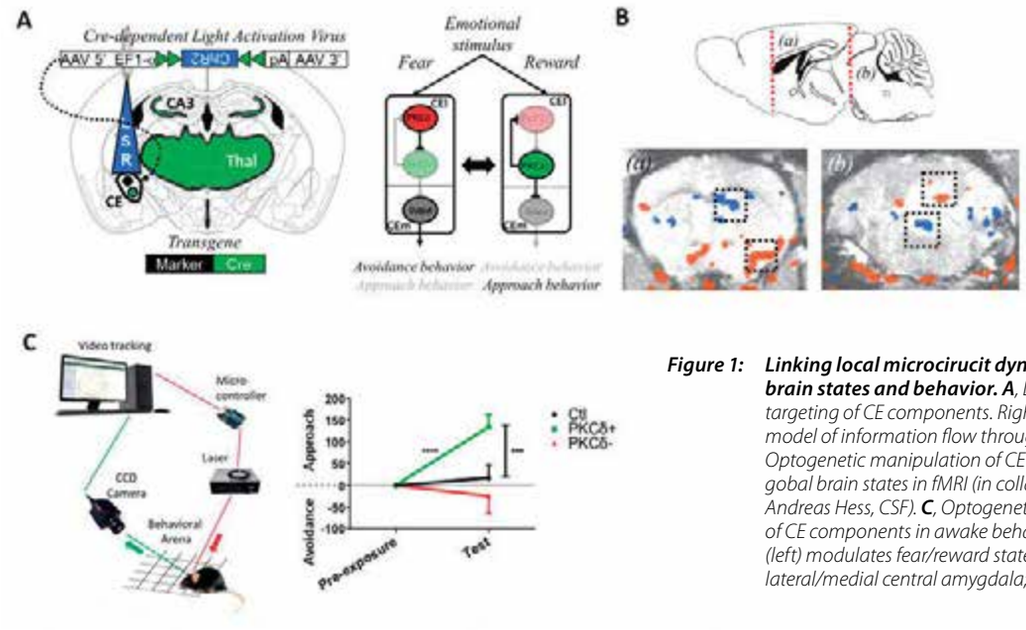

Figure 1: ***Linking local microcirucit dynamics, global brain states and behavior. A****, Left, Optogenetic targeting of CE components. Right, Neural circuit model of information flow through the CE.* ***B****, Optogenetic manipulation of CE circuitry affects gobal brain states in fMRI (in collaboration with Andreas Hess, CSF).* ***C****, Optogenetic manipulation of CE components in awake behaving animals (left) modulates fear/reward states (right). CEl/m, lateral/medial central amygdala; Thal, thalamus.*

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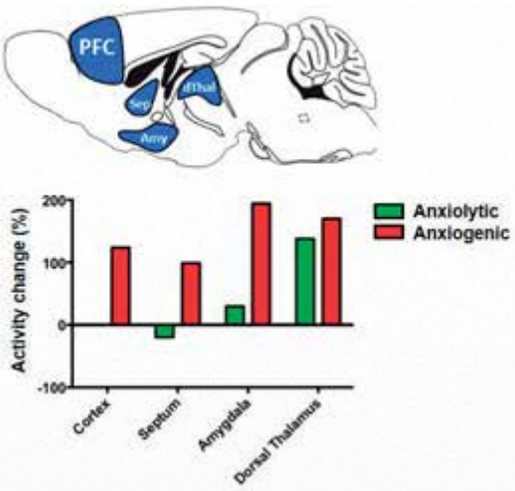

Figure 2: ***Mapping targets of psychoactive drugs.*** *Drug induced changes in neural activity across the forebrain.*

Psychopharmacology of emotion circuits

While the molecular mechanics by which genes and psychoactive drugs control neural activity at the cellular level have been worked out in great detail, the circuit mechanics by which this translates into behavioral changes have not yet been resolved. The circuits identified above provide an ideal substrate to study this problem. We therefore investigated drug effects (anxiolytic and anxiogenic drugs) on the activity of emotion circuits (Figure 2) and how these changes in activity modulate emotional states and behavior. Preliminary results suggest that the benzodiazepine (BZD) anxiolytic effect results from a balance shift of neural activity in the EA network.

Taken together, we hope to disclose principles of stimulus behavior transformations and the neural organization of emotions. Moreover, our research will provide a framework for genetics and the psychopharmacology of emotions in health as well as diseases such as anxiety disorders and addiction.

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DAVID KEAYS

Neuronal migration and magnetoreception

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The Keays lab aims to understand two biological phenomena: 1) How do neurons migrate in the developing brain? 2) How do animals detect magnetic fields? In tackling these two questions we adopt an interdisciplinary approach, employing a wide range of genetic, molecular, cellular, and behavioral tools.

Tubulins in neuronal migration and disease

Neuronal migration underlies the organization of the mammalian brain. All neurons that are born in the proliferative ventricular zones migrate to their final destination by extending their primary neurites and translocating their nuclei. This migration is crucial because it not only determines the destination of a given neuron, but also the circuit in which it operates. Moreover, a host of neurodevelopmental diseases are known to be linked with defective neuronal migration. One example is lissencephaly, a disease that is characterized by a "smooth brain", epilepsy, and mental retardation. We have shown that mutations in the alpha tubulin gene *TUBA1A* cause lissencephaly in humans, and neuronal migration abnormalities in mice (Keays et al., 2007) (Figure 1). The importance of the tubulin gene family in cortical development is further illustrated by our finding that mutations in the beta tubulin gene *TUBB2B* cause polymicrogyria, a disease characterized by excessive folding of the cortex (Jalgin et al., 2009). Most recently we have shown that mutations in *TUBB5*, cause a reduction in brain size in humans, and perturb both migration and neurogenic division in mice (Breuss et al., 2012).

To gain insight into the role of different tubulin genes, how they cause disease, and the molecular mechanisms underlying the migration of neurons, the Keays lab is employing the mouse as a model system. We use ENU mutagenesis, as well as transgenic methods to generate new models for human disease. To complement these murine studies we are taking advantage of next-generation sequencing, and in collaboration with a network of clinical colleagues, sequencing the exomes of patients with sporadic neuronal migration disorders. These genetic studies have already identified a number of new disease-causing genes which are currently being functionally interrogated.

Molecular and cellular basis of magnetoreception

Many species on the planet, whether they be birds, fish or insects, rely on the Earth's magnetic field to guide migration or assist navigation. This remarkable sense is known as magnetoreception. One idea that aims to explain how animals detect magnetic fields is known as the magnetite-based theory of magnetoreception. This theory holds that mechanosensitive ion channels coupled to an intracellular compass made of an iron oxide known as magnetite (Fe_3O_4) transduce local magnetic information into a neuronal impulse. We are using the rock pigeon *Columbia livia* as a model (Figure 2). Previous studies have asserted that, pigeons employ a magnetite-containing sensory apparatus located at six specific loci in the skin of the beak. We have shown that this established dogma is false, and in reality clusters of iron-rich cells in the beak of pigeons are macrophages; they are not magnetosensitive neurons (Treiber, Salzer et al., 2012). The field is now engaged in a renewed search for the true magnetoreceptive cells. Where are the magnetosensory cells in avian species?

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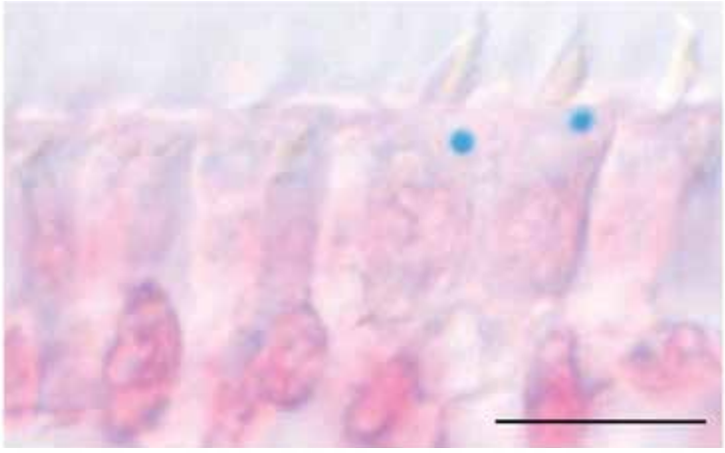

Figure 1: *The Jenna mutant mouse. This mouse harbors a S140G mutation in the Tuba1a gene, which results in defective neuronal migration during development. As a result, the Jenna mouse is characterised by abnormal lamination of the hippocampus, accompanied by hyperactivity and deficits in cognitive tasks.*

Figure 2: *Pigeons: our model system for studying how animals detect the Earth's magnetic field.*

Figure 3: *Pigeon hair cells stained with Prussian blue, (which labels ferric iron), and nuclear fast red. A single iron-rich organelle is found per cell, and is located beneath the stereocilia. The scale bar shows 10 µm.*

Recent electrophysiological studies have identified a population of neurons in the vestibular nuclei of pigeons that respond to magnetic vectors of a specific orientation and intensity (Wu and Dickman, 2012). These results strongly suggest that a population of unidentified magnetosensory cells lie in the inner ear of pigeons. Employing histological techniques, we identified sensory hair cells that contain a single iron-rich organelle (Figure 3). This organelle is located directly beneath the stereocilia and is embedded in the actin-rich cuticular plate. We have shown that this structure is present in a wide range of avian species, but is not found in rodents, fish, or humans (Lauwers, Pichler et al., 2013). Our current work is focused on elucidating the function of this organelle, specifically whether it plays a direct or indirect role in magnetoreception.

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KRYSTYNA KELEMAN

Molecular and cellular basis of learning and memory in *Drosophila*

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To survive and reproduce in an ever-changing environment, animals need to be able to modulate their behaviour upon experience. We seek to understand this process of learning and memory by studying a specific form of behavioural plasticity in Drosophila at the molecular, cellular, and circuit levels.

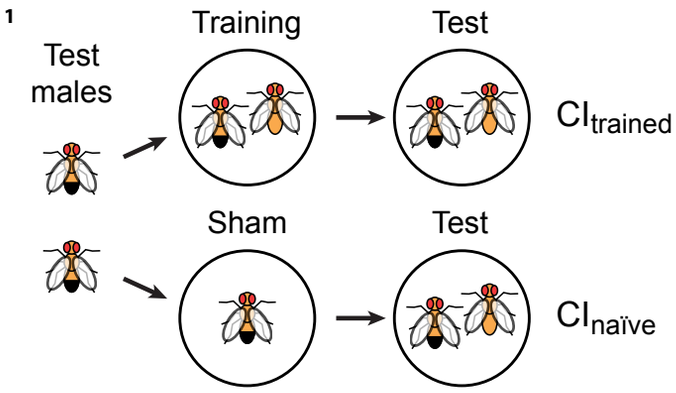
The learning paradigm we have chosen to investigate is courtship conditioning in *Drosophila*. This is naturally occurring form of learning in which males learn to choose appropriate females as courtship objects. Naïve *Drosophila* males court both mated and virgin females, yet only virgins will be receptive to their mating attempts. Mated females actively reject courting males. Having experienced such rejection, a male is subsequently less inclined to court other mated females, but will still court virgin females as vigorously as a socially naive male (Fig.1). As with many other learning paradigms, this behavioural modification can be of either short or long duration, depending on the training regimen. We aim to understand the molecular, cellular and circuit mechanisms that underlie this robust and powerful form of memory.

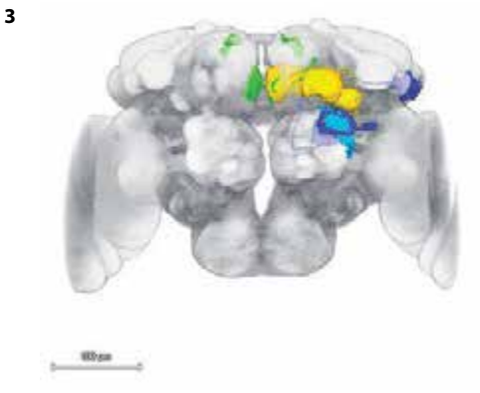
Molecular mechanisms of courtship memory

Despite the many molecular factors and even whole molecular pathways that have been implicated in learning and memory over the last thirty years, there are clearly still many more essential molecular components of memory formation to be discovered. With the powerful new genetic tools available in *Drosophila*, we can now use genome-wide transgenic RNAi to systematically test the function of every gene in the Drosophila genome. We are currently conducting such a genome-wide RNAi screen to identify the genes required for courtship memory. We hope here to both discover new genes and pathways involved in courtship conditioning, and to gain further insight into those that have already been implicated in this form of learning and memory.

One phylogenetically conserved class of proteins already known to function in learning and memory are the CPEB proteins. CPEB proteins are thought to regulate RNA trafficking and/or translation, and might thus contribute to local protein synthesis in activated synapses. We have shown that the *Drosophila* CPEB, Orb2, is acutely required for long-term memory [1]. Specifically, we have established that its glutamine-rich domain (Q domain) is essential for its function in memory formation. In order to further investigate the molecular mechanism underlying how CPEB proteins in general, and Orb2 in particular, function in this process, we have generated a new *orb2*^{attp} allele (Fig.2). This allele allowed us to rapidly introduce any modification of the endogenous Orb2 protein in the tissue selective manner and test its role in courtship conditioning.

We have uncovered a novel mechanism of how Orb2, and likely other members of the CPEB family of proteins, might function in memory. We have established that the two Orb2 isoforms, Orb2A and Orb2B, while both being essential and containing identical Q domain and the RNA binding (RBD) domain, function in memory formation by distinct mechanisms. Orb2A uniquely requires its Q domain whereas Orb2B its RBD. Furthermore, Orb2A induces complexes with Orb2B in the Q domain and neuronal activity dependent manner. We propose that Orb2B acts as a conventional CPEB to regulate transport and/or translation of the specific mRNAs, whereas Orb2A acts in an unconventional manner to form stable complexes essential for memory to persist [2].





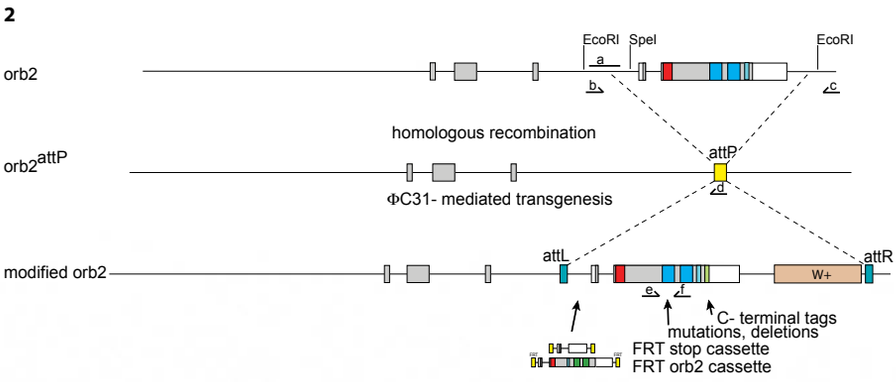


Figure 1: *Courtship conditioning. When tested with unreceptive mated females, males previously exposed to mated females court less than naïve (sham-trained) males (CI = courtship index; $CI_{trained} < CI_{naïve}$)*

Figure 2: *Strategy to modify orb2 endogenous locus. An attP allele of orb2 was generated by homologous recombination. Then using the phiC31 site specific transgenesis system, any desired modification was introduced into the orb2 endogenous locus.*

Figure 3: *Dopaminergic aSP13 neuron innervate the MB gamma lobe. After experience with an unreceptive mated female, the dopaminergic neuron aSP13 (green) releases dopamine on the MB gamma lobe (yellow), which enhances the behavioural response of the male to the pheromone cVA transmitted by the DA1 PNs (dark blue) from the DA1 glomerulus in the antennal lobe (light blue) and its ability to discriminate between virgin and mated females.*

Circuit mechanisms of courtship learning

To understand how courtship conditioning is implemented in the Drosophila brain, we also need to delineate the underlying neuronal circuit at the cellular resolution. What are the sensory cues important for this learning, how are they processed, how is this processing modulated by experience, and how does plasticity at the circuit level translate into behavioural plasticity?

We recently uncovered a simple learning rule and identified key components of the neuronal circuit of courtship conditioning. Courtship learning reflects an enhanced behavioural response to the male pheromone cis-vaccenyl acetate (cVA), which remains on females after mating and distinguishes them from virgins. We have identified a specific class of dopaminergic neuron in the protocerebrum that is critical for courtship learning and provides input to the mushroom body (MB) gamma lobe (Fig.3). We postulate that, during learning, dopamine modulates the processing of cVA signals within the gamma lobe neurons, resulting in an increased sensitivity to cVA and hence an increased ability to discriminate mated females from virgins [3]. Our ongoing efforts are aimed at identifying additional components of this circuit, and to determine how cVA signals are processed within it, with the ultimate goal of understanding how courtship conditioning is implemented in the Drosophila brain.

The long-term goal of these studies is to provide a complete picture of how the specific experience of courtship rejection modulates the Drosophila male's subsequent mating strategy. We hope that this will serve as a paradigm for more complex forms of learning and memory in the fly and other species.

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Molecular Machines

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[1] *from December 2013*

Membrane-associated processes are a fundamental characteristic of all living cells. They ensure that the cells are able to effectively communicate with, and adapt to, their environment. The cells achieve this by either physically translocating molecules to the opposite site of a membrane, or by receiving, transmitting, and amplifying incoming signals. Our laboratory is interested in understanding the molecular mechanism underlying such processes. Specifically, we focus on machineries capable of translocating bacterial toxins into eukaryotic cells.

Microbial pathogenesis

Gram-negative pathogens such *Yersinia, Shigella, Pseudomonas, enteropathogenic/enterohemorrhagic E. coli* (*EPEC/EHEC*) and *Salmonella* as well as *Erwinia, Ralstonia* and *Xanthomona* are causative agents for many diseases in animals, humans, and plants. They range from mild to deadly outcomes, and include food-borne diseases such as diarrhea or bubonic plaque, or induced cell necrosis in plants. A central aspect of pathogenicity are bacterial toxins ('effectors'), which are delivered via the type-III secretion system, a large membrane-embedded machinery, from the bacterium to its host cell. As a consequence, translocated effector proteins have the remarkable capacity to modulate various host-cell pathways, including endocytic trafficking, gene expression, programmed cell death, or cytoskeleton dynamics that induce membrane ruffling and subsequently render the host accessible to bacterial infection.

Unfolded protein transport across membranes?

The hallmark function of all type-III secretion is the safe and directional transport of effector proteins across membranes. Our recent structural analysis (Schraidt and Marlovits, Science 2011) of the injectisome, the most prominent and cylindrical structure of the type-III secretion system, revealed a potential secretion path through the central part of the membrane-embedded complex. However, the inner diameter of this path is too small to accommodate a fully folded effector protein, suggesting that either the injectisome must undergo large conformational changes during transport, or effector proteins need to be unfolded. Thus, during the last year we focused on the following: a) determining the secretion path of injectisomes, b) understanding the mechanism of transport, and c) visualizing protein transport in situ. To address these questions, we first analyzed the requirements for substrate association with, transport through, and exit from the injectisome. To our surprise we found that the size and length of novel substrates does not have a major impact on their secretability. We learned that the fusion of thermodynamically stable protein domains to otherwise secreted substrates does not influence successful engagement to the injectisome, but prevents complete transport across membranes. Such designed and trapped substrates are highly associated with injectiosmes. We discovered that such substrates are inserted into the secretion path in a polar fashion - N-terminal regions first - suggesting that other substrates with a similar domain organization follow the same principle. Our structural analysis of trapped substrates clearly revealed for the first time that they are in an unfolded state during transport, suggesting that the type-III-specific ATPase acts as an unfoldase. In contrast, injectisomes stay largely invariant during protein transport. To understand, whether such behavior is in fact observed in situ, we performed cryo-electron tomography.

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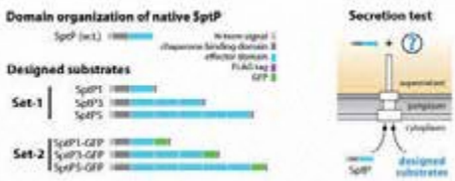

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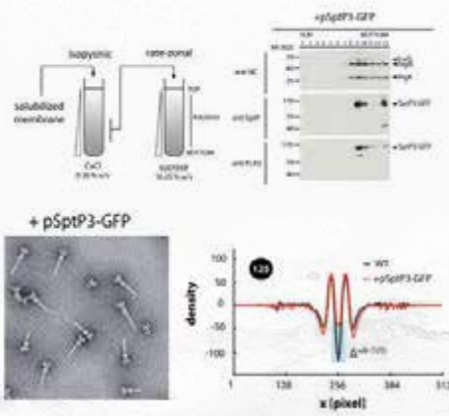

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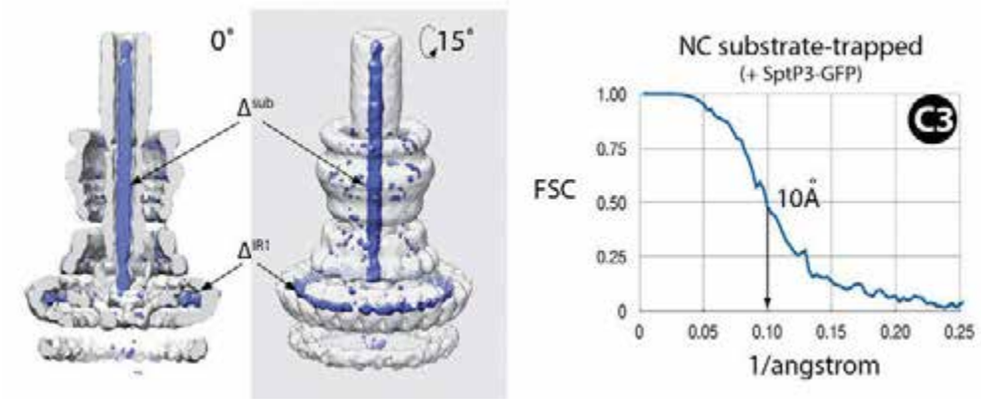

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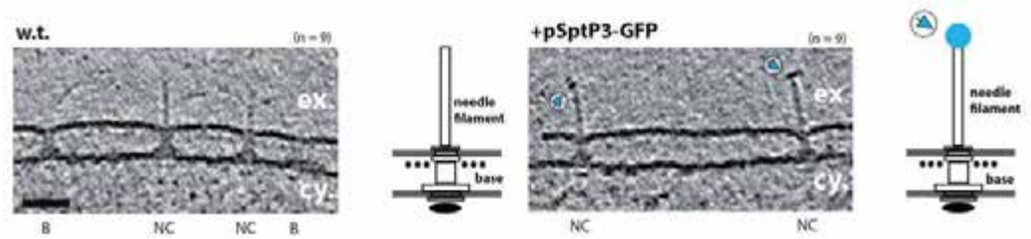

Figure 1: *Design of novel type-III secretion substrates and experimental set-up*

Figure 2: *Purification and analysis of substrate-trapped injectisomes*

Figure 3: *Structural analysis of substrate-trapped injectisomes*

Figure 4: *Unfolded protein transport across membranes revealed by cryo-electron tomography*

This method permits the investigation of molecular structures within cells in a spatiotemporal manner and in a near-native state. For the first time we were able to visualize pathogenic type-III secretion systems from Salmonella in action and - more generally - protein transport across several membranes.

By understanding the molecular mechanism of TTSS-mediated protein transport, we hope to provide a basis for the development of novel therapeutic strategies that will either inhibit its activity or modify the system for targeted drug delivery.

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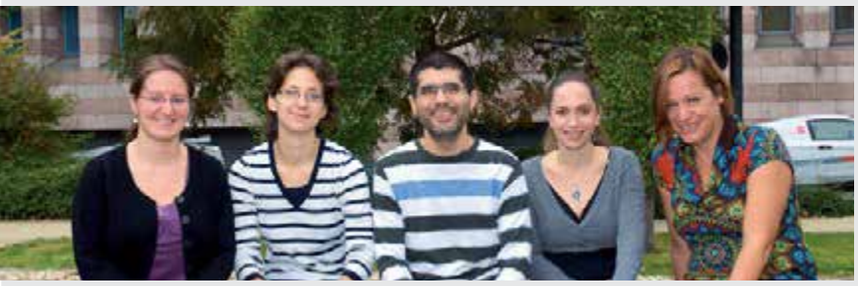

RUSHAD PAVRI

Molecular mechanisms of antibody diversification

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In our lab, we study the phenomenon of antibody gene diversification in B lymphocytes - the process by which the immune system generates a vast repertoire of high-affinity antibodies essential to combat pathogenic challenges. We focus on the transcriptional and co-transcriptional mechanisms underlying these events using a combination of RNAi screening, genetic models, and genomic analysis.

Research activities

Antibody diversification occurs at the immunoglobulin (*Ig*) loci and consists of two processes: somatic hypermutation (SHM) which modulates the antigen-binding properties of the antibody, and class switch recombination (CSR) which generates various antibody isotypes (Figure 1). Antibody maturation is triggered by activation-induced deaminase (AID), which generates mismatches in the *Ig* DNA sequence by converting cytosines into uracils on single-stranded DNA. AID can also target non-*Ig* genes, such as the oncogenes, *c-myc* and *Bcl6*, albeit to a much lesser extent (Figure 1). These lesions are then processed by various means, involving error-prone repair, double strand breaks (DSBs) and non-homologous end-joining (NHEJ), resulting in SHM and CSR (Figure 1). AID-induced DSBs at non-*Ig* genes like *c-myc* may also lead to *c-myc/IgH* translocations and lymphoid malignancies (Figure 1). In our lab, we are interested in understanding the role of transcription and co-transcriptional factors involved in AID targeting and mutagenesis, and we determine how AID targeting is restricted to specific loci in B cells.

Transcriptional regulation of AID targeting

We have shown that the RNA polymerase II (Pol II) stalling and elongation factor, Spt5, is required for CSR and AID targeting. Based on our findings we proposed a model for AID targeting via Spt5 and Pol II stalling (Figure 2), wherein AID is preferentially targeted to regions of Pol II stalling through direct interaction with Spt5. Stalling provides AID with long residence times at, and access to, its ssDNA substrate within the transcription bubble. In this manner, mutations are focused on specific genomic regions which coincide with the known mutation profiles of AID as well as hotspots of AID-dependent translocations (Figure 2). We are currently developing tools and genetic models to further study the impact of Pol II stalling and Spt5 function in diversifying B cells. Our preliminary findings suggest entirely new modes of gene regulation by Spt5 during B cell activation. We are currently investigating how this influences AID regulation, Ig gene expression, and AID targeting during CSR and SHM.

Cell cycle regulation of antibody diversification

Antibody diversification, both *in vivo* and *in vitro*, occurs in highly proliferating B cells. *In vitro* studies clearly show a correlation between the magnitude of CSR and cell division. The reasons for this are unclear. Cell cycle regulation and/or DNA replication have not been implicated in CSR and SHM so far. Using RNAi screening in B cells, we disclosed the role of cell cycle and DNA replication factors in CSR. Preliminary findings show that these factors associate with AID and Spt5, both *in vitro* and *in vivo*, and are localized to AID target genes on a genome-wide basis. We are currently investigating the mechanisms by which these factors regulate CSR, and how CSR is coordinated during the cell cycle in proliferating and hypermutating B cells.

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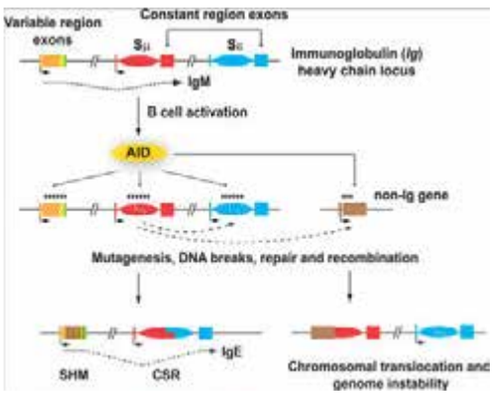

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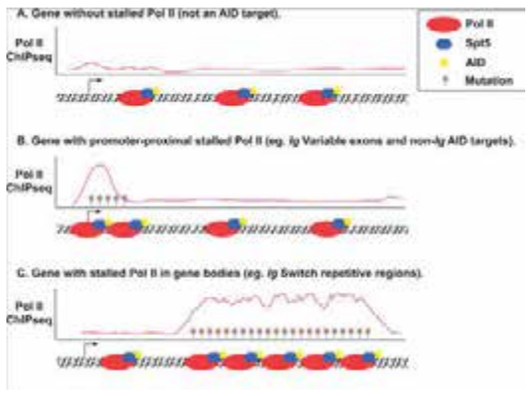

Figure 1: *Antibody diversification at the Ig heavy chain locus. B cell activation triggers AID expression, which generates mutations (arrowheads) in specific regions. These lesions are processed through various DNA repair pathways, resulting in SHM and CSR. Mutations at non-Ig genes results in reciprocal translocations between these genes and the Ig locus, leading to genome instability and tumorigenesis.*

Figure 2: *Model for AID targeting via transcriptional stalling (see text for details). Spt5 recruits AID to the Pol II complex. Stalling at promoter-proximal regions results in AID-mediated mutations in these regions (variable genes and non-Ig genes). Stalling within switch-repetitive regions results in mutations within the regions associated with CSR.*

Identification and characterization of novel factors in CSR and SHM

To gain insights into the mechanism of CSR, we performed an RNAi screen for the purpose of identifying new factors involved in this process. We are currently investigating interesting candidates determined from this screen, using mouse genetics, biochemical analyses, and genomics. In addition, we are developing new assays to quantitatively test for SHM in B cells, which will allow us to perform RNAi studies to address the precise role of various putative factors involved in SHM and identify new players in this process. Our long-term goal is to determine how AID targeting is tightly restricted to specific regions within the transcribed *Ig* locus, especially during SHM. This enigmatic aspect of the phenomenon eludes a clear explanation, but is critical for the immune response.

JAN-MICHAEL PETERS

Mitosis and chromosome biology

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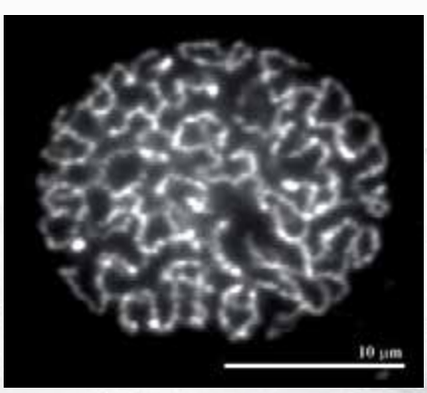

To pass the genome from one generation to the next, eukaryotic cells first replicate their DNA, then biorient chromosomes on the mitotic spindle and finally separate their sister chromatids, thus enabling the division of one cell into two genetically identical daughter cells. We are interested in understanding these processes at the molecular level.

How is sister chromatid cohesion established and maintained?

Numerous sites in the genome are bound by cohesin complexes. During DNA replication, these complexes establish physical connections between the newly synthesized sister chromatids. The resulting cohesion is known to be essential for chromosome segregation and DNA damage repair, but how cohesion is established and maintained is poorly understood. Our work indicates that cohesin is converted into a 'cohesive' form by the protein Sororin, which associates with cohesin during DNA replication, inhibits the cohesin release factor Wapl, thus stabilizes cohesin on DNA, and enables cohesin to maintain cohesion. Our current work aims at understanding how Wapl releases cohesin from DNA, how Sororin prevents this, and how these proteins are regulated in mammalian oocytes where cohesion has to be maintained for long periods of time. In humans this is required for many years.

How does cohesin control chromatin structure and gene regulation?

Although cohesin can establish cohesion only during DNA replication, cohesin also associates with unreplicated DNA in the G1 phase and in post-mitotic cells. In these situations cohesin is not stabilized on DNA by Sororin, but can instead be released again by Wapl. We suspect that cohesin binds to unreplicated DNA in a dynamic manner because it also has important functions in structuring chromatin and gene regulation. Supporting this hypothesis, we found that cohesin co-localizes with the transcriptional insulator protein CTCF in mammalian genomes, is required together with CTCF for gene regulation, and causes major changes in chromatin compaction when it is stabilized on DNA by the depletion of Wapl. In the future we aim to understand how cohesin controls chromatin structure and gene regulation, and how Wapl might regulate these functions.

How is sister chromatid cohesion dissolved during mitosis?

Sister chromatid separation in anaphase depends on the removal of cohesin from chromosomes. This is mediated by Wapl, which is activated in prophase and releases cohesin from chromosome arms, whereas centromeric cohesin is only removed from chromosomes in metaphase by the protease separase. We recently discovered that the Wapl-dependent prophase pathway of cohesin release is important for complete separation of chromosome arms in anaphase, and simultaneously protects most cohesin complexes from cleavage by the protease separase. This protection might be important for the next cell cycle, in which intact cohesin complexes are needed to re-establish a proper chromatin structure and gene expression programs.

How does the APC/C initiate anaphase?

In metaphase, when all chromosomes have been bioriented, the anaphase-promoting complex/cyclosome (APC/C) is activated. The APC/C is a 1.5 MDa complex which assembles ubiquitin chains on securin and cyclin B. The subsequent destruction of these proteins by

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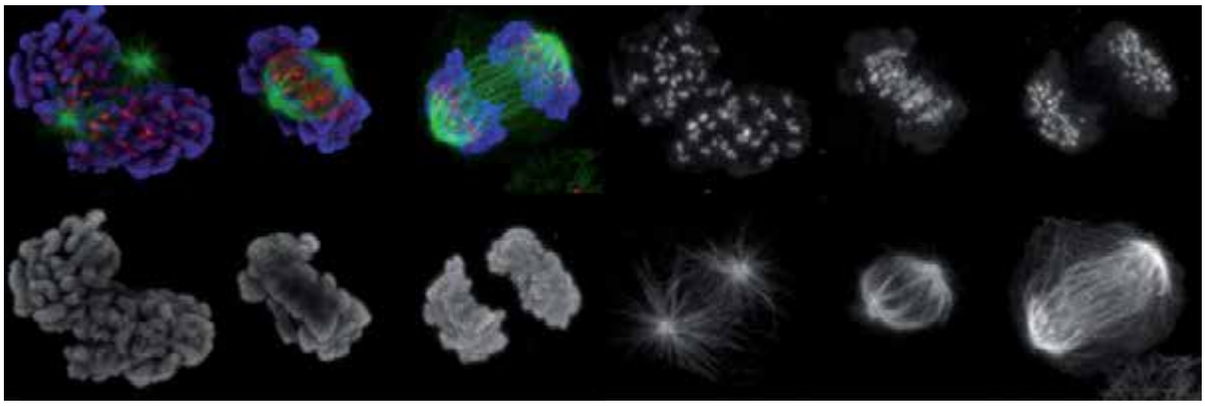

Figure 1: *Mouse embryonic fibroblasts depleted of the cohesin release factor Wapl and stained for cohesin. Wapl depletion reveals that cohesin is located in elongated structures, called 'vermicelli', which are present in interphase chromosome territories. These axial chromosomal elements might organize chromatin structure, perhaps by anchoring chromatin loops. For details see Tedeschi et al., Nature 501, 564-568. Courtesy of Antonio Tedeschi.*

Figure 2: *Human cells (HeLa) in prometaphase (left), metaphase (middle) and anaphase (right) stained for histone H3 phosphorylated on serine 10 (2nd row; blue in the merged images in the top row), a centromere protein (3rd row, red in the merged images in the top row) and α-tubulin (bottom row; green in the merged images in the top row). Courtesy of Rene Ladurner.*

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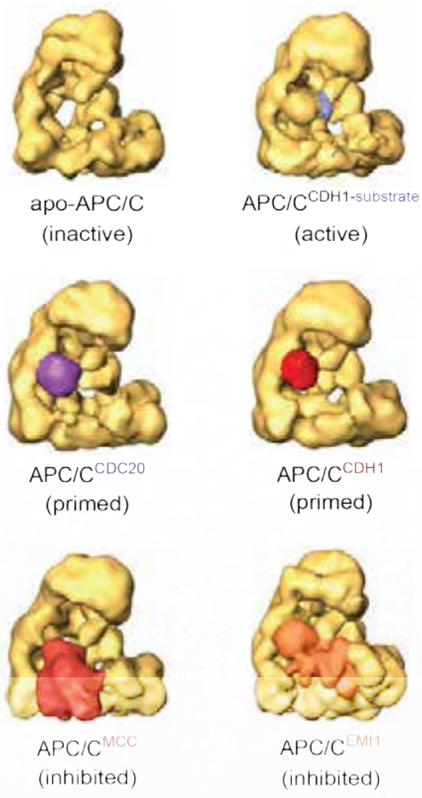

Figure 3: *Structure of human APC/C associated with different coactivators (Cdc20, Cdh1), inhibitors (mitotic checkpoint complex MCC, Emi1), or Cdh1 and a substrate protein (Hsl1). For details, see Herzog et al., Science 323, 1477-1481, and Buschhorn and Petzold et al., Nat. Struct. Mol. Biol. 18, 6-13, 2011, and Frye at al., Nat. Struct. Mol. Biol. 20, 827-835, 2013. Courtesy of Georg Petzold.*

the 26S proteasome permits activation of separase, cleavage of centromeric cohesin, and sister chromatid separation. Until chromosome biorientation is complete, the APC/C is inhibited by the spindle assembly checkpoint (SAC). The SAC ensures that sister chromatids are only separated once chromosomes have been attached to both spindle poles. Despite the importance of the APC/C, it is poorly understood how this complex is inhibited by the SAC, how the inhibition is relieved in metaphase, and how active APC/C recruits and ubiquitylates its substrates. We are using biochemical assays and electron microscopic analyses of the APC/C in different functional states to address these questions.

MitoCheck /MitoSys

Although mitosis has been studied for more than a century, our molecular understanding of this complex process is far from complete. From 2004 to 2009, the MitoCheck consortium funded by the European Union developed and applied genomic and proteomic approaches to study mitosis. The consortium used RNA interference screens to identify proteins required for mitosis in human cells, tagging of genes in bacterial artificial chromosomes (BACs) to enable intracellular localization and affinity purification of these proteins, and mass spectrometry to identify protein complexes and mitosis-specific phosphorylation sites on these. This work led to the identification of about 100 human protein complexes, many of which had been previously unknown or poorly characterized. Importantly, the approaches developed by MitoCheck will be generally applicable to high throughput analyses of other processes in mammalian cells. In a new project funded by the European Union, known as MitoSys (2010 to 2015), we are developing quantitative assays for mitosis.

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SIMON RUMPEL

Auditory perception and memory in the neocortex

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Our lab focuses on the function and plasticity of neuronal circuits. Specifically, we would like to know how sounds are represented in the auditory cortex and how these representations are transformed into long-term memory. This is fundamental in understanding the mind: memories of past experiences shape our personalities and influence our current perception.

A look into the brain

Long-term storage of information about relevant experiences is essential for successful adaptation of human and animal behavior in a changing environment. A current model of memory formation suggests plastic adaptations in neuronal connections (synapses) caused by relevant experiences. Yet, how such changes in synaptic connectivity lead to the formation of a memory trace remains elusive. How is the processing of external stimuli altered after the formation of a memory? How are we able to continuously store novel memories in a given neuronal circuit without corrupting previously stored memories? In order to understand the mechanisms by which the auditory cortex mediates the processing of sounds and is involved in the formation of memories, we are currently applying *in vivo* imaging techniques in mice.

Two-photon laser scanning microscopy in transgenic animals expressing green fluorescent protein in just a small subset of cells permits the same neurons, and even the same individual synapses, to be revisited day after day. This is truly remarkable because we estimate that the brain comprises about 10 trillion (10^{13}) synapses. We find that neocortical circuits are highly dynamic: remodeling occurs by the formation/elimination of synaptic connections as well as adaptations in the strength of existing connections. In addition, we observe that memory formation induced by classical conditioning of a sound stimulus is correlated with a transient increase in spine formation that leaves a long-lasting trace in the network. Interestingly, memory recall does not lead to the recapitulation of structural remodeling as observed in the formation of initial memory. Our findings provide a potential synaptic mechanism underlying previously reported functional changes in the auditory cortex after fear conditioning and challenge models, in which memory traces are modified upon memory retrieval.

In vivo imaging permits analysis of synaptic connections as well as monitoring of neuronal activity in tens of neurons simultaneously. Action potential-mediated increases in calcium levels can be detected as changes in fluorescence of calcium indicators. We are investigating activity patterns elicited by various sounds in neuronal populations of the auditory cortex in order to learn about the principles how sounds are encoded and recognized in the brain. We observe that activity in layer 2/3 neuronal ensembles is rather strongly constrained into a few response modes. Interestingly, these discrete activity modes may serve as a representational basis to predict generalization behavior in an auditory discrimination task. Our findings point toward a model of neocortical function in which external stimuli are represented in a broad basis set of spontaneous associations into common activity patterns, and classified by sharp transition across the activity patterns. We are currently investigating the circuit mechanisms that lead to the generation of sound representations in discrete activity modes, and to what extent auditory learning paradigms cause changes in these neuronal representations of memorized sounds. To this end, we utilize adeno-

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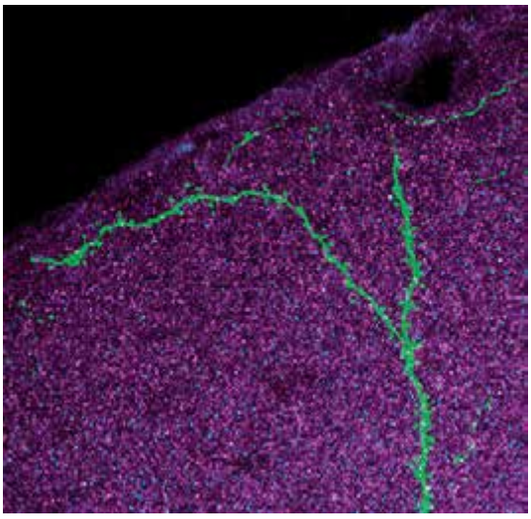

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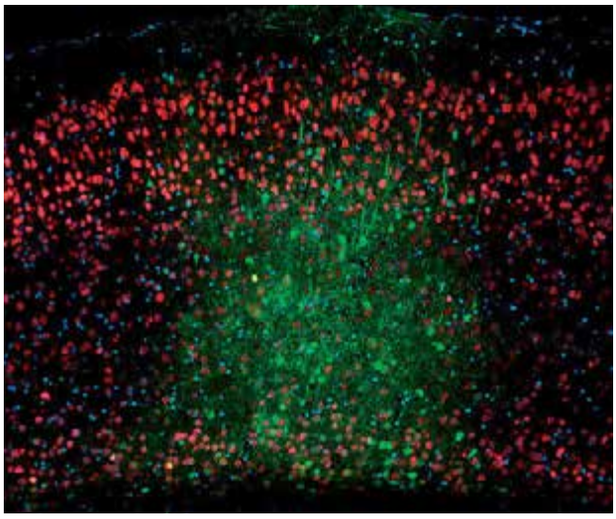

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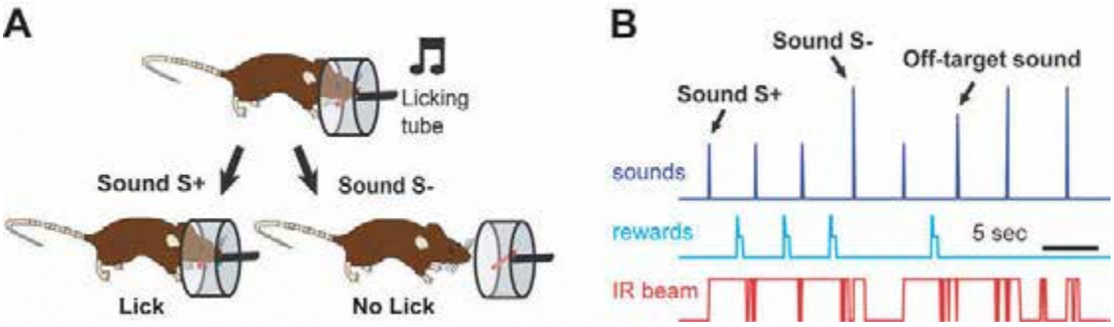

Figure 1: ***Tracing the dynamics of dendritic spines.*** *Cross-section of the auditory cortex of a transgenic mouse used for in vivo spine imaging. A single neuron is highlighted by green fluorescent protein. The dendritic processes are decorated with spines, the morphological correlate of excitatory synapses. Counterstaining for synaptic marker proteins ErbB4 (cyan) and Synapsin1 (magenta).*

Figure 2: ***Measuring auditory perception in mice.*** *(A) Mice are trained to discriminate two sound stimuli: S+ predicting the availability of a water reward when the mouse keeps licking on a water spout; S- predicting a mildly aversive air puff that can be avoided when the mouse suppresses licking. (B) Example of a sequence of trials from a well-trained mouse. Positively (S+) and negatively (S-) reinforced sounds are shown as short and long spikes on the dark-blue trace. The mouse stays on the licking spout (IR beam break) selectively after the positive sound to obtain the water reward. Mice spontaneously categorize non-reinforced off-target sounds and thus indicate whether the sound is perceived as being more similar to S+ or S-.*

Figure 3: ***Using viruses to genetically target neurons in the brain.*** *Coronal section of the auditory cortex of a mouse transduced with an adeno-associated virus driving expression of GFP (green). The sample was labeled with DAPI highlighting nuclei (blue) and immunostained for a neuronal marker (NeuN, red).*

associated viruses to drive the expression of genetically encoded calcium indicators in the auditory cortex. This approach allows us to monitor the dynamics of spontaneous and sound-evoked activity patterns in the same ensembles of neurons over weeks.

Jointly, these approaches will pave the way for a series of novel experiments addressing the storage of information in living neuronal networks - a field of research that has been almost exclusively the domain of theoretical neuroscientists.

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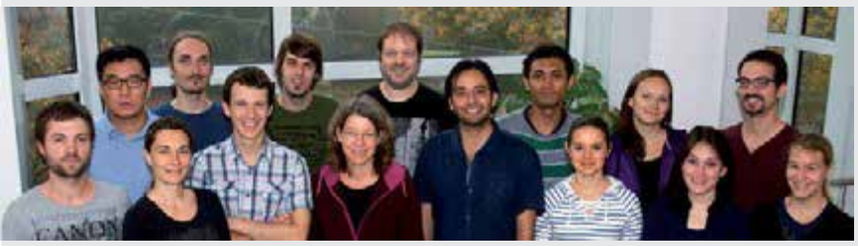

ALEXANDER STARK

Systems biology of regulatory motifs and networks – towards understanding gene expression from the DNA sequence

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The regulation of gene expression in response to developmental or environmental stimuli is a crucial mechanism in all organisms. Transcription is regulated by trans-acting transcription factors that recognize cis-regulatory DNA elements (CRMs or enhancers), and function in a combinatorial fashion. Enhancers retain their activity even when placed in artificial contexts (such as in reporter gene assays), but the exact requirements for enhancer function, i.e. a regulatory code, remain unknown. Enhancer activity cannot be predicted from the DNA sequence. Employing an interdisciplinary approach, we use both bioinformatics and molecular-biology-based methods to achieve a systematic understanding of the structure and function of enhancers. Our goal is to "crack" the regulatory code, predict enhancer activity from the DNA sequence, and understand how transcriptional networks define cellular and developmental programs.

The regulatory code of context-specific transcription factor binding

Transcription factors are employed in different contexts (such as different tissues or developmental stages), and typically regulate context-specific targets that are determined by the respective enhancer sequences and transcription factor combinatorics. We use tissue-specific ChIP-Seq, bioinformatics, and machine learning to determine the sequence determinants of context-specific transcription factor binding in *Drosophila*. For example, transcription factor binding during embryonic mesoderm and muscle development is highly stage dependent (Zinzen et al., 2009). We were able to predict and validate that the transcription factor vielfaltig/zelda is an important determinant of transcription factor binding in the early embryo (Yáñez-Cuna et al., 2012). We also determined the context-specific targets of the circadian clock factors CLOCK and CYCLE, and identified two factors that help these factors to specify their targets in different contexts (Meireles-Filho et al., 2014). In collaboration with the Zeitlinger group (Stowers Institute), we studied *in vivo* transcription factor binding in 6 *Drosophila* species and found that transcription factor binding is highly conserved in species as distant from *D. melanogaster* as platypus or chicken from human (He & Bardet et al., 2011).

Collections of enhancers that function similarly across cell types would be an invaluable resource to study the sequence basis of enhancer activity. We therefore focus on the large-scale discovery of enhancers *in vivo* and *in vitro*.

In vivo enhancer screens

The Vienna Tiles (VT) library consists of ~8000 transcriptional reporter constructs integrated at a single defined genomic position in transgenic *Drosophila* lines, each bearing a distinct ~2kb-long candidate DNA fragment. Together, these fragments cover ~14% of the *Drosophila* non-coding, non-repetitive genome. We are determining the temporal and spatial enhancer activity of these fragments in transgenic *Drosophila* embryos by *in situ* hybridization. About one half of all fragments showed specific activity patterns during embryogenesis, indicating that the fly genome is densely packed with enhancers (Figure 1). Interestingly, the activity rate increases from about 7% in early embryos to about 35% in late embryos, reflecting the increasing complexity of the embryo with a rising number of distinct tissues and cell types (Kvon et al., in revision).

Enhancer activity and gene expression analysis by automatic image processing

We are developing computational tools to automatically find and extract embryos from whole-mount *in situ* images as obtained during our *in vivo* enhancer screen (Figure 1). In collaboration with Christoph Lampert's group (IST Austria), we developed a method to automatically segment images of entire microscopy slides and predict the developmental stage of individual embryos. The method combines general features that are invariant to

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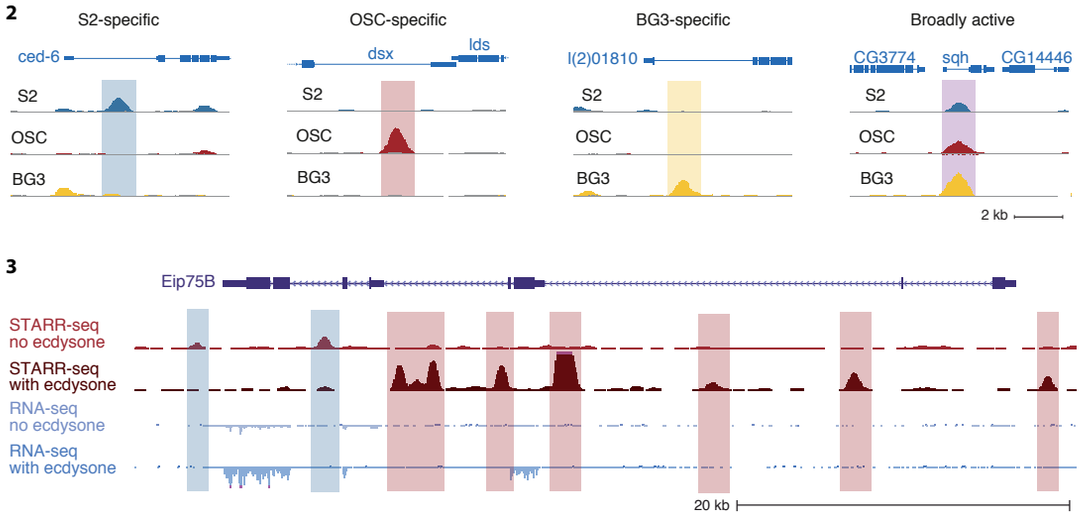

Figure 1: *Enhancer screen in transgenic Drosophila embryos. We show here a sample of embryos that stain positively in our enhancer assay and display diverse activity patterns. The embryos are in different stages, and are shown in lateral orientation, anterior to the left. The inset shows a magnification of a blastoderm embryo with an enhancer activity signal in the dorsal ectoderm (Kvon et al., in revision).*

Figure 2: *STARR-seq fragment densities for enhancers that are specifically active in one of three Drosophila cell lines (S2: embryonic, OSC: adult ovarian, BG3: larval neuronal), and an enhancer that is active in all three cell types (Yáñez-Cuna et al., submitted).*

Figure 3: *STARR-seq fragment densities in the Eip75 gene locus before and after treatment of Drosophila S2 cells with ecdysone. Induced peaks are shaded red while repressed peaks are shaded blue (Shlyueva et al., in revision).*

the tested enhancer candidate (such as the shape of the embryo) with features that are specific to each candidate (for instance its activity pattern) in a principled framework of label propagation (Kazmar et al., 2013).

In vitro enhancer screens (STARR-seq)

We have established a high-throughput method based on next-generation sequencing that allows us to assess enhancer activity in different cell types quantitatively and on a genome-wide basis (STARR-seq; Arnold et al., 2013). Its application to three different *Drosophila* cell lines identified thousands of cell-type-specific enhancers across a wide continuum of strengths (Figure 2). Using bioinformatics and machine learning, we found that cell-type-specific enhancers show distinct motif signatures. Moreover, we identified a novel class of enhancer elements known as dinucleotide repeat motifs (DRMs), which are highly enriched in enhancers (Yáñez-Cuna et al., submitted).

We are also using STARR-seq to assess the evolution of enhancer function across closely related *Drosophila* species in *D. melanogaster* S2 cells, i.e. a constant trans-regulatory environment. This revealed high functional and positional conservation of enhancer activity, which is not reflected by overall enhancer sequence similarity but by the conservation and turnover of functionally important transcription factor motifs (Arnold et al., in revision).

To study hormone-mediated gene regulation, we applied STARR-seq to ecdysone signaling in different *Drosophila* cell types and derived the first genome-wide map of hormone-dependent enhancer activity (Figure 3). We found hundreds of enhancers that are activated or repressed by ecdysone, and showed that they contain characteristic cis-regulatory motifs that are predictive and required for enhancer activation (Shlyueva et al., in revision). This has been an important pilot step to demonstrate the applicability of STARR-seq to study enhancers downstream of cellular signaling pathways, which we are now exploring further.

We are applying STARR-seq to several other questions in both, *Drosophila* and human systems.

Novel methods based on next-generation sequencing (NGS)

High-throughput next-generation sequencing has become the basis of many novel methods. We are establishing computational tools to analyze NGS data for RNA sequencing, RNA cross-linking and immunoprecipitation (CLIP), haploid genetic screens, and chromatin-immunoprecipitation coupled to NGS (ChIP-Seq; Bardet & Steinmann et al., 2013). We are collaborating with many experimental groups on the campus and abroad.

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Neural circuits for vision

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Circuit neuroscience has been revolutionized in the last few years by the use of genetics to express proteins such as channelrhodopsin or GCaMP in molecularly defined neuronal classes. Concurrently, a recent trend in cognitive science is to model the mind within a quantitative framework in which the purpose of perception, decision-making and learning is framed in the context of Bayesian inference. Visually guided behavior of the fruit fly is an ideal experimental system for linking these approaches to establish a mechanistic understanding of neuronal circuit function as well as the computational purpose of behavior. This is the focus of our work.

Engineering tools for quantitative behavior

A major challenge in modern neuroscience is to understand how behavior emerges from the interplay of multiple physiological processes and an animal's own environmental surroundings. As the circumstances under which animals evolve are very different from most laboratory experimental paradigms, it is difficult to relate the results of many behavioral assays to behaviors shaped by natural selection. This makes it difficult to reason about the purpose of a particular behavior. We address this problem by creating novel experimental devices that allow laboratory-level rigor in experiments performed under naturalistic conditions.

One set of our technical innovations consisted of a virtual reality system capable of simulating arbitrary visual scenes. By utilizing live animal tracking, a tethered or freely moving animal is placed in a virtual environment rendered physical by means of multiple computer displays. We made use of recent advances in computer graphics technology, such as GPU shaders, to achieve real-time luminance blending across multiple projection paths and onto arbitrarily shaped projection surfaces.

Mapping neural circuits for visual locomotor guidance in *Drosophila*

In one set of related ongoing projects, we are performing genetic manipulations to silence small numbers of neurons and measuring behavioral deficits in response to precisely defined visual stimuli. Specificity down to single cell types is attained with recent genetic techniques. A variety of effector molecules that kill cells, hyperpolarize them, or block chemical synaptic transmission are used. A completely automated free-flight virtual reality arena allows us to perform visual psychophysics experiments in high throughput. A tethered flight apparatus enables us to measure and stimulate with high spatial and temporal resolution. As this setup is similar to the type required for electro- and opto-physiology, it permits us to make direct predictions for such work. The basic idea of these experiments is simple: to eliminate a group of cells and measure associated behavioral deficits.

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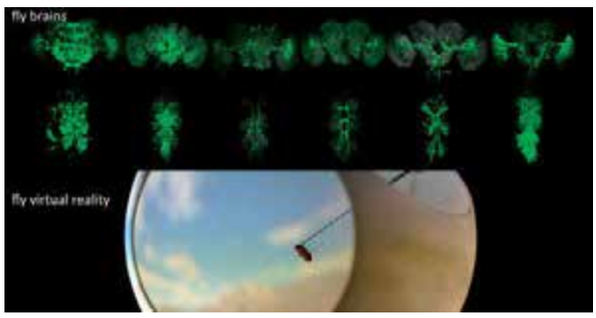

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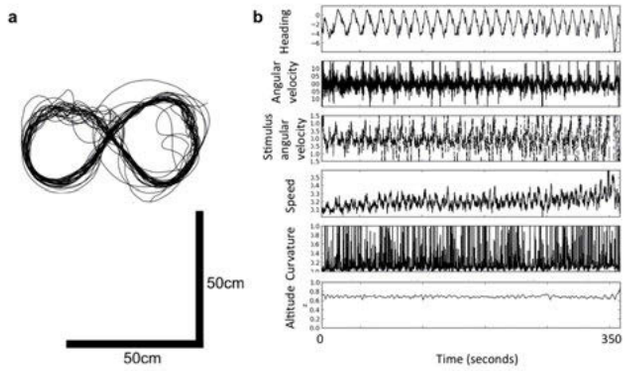

Figure 1: *Genetic advances, such as the Vienna Tiles GAL4 library, allow targeted expression of specific molecules in defined neurons. Combined with virtual reality experiments, we reverse-engineer the mechanisms and purpose of the fly eye.*

Figure 2: *By modifying the visual feedback supplied to freely flying flies, we can make them follow arbitrary trajectories. This permits us to record extremely long trajectories in a confined experimental space, and quantify several aspects of sensory-motor performance.*

How flies resolve conflicting visual information – cells and models

Several fundamental visual behavioral modules, such as wide-field rotational stabilization and object approach, shape a fly's trajectory as it flies through its habitat. How do these modules interact? From basic principles it is clear that any organism should select one stimulus in those situations in which attempting to simultaneously respond to both would be counter-productive. We are now dissecting the algorithmic and neural basis of such situations and the resulting higher-level visual behaviors that necessarily arise as a fly travels through a more realistic natural environment. The neural substrate of these computations is being mapped with the strategy described above. We are interested not only in the relevant circuits, but also in the conceptual and algorithmic components of the problem.

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ALIPASHA VAZIRI

Dynamics of coupled biological systems: methods and phenomena

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Our goal is to understand how stochasticity, non-linearity, correlations, and coupled excited state dynamics of biological systems and networks contribute to their function. We investigate these questions in different systems and at different levels, from the fundamental principles of protein and bio-molecular dynamics on the nanoscale to dynamic interactions in cellular networks, giving rise to physiological function.

Description of recent research activities

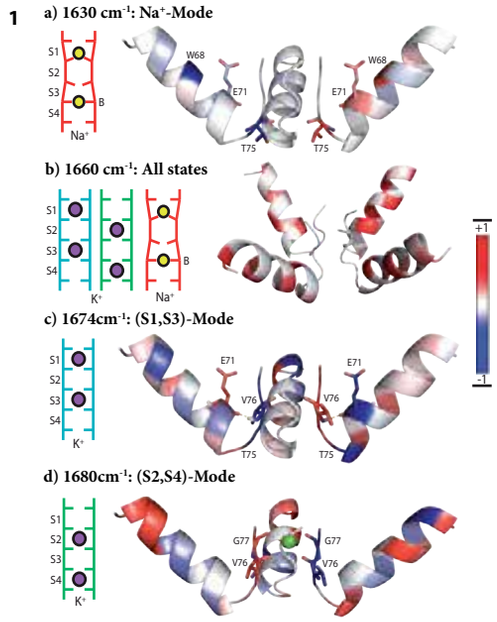
Our goal is to understand how stochasticity, non-linearity, correlations, and coupled excited state dynamics of biological systems and networks contribute to their function. We investigate these questions in different systems and at different levels, from the fundamental principles of protein and bio-molecular dynamics on the nanoscale to dynamic interactions in cellular networks, giving rise to physiological function.
To address these questions we adopt a multidisciplinary approach and develop new methods and technologies, such as advanced imaging and spectroscopy techniques based on ultrafast and quantum optics, and combine these with molecular biology, optogenetics, and electrophysiology. We are currently pursuing three areas of research.

Optogenetics and dynamics of neuronal circuits

Wide-field temporal focusing (WF-TEFO) is a two-photon imaging technique based on light sculpting. It effectively decouples the parameters governing the lateral size of a light beam and its axial resolution. Thus, the technique permits the investigator to excite a large area in the lateral dimension while retaining exceptional resolution in axial direction. In an actual setup, this is akin to creating a thin "disc" of excitation light. WF-TEFO is well suited for fast volumetric imaging, as scanning is reduced to a single dimension. We developed a two-photon technique for brain-wide calcium imaging in *C. elegans* using wide-field temporal focusing (WF-TEFO). A crucial aspect of our results is the use of a nuclear-localized, genetically encoded calcium indicator (NLS-GCaMP5K), which permits unambiguous discrimination of individual neurons within the densely packed head ganglia of *C. elegans*. We demonstrate near-simultaneous recording of the activity of as many as 70% of all head neurons. In combination with a lab-on-a-chip device for stimulus delivery, this method provides an enabling platform for establishing functional maps of neuronal networks.

Coupled dynamics and protein function

We developed a combined optical and electrophysiological approach to investigate the influence of structural flexibility and vibrational modes on the selectivity and function of the prototype potassium channel KcsA. We used femtosecond 2DIR spectroscopy to map changes in the flexibility of the selectivity filter of the potassium channel KcsA in response to different ions and binding states by using Fourier transform infrared spectroscopy (FT IR) and two-dimensional infrared spectroscopy (2D IR) in combination with spectral modeling. We are able spectroscopically identify the signatures of different ion binding states, and found that they are not restricted to the filter region but also lead to delocalized vibrations of the adjacent pore helices of all subunits. In addition, high couplings between some of these modes are observed on spectroscopy. Our results show that local ion-induced changes in structure and flexibility can be mapped with single



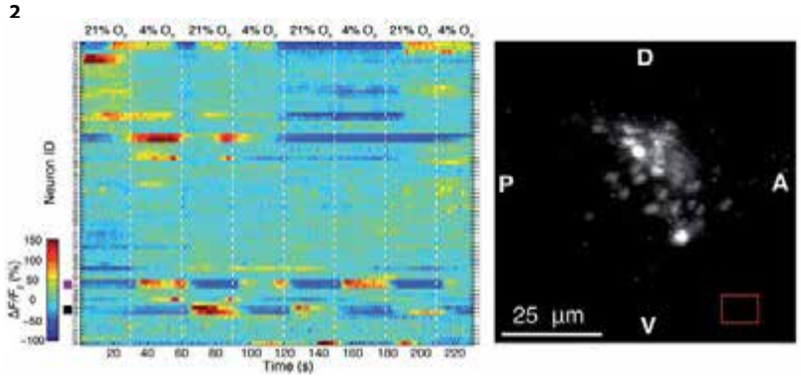


Figure 1: *WF-TEFO.ai: The left side shows activity patterns of individual neurons over time, reconstructed from 3D videos of entire worm brain activity shown on the right.*

Figure 2: *KcsA vibrational modes.ai: Delocalized vibrations induced by different ions binding to the selectivity filter of KcsA spread out on adjacent alpha helices. Color shows the phase and intensity of the amplitude of motion.*

residue resolution in a large membrane protein such as the KcsA. This capability paves the way for a wide range of future studies to investigate the functional role of vibrational dynamics and structural plasticity in proteins. Electrophysiology is used to investigate the potential functional impact of vibrational modes on conductance and selectivity by changing the vibrational landscape of the channel via mutations, radio frequency fields, stimulated Raman spectroscopy, or Isotope labels.

Optical tweezers

Optical tweezers have revolutionized our understanding of how molecular motors function, and permitted us to raise questions that were previously unanswerable by other methods. However, there is still a big gap between our ability to apply controlled forces to single molecules and our understanding of how this force affects states of the molecular motor and conclusions we can draw about the molecular mechanism of its action. We are currently working on two aspects: first, we are interested in designing new methods based on optical tweezers that will allow us to obtain additional information about molecular motors; second, we are applying our tools to study DNA polymerase and other molecular motors such as kinesins.

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Kinetochores and the Microtubule Cytoskeleton

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Eukaryotic cells have evolved complex and extremely precise chromosome segregation mechanisms which ensure that genetic information is passed on correctly from one generation to the next. In order to understand how the cell moves chromosomes during mitosis, our group is studying the structure, function, and regulation of the kinetochore, a complex multi-protein assembly that connects chromosomes to dynamic microtubules. In a reductional biochemical approach we aim to study and assemble functional kinetochores from their constituent parts in vitro. In addition, we use yeast genetics to manipulate and engineer simple chromosome segregation systems in vivo.

Towards a mechanistic understanding of the chromosome segregation machinery

An understanding of kinetochore function requires the study of its constituent parts, the assembly of these parts into higher-order structures, and ultimately the reconstitution of kinetochore function *in vitro*. The kinetochore is a complex macromolecular machine that hierarchically assembles from a set of conserved multi-protein complexes. We have reconstituted a number of these complexes by co-expressing multiple subunits in bacteria, and studied their biochemical properties. This analysis has already yielded a number of important insights: the Dam1 complex, a specialized microtubule-binding component of the budding yeast kinetochore (**Figure 1**), oligomerizes to form a ring around microtubules *in vitro*. This ring slides along the microtubule lattice and remains attached to the plus-end even during microtubule disassembly. These properties make the Dam1 ring a very efficient force coupler at the kinetochore. A challenge for the future is to understand how the Dam1 ring is connected to the rest of the kinetochore, visualize the structure of the fully assembled interface, and analyze how it is regulated, for example, by mitotic kinases. Our investigations into kinetochore assembly have led to the identification of a conserved receptor molecule for the microtubule-binding Ndc80 complex. We have resolved the crystal structure of the interface between Ndc80 and the histone-fold protein Cnn1 (**Figure 2**), and are further investigating how the cell employs different Ndc80 receptors to promote chromosome segregation.

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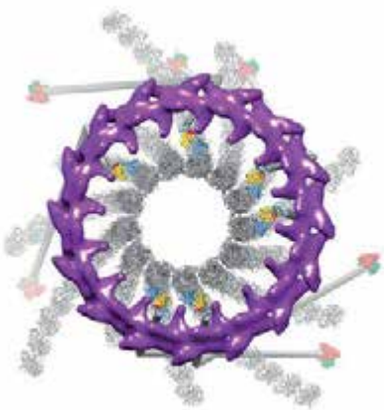
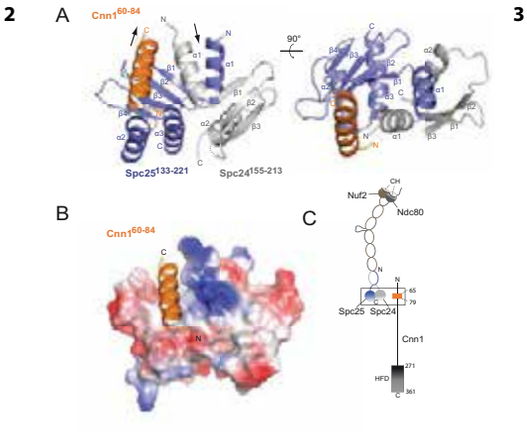


Engineering simplified chromosome segregation systems in the cell

To define functional modules within the complex kinetochore architecture, we have adopted a reductional approach to design simple kinetochores *in vivo*. By artificially recruiting individual kinetochore components to engineered binding sites on circular plasmids and native yeast chromosomes, we demonstrated that the Dam1 complex is not only necessary but also sufficient to generate an interface that supports chromosome segregation. In the future we will further characterize the protein composition and precise function of these "artificial" kinetochores.
Another challenge for the future is to understand how kinetochore structure and function is modulated throughout the course of the cell cycle. The basic signals that couple cell cycle progression with the regulation of kinetochore function have remained elusive. Combining time-resolved analysis of post-translational modifications with yeast genetics should enable us to determine general principles of regulation.



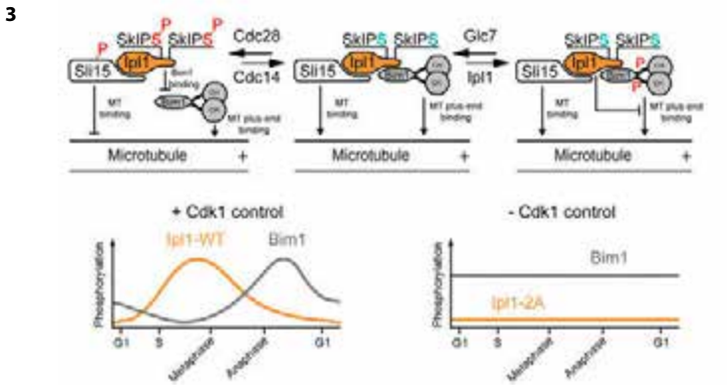


Figure 1: *Model of the Dam1-Ndc80 interface. A Dam1 ring (purple) seen in "end-on view" associating with six Ndc80 complexes (grey). The microtubule-binding CH domains of the Ndc80 complex are shown in yellow and blue.*

Figure 2: *Crystal structure of the Ndc80-Cnn1 interface. **A**. A complex between the conserved receptor motif of Cnn1/CENP-T (orange) and the globular Spc24-25 heterodimer (blue and silver). **B**. Surface representation showing that the receptor helix is buried in a hydrophobic groove. **C**. Overall arrangement of the Ndc80 complex and Cnn1/CENP-T).*

Figure 3: *Model for Cdk1 regulation of the Ipl1-Sli15 complex. During metaphase Cdk1 phosphorylates Sli15 and inhibits its MT-binding domain, whereas by acting directly on Ipl1 it prevents association with Bim1. Cdk1 controls a cascade of phosphorylation events in which Ipl1 phosphorylation is temporally separated from Bim1 phosphorylation.*

Single molecule analysis of microtubule-associated proteins and motors

A defining feature of kinetochores is their ability to interact with microtubule plus-ends through multiple rounds of polymerization and depolymerization. How does the kinetochore achieve this remarkable task? What features enable it to follow a polymerizing microtubule end, and also stay connected during disassembly? How does the kinetochore modulate microtubule dynamics? To analyze this process we have reconstituted dynamic microtubules *in vitro* and visualized the interaction of individual kinetochore components using total internal reflection fluorescence (TIRF) microscopy. This technique allows the observation of individual kinetochore complexes and microtubule-binding proteins with single-molecule sensitivity, and thus reveals their mode of interaction with dynamic plus-ends. We have recently started to investigate motor proteins involved in kinetochore transport. We hope to achieve a mechanistic understanding of this process, and also explore the type of features that allow translocation along microtubules.

Dissecting microtubule plus-end interaction networks

While kinetochores are stable and strictly defined structures that establish connections between chromosomes and the spindle, the plus-ends of microtubules are also sites of dynamic assemblies of protein networks with important roles in cellular morphogenesis and signaling. At the core of these protein networks is the microtubule plus-end-binding protein EB1 (Bim1 in budding yeast), which controls the association of many diverse proteins with microtubules (**Figure 3**). We have started to systematically analyze the composition of EB1-based protein interaction networks in yeast. We follow their changing composition over the cell cycle using quantitative mass spectrometry, investigate how these changes in composition are established on a molecular level, and determine their functional consequences.

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The neural basis of behavior

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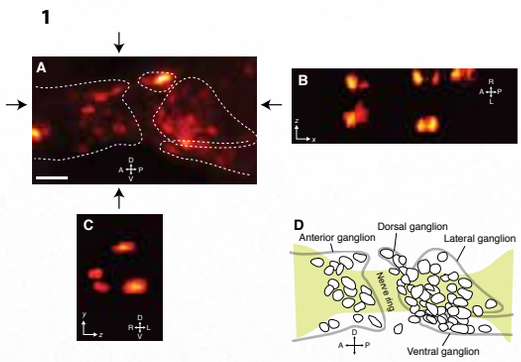


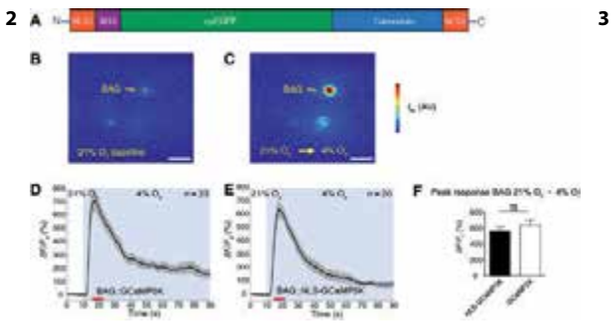
One of the prime goals pursued by current neuroscientists is to gain a comprehensive understanding of how networks of neurons operate as a single brain to produce sensations, thoughts, and behavior. This is a challenging endeavor because of the sheer complexity of mammalian nervous systems. To address this problem, at our lab we study the nematode C. elegans, which is equipped with a simple and anatomically well defined nervous system of just 302 neurons. Specifically, we combine worm genetics, quantitative behavioral assays, novel functional imaging techniques, and theoretical modeling to elucidate the neural circuits that control locomotion and chemosensory behaviors.

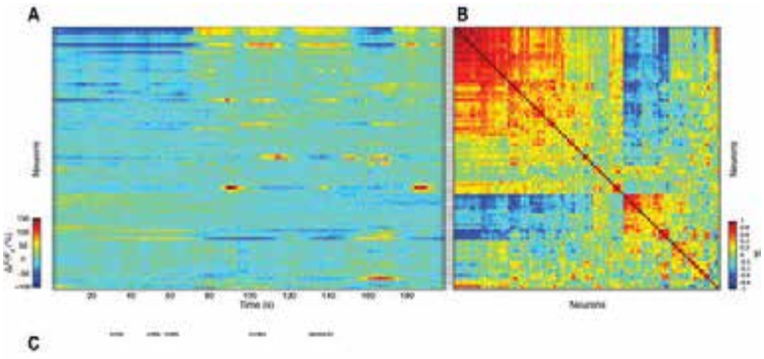
Brain-wide calcium imaging in *C. elegans*

A better understanding of how neural circuits in the brain process information and generate behavior requires detailed knowledge of both anatomical neuronal wiring, as well as the dynamic rules governing the activity of neurons and their connections. The complete connectome of the *C. elegans* nervous system was reported more than 25 years ago. However, this has not been sufficient to explain how behaviors arise. The major impediment is the dense interconnectedness of neurons, with the consequence that there are no obvious anatomically identifiable pathways from sensory input down to motor output. These and other findings in different animals led to the current view among neuroscientists that many sensory functions and behavioral states of animals are represented in a flexible and distributed fashion across neuronal networks. The current challenge in systems neuroscience is to identify the nature of, and decipher the mechanisms responsible for, the computations and algorithms that are performed by these neural networks.

Research in *C. elegans* focuses largely on isolated neurons and small circuits. This is mainly because of technical limitations in electrophysiology and functional imaging: *C. elegans* neurons are small and densely packed into head and tail ganglia and closely surrounded by neuropil, which makes them inaccessible to multi-electrode arrays and hard to track with image segmentation methods when pan-neuronally expressed genetically encoded calcium indicators are used. To overcome these limitations, the Vaziri lab developed wide-field temporal focusing (WF-TeFo), a 2-photon excitation based high-resolution imaging technique which is capable of unbiased and fast volumetric imaging of neurons. In addition, we designed a novel genetically encoded calcium reporter which is localized to the cell nucleus (NLS-GCaMP5K). In combination, the approaches allowed unambiguous anatomical identification of neurons and their temporal calcium signals (**Figure 1**). We confirmed that NLS-GCaMP5K reliably reports neural activity by comparing nuclear with cytoplasmic calcium responses in oxygen-sensing neurons (**Figure 2**).

Using WF-TeFo microscopy, we were able to capture calcium signals from most of the neurons in the worm's brain at a volume acquisition rate of 5 volumes per second. **Figure 3** shows the result of a typical recording. We found that at least 50% of neurons in the brain are active under our experimental conditions. Correlation analysis revealed a cluster of up to 20 neurons engaged in highly correlated activity, which is also anti-correlated to other smaller clusters of neurons (**Figure 3C**). In nematodes, cell positions and identities are stereotypic. This enables us to identify, on our images, a subset of neurons with prominent activity patterns. The large cluster of correlated neurons is represented by pre-motor interneuron classes, which have previously been implicated in decision-making during *C. elegans* locomotion. When foraging, the animals explore the environment by occasionally changing the direction of locomotion. The initiation of these maneuvers is reported to be associated with enhanced activity of pre-motor interneurons such as AVA and RIM.





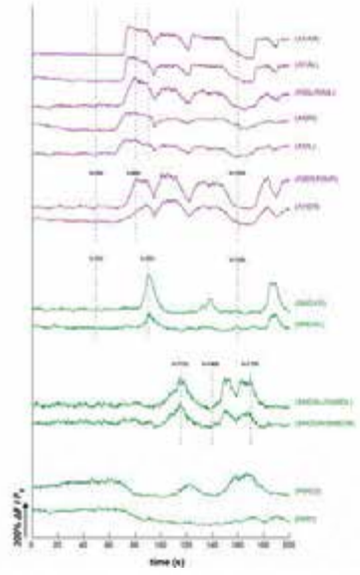


Figure 1: ***WF-TeFo imaging of the head region of a worm with pan-neuronal NLS-GCaMP5K expression***. *(**A**) Maximum intensity projection of 14 z-planes. Dashed lines outline the locations of head ganglia as shown in (D). Scale bar represents 10 µm. Arrows indicate the positions of y-z and x-z cross-sections shown in (**B**) and (**C**) respectively. (**D**) Schematic diagram of the left anterior head ganglia for comparison with (A).*

Figure 2: ***In vivo characterization of NLS-GCaMP5K.*** *(**A**) Schematic diagram of NLS-GCaMP5K. (**B-F**) Calcium imaging of oxygen-sensing BAG neurons by epifluorescence microscopy. (**B-C**) NLS-GCaMP5K fluorescence in stimulated BAG neurons. (**B**) Baseline (21% oxygen) fluorescence levels. (**C**) Oxygen downshift evoked response in BAG. (**D-E**) Averaged calcium transients in BAG expressing cytoplasmic (**D**) or nuclear (**E**) G-GAMP5K, and quantification of mean peak responses (**F**). Scale bars represent 10 µm.*

Figure 3: ***Activity of the C. elegans brain***. *(**A**) Activity of 99 neurons from the same worm as in Figure 1, imaged volumetrically at 5 Hz for 200 s. Each row shows a heat plot of the NLS-GCaMP5K fluorescence time series. (**B**) Matrix showing the correlation coefficient (R) calculated from all time series shown in (A). Color indicates the degree of correlation. The data in A-B are grouped by agglomerative hierarchical clustering. (**C**) Selected traces of neurons. Purple: pre-motor interneurons. Green: head motor neurons.*

These activities are shown in **Figure 3C**. Smaller clusters of neurons that appear anti-correlated with pre-motor interneurons were identified as head motor neurons. These are believed to control the undulatory motion of the worm.

Our results reveal unexpected system-wide synchrony and antagonistic complexes in the pre-motor circuits of the *C. elegans* brain. Our data suggest that decision-making, i.e. the initiation of locomotion maneuvers, can be represented by transitions between network attractors. We are currently testing this hypothesis by combining our novel imaging approach with genetics, behavioral analysis, and theoretical modeling.

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Finding and probing cancer drug targets using advanced RNAi technologies

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The genetic complexity, heterogeneity, and plasticity of human cancers pose a daunting challenge for the development of effective targeted therapies. Despite their diversity, individual mutations converge at the functional level to dysregulate a limited number of cellular processes, which not only promote malignant growth but are thought to result in cancer-specific dependencies that can be exploited for therapeutic purposes. To systematically explore such "non-oncogene addictions" and study them in a physiologically relevant setting, our lab combines genetically engineered mouse models (GEMMs) and advanced miRNA-based shRNA (shRNAmir) technologies optimized for multiplexed screening and studies in vivo.

An optimized microRNA backbone for effective single-copy RNAi

Due to our incomplete understanding of miRNA biogenesis factors, available shRNAmir reagents remain far from perfect and often fail to trigger potent target knockdown. Following recent advances in the design of the synthetic stem, over the past year we have established an optimized miRNA backbone termed miR-E (Figure 1), which greatly improves knockdown potency by enhancing pri-miRNA processing (~10-fold). When combined with up-to-date design algorithms and improved expression vectors, miR-E predominantly yields single-copy effective shRNAs required for multiplexed screening, and thereby overcomes a key limitation of previous reagents. To implement these improvements in new shRNA libraries, we have established a robotics/deep-sequencing-supported cloning pipeline which permits rapid construction of custom-optimized shRNA libraries.

Exploring new therapeutic targets in high-risk AML

Despite our advanced genetic understanding, acute myeloid leukemia (AML) remains incurable in more than 70% of patients. While many targeted therapies (mainly inhibitors of pro-proliferative "type-I" mutations) have failed in clinical studies, all-trans-retinoic acid (ATRA) targeting PML/RARA (a self-renewal promoting "type-II" mutation) has turned a deadly AML subtype into a curable disease. To systematically search entry points for similarly effective targeted therapies, we are pursuing two approaches: a) Using a series of AML mouse models harboring regulatable "type-II" mutations and focused RNAi studies, we seek to gain insight into common factors and pathways involved in maintaining aberrant self-renewal; and b) Using an established model of high-risk MLL-AF9;Nras-driven AML, we have performed a multiplexed RNAi screen targeting ~1000 druggable and MLL-regulated candidate genes, which has revealed druggable leukemia-specific dependencies including several metabolic regulators, which we focus on in mechanistic follow-up studies.

A systematic survey of chromatin-associated cancer dependencies

Altered chromatin landscapes in cancer are believed to open major therapeutic opportunities because epigenetic aberrations are, in principle, reversible and controlled by a machinery that is amenable to drug modulation. To systematically explore this promising target space, we have constructed a comprehensive shRNA library (>5000 miR-E shRNAs) targeting 650

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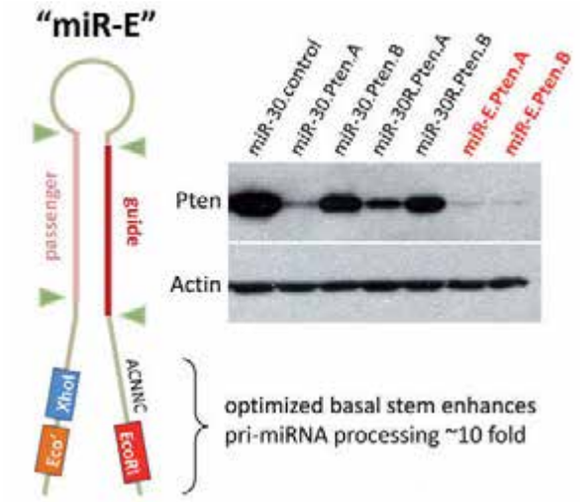

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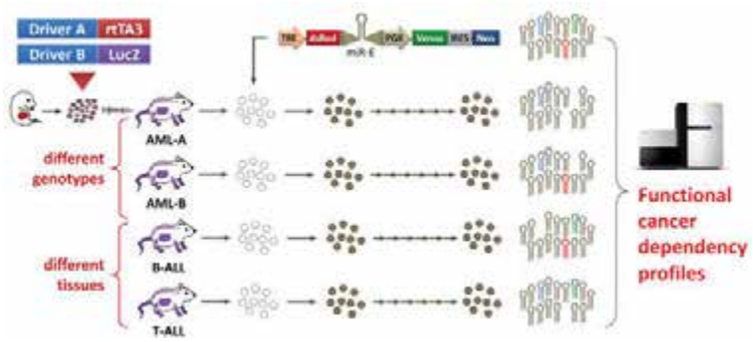

Figure 1: ***The optimized miR-E shRNA backbone for effective single-copy RNAi.*** *The miR-E backbone harbors a modified basal context featuring repositioned shRNA cloning sites and an ACNNC motif, which strongly enhances pri-miRNA processing. The resulting improvement in knockdown is exemplified by immunoblotting, which shows Pten protein levels in NIH3T3 fibroblasts expressing Pten shRNAs (A and B) in different shRNAmir backbones (miR-30, miR-30R, miR-E) from a single viral integration.*

Figure 2: ***Comparative multiplexed shRNA screening in defined leukemia contexts.*** *Genetically defined leukemia models can be rapidly generated by co-introducing driver mutations into fetal-liver-derived hematopoietic progenitor cells. Subsequently, leukemia cells are single-copy transduced with custom-optimized shRNA libraries, which are screened in a multiplexed format. shRNAs targeting genes required for leukemia cell survival will be depleted from the population, which can be quantified using next-generation sequencing of genomic shRNA guide strands. By comparing profiles in different cancer contexts and normal cells, we intend to establish cancer- and context-specific dependency profiles.*

chromatin-associated genes, which we are screening comparatively in several leukemia models (Figure 2). Using this approach we intend to establish functional-genetic dependency profiles that, similar to expression profiles developed a decade ago, will provide a new layer of cancer classification with direct translational implications.

Mechanisms of sensitivity and resistance to BRD4 inhibition

In a pioneering screen using an incomplete chromatin shRNA library (242 genes, 1072 shRNAs), we previously identified the epigenetic reader BRD4 as a promising therapeutic target in AML. While several BET bromodomain inhibitors have now entered clinical phase-I trials, the mechanistic basis underlying sensitivity and resistance to BRD4 suppression remains unclear. To explore genetic and epigenetic determinants of BRD4 dependency, we profiled sensitive and resistant cancers of different tissue origins in great detail and performed multiplexed RNAi screens to systematically probe chromatin regulators for a potential role in modulating the response to BRD4 suppression. From a translational perspective, we hope these studies will help to identify reliable biomarkers which are urgently needed to further develop BRD4 inhibitors in the clinic.

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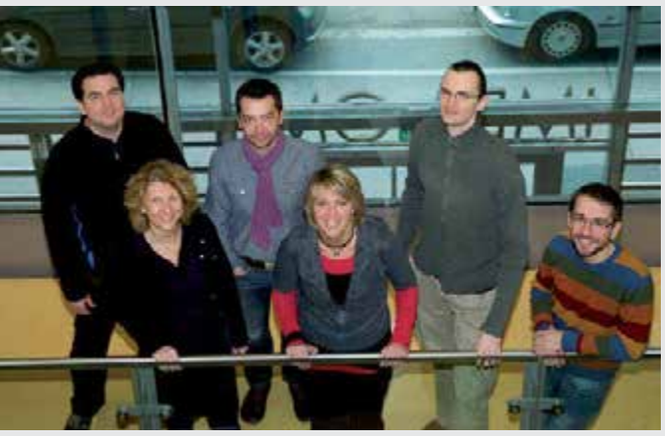

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The services offered by the BioOptics Facility to researchers at IMP, IMBA and GMI encompass analytical flow cytometry and cell sorting, as well as a large variety of microscopy techniques, image processing and analysis.

Flow Cytometry

We provide instrumentation, education and expertise for all flow cytometry requiring experiments, and offer high-speed cell sorting, as well as advanced and general flow cytometry analysis. Users are given guidance and support with the planning of experiments and implementation of new fluorophore combinations. They are trained in the use of hardware and software for all of the three available state-of-the-art flow cytometers, as well as in data processing and analysis. Two cell sorters are operated by the staff of the facility. Four cell populations can be sorted simultaneously in bulk, or single cell sorting can be performed.

Microscopy

The BioOptics Facility currently manages more than twenty microcopy systems, including wide-field microscopy, confocal laser scanning microscopy (CLSM), two-photon (2P) microscopy, total internal reflection (TIRF) microscopy techniques, and automated slide scanner for samples with or without fluorescence. Most of the systems are motorized - thus providing automation for higher throughput - and are suitable for fixed samples as well as live cell experiments. The facility provides assisted use and training on instrumentation and consultation concerning all microscopy-related subjects, including project planning, staining, microscope selection, etc. Additionally intense basic as well as advanced practical microscopy courses are organized including hands-on sessions as well as lectures by internal and external faculty.

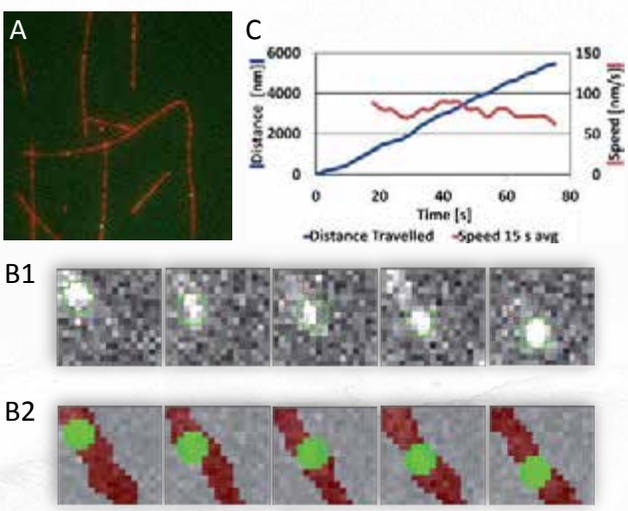


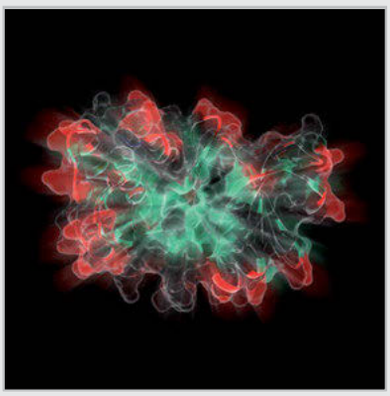
Image Processing and Analysis

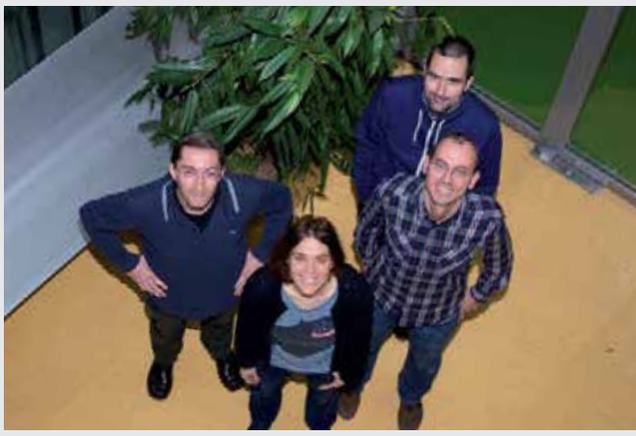
Five state-of-the-art computer workstations are available at the BioOptics Facility, operating most of the common commercial image processing and visualization software. A server solution with a Web-based interface has been set up to enable deconvolution of microscopy images. The server permits efficient, multi-user, parallel, batch deconvolution that can easily be started from the individual scientist's computer. Users are trained in the use of specific software, depending on their demands or are trained in an annual course on image processing and analysis with lectures and hands-on sessions by the BioOptics staff. Several image analysis algorithms are available, such as object tracking and tracing, determination of measurement parameters like intensity, distance, area, volume and co-localization. Customized classification and measuring algorithms are developed at the facility for advanced image analysis and automated object recognition.

For more information please visit http://cores.imp.ac.at/biooptics/

Figure: *Motor proteins moving along microtubules were imaged using a TIRF microscope (A), automatically identified, classified and tracked through direct overlap (B1; B2). Information on distance travelled and speed of the tracked objects was acquired (C).*

Legend for Fig. B1 and B2: *Motor protein* *Microtubules*





BIOINFORMATICS

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The Bioinformatics unit assists research groups in molecular biology-related fields by providing sequence analysis services, scientific data mining, hardware and software infrastructure, and training in bioinformatics.

Benjamin Almeida [1] / Software Engineer
Wolfgang Lugmayr [2] / Software Engineer
Hannes Schabauer [3] / Software Engineer
Thomas Burkard / Computational Biologist
Maria Novatchkova / Computational Biologist
Alexander Schleiffer / Computational Biologist

[1] *from October*
[2] *until September*
[3] *from December*

Sequence analysis

One of the principal areas of expertise at IMP-IMBA Bioinformatics is sequence analysis. Typical tasks include functional and structural characterization of proteins and genomic regions using methods such as pattern matching, complexity analysis, and homology searches. As conclusions in bioinformatics are achieved by synthesizing the results of multiple algorithms, we maintain and develop a set of specialized software tools to support this type of meta-analysis. Web access is provided for widely used scientific applications related to protein motif analysis (in-house development), similarity searching (BLAST, PSI-BLAST, FASTA), whole-genome viewing (GBrowse, UCSC browser), and various sequence manipulation and exploration tasks (EMBOSS).

Large-scale data analysis

Additional demands arise from the investigation of large functional genomics or high-throughput biological datasets. Assistance is provided in experimental design and subsequent analysis of next generation sequencing, microarray, and mass-spectrometry-based proteomics experiments. We also engage in custom software and database development, and design computational and mathematical solutions that can cope with higher loads and memory requirements. To perform complex sequence analysis tasks we maintain the IMP ANNOTATOR, which is a user-friendly Web application and a high-throughput protein annotation system. Local instances of integrated model organism databases (Wormbase) and genome annotation portals permit visualization and analysis of in-house data with dedicated resources and additional privacy. User-driven data exploration is supported by the Ingenuity Pathway Analysis System.
For heterogeneous computational tasks, we maintain a high-performance computing cluster in which dedicated software is adapted to run in a batch and parallel computing environment. This includes tools for statistical computing (e.g. R, Bioconductor), motif discovery and analysis (e.g. AlignAce, MDscan, MEME, Weeder), structural biology (e.g. VMD, pyMOL, HKL2000), image processing (e.g. Xmipp), a wide range of sequence analysis, assembly, mapping and classification tasks (e.g. RNAhybrid, phylip, HMMer), and others.

Training

We provide hands-on training courses on the ANNOTATOR, at which attendees learn the basic principles and limitations of sequence analysis and data integration. To enable researchers to use our server environment in an optimal way, we also provide training in BASH and Unix command line tools specific to the IMP/IMBA infrastructure.

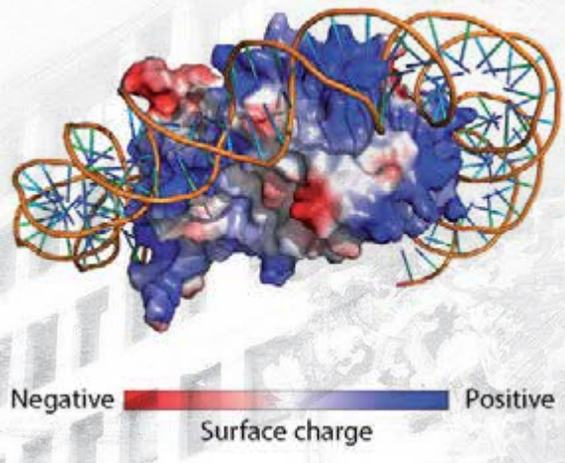

Figure: *Hypothetical model of the non-canonical histone fold proteins CENP-T/W/S/X enclosed by a 73bp DNA fragment. The model is based on crystal structures of the CENP-T/W/S/X heterotetramer (PDB: 3VH5) superimposed on one half of the nucleosome core particle (only the DNA fragment is shown, PDB: 1KX5). The CENP-T/W/S/X heterotetramer is plotted as electrostatic surface potentials, illustrating the high density of positively charged surface residues (blue) that form the putative DNA interface.*

PROTEIN CHEMISTRY

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Karl Mechtler / Head of Facility

Zuzana Demianova / Postdoc
Christoph Jüschke [1] / Postdoc
Thomas Köcher / Postdoc
Nikolai Mischerikow / Postdoc
Evelyn Rampler / Postdoc
Johannes Stadlmann / Postdoc
Werner Straube / Postdoc
Gerhard Dürnberger / Bioinformatician
Sergey Maltsev / Bioinformatician
Thomas Stranzl / Bioinformatician
Thomas Taus / Bioinformatician
Debora Broch Trentini / Phd Student
Johannes Fuchs / Technical Assistant
Otto Hudecz / Technical Assistant
Richard Imre / Technical Assistant
Gabriela Krssakova / Technical Assistant
Mathias Madalinski / Technical Assistant
Michael Mazanek [2] / Technical Assistant
Susanne Opravil / Technical Assistant
Elisabeth Roitinger [2] / Technical Assistant
Michael Schutzbier / Technical Assistant
Ines Steinmacher / Technical Assistant
Etienne Beltzung / Trainee
Dominik Mayer / Trainee
Florian Stanek / Trainee

[1] *until 31.12.2013*
[2] *Part Time*

At the Mechtler laboratory we analyze biological samples with mass spectrometric (MS) methods. We also develop novel MS-based methods to answer fundamental biological questions.

Glycoproteomics

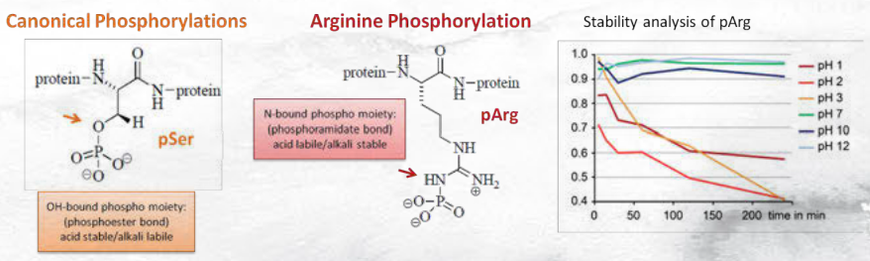
Glycosylation is an abundant - yet functionally not well characterized - post-translational modification of proteins. Due to the structural complexity and the unfavorable fragmentation behavior of glycopeptides, the carbohydrates are removed from the peptide prior to MS analysis. As a consequence, the majority of MS-based glycoproteomic strategies do not yield information on glycan structures. Using our newly developed glycoproteomic workflow comprising a number of novel in-house software tools, we are able to provide comprehensive information about the primary structure of glycopeptides. In collaboration with the Penninger group we use the methodology for the characterization of glycoproteins from murine disease models.

Stoichiometry of the kinetochore protein complex

Targeted proteomics approaches are widely used to quantify sets of predefined proteins in biological samples. In addition to selected reaction monitoring (SRM), a recently introduced method can be used to quantify a potentially larger number of proteins. SWATH-MS tries to combine the advantages of SRM and untargeted MS, potentially monitoring fragment ions of all precursor ions simultaneously. We recently established the novel method in our laboratory. In collaboration with the Westermann group, we use the approach to study dynamic changes in the stoichiometry of the kinetochore complex during the cell cycle.

Systematic detection of N-phosphorylation in bacteria and higher organisms

Across all life forms, the reversible phosphorylation of proteins is a universal regulator of biological systems. Protein phosphorylation analysis has therefore become one of the main targets for the development of MS methods. Today we have a wealth of information about serine, threonine and tyrosine phosphorylation sites. In contrast, very little is known about N-phosphorylation, modifying the side chain of histidine, arginine and lysine. The phosphoramidate bond is unstable at low pH – a condition commonly used in standard proteomic methods. The main focus of our work is to improve the MS-based toolbox for the study of arginine phosphorylation sites. Recently the Clausen group discovered the first known protein arginine kinase, McsB, acting in the stress response of Gram-positive bacteria. Using this kinase for the generation of arginine-phosphorylated samples, we studied the stability of phosphoarginine (Figure 1) and adapted MS protocols accordingly. In collaboration with the Clausen laboratory, we apply the optimized methods to study the role of arginine phosphorylation in the stress response system of Bacillus subtilis. Furthermore, we work on developing specific enrichment procedures for phosphoarginine-containing peptides, which will permit the analysis of more complex biological systems.



CORE FACILITIES

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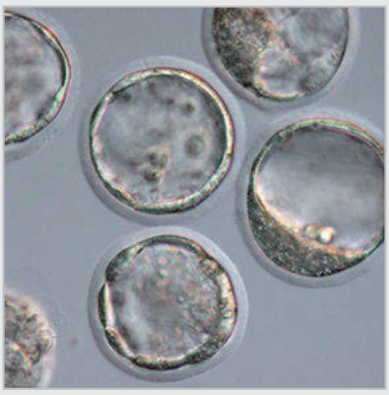

COMPARATIVE MEDICINE

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TRANSGENIC SERVICE

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Comparative Medicine

Scientific work at the IMP and IMBA depends to a high degree on the use of model organisms. IMP and IMBA acknowledge and accept responsibility for the care and use of those animals according to the highest ethical standards. The institute ensures that all employees dealing with the animals understand their individual and collective responsibilities for compliance with Austrian laboratory animal law as well as all relevant regulations and rules concerning laboratory animal husbandries. In accordance with this institutional policy the animal house group - trained and highly qualified animal attandants - provides husbandry of animals and services for the various research groups.

Husbandry:

The largest area of the animal house is the mouse section, which comprises breeding colonies, stock and experimental animals including many transgenic and knock-out mouse lines. To provide a constant supply of mice for the various projects, commonly used standard strains are routinely bred in-house.

Comparative Medicine Services:

Veterinary services, such as monitoring of the facility's health-status (sentinel-program etc.), experimental procedures in animals such as collection of blood, implantation of tumor cells and administration of substances. All procedures are performed to a high standard under appropriate anaesthetic regimes and in conjunction with the necessary project licenses.
Animal procurement, such as ordering of mice from external breeding companies, organizing and handling of incoming and outgoing mouse-shipments per year.
Administration of regulatory affairs in accordance with the Austrian laboratory animal law, which includes record-keeping and updating of laboratory animal statistics, specific documentation of laboratory animal experiments.

Transgenic Service

The Transgenic Service Department was set up in the beginning of 1998 to cope with the increasing demand for mouse studies and generation of transgenic mice. The Transgenic Service Department is shared by the IMP and IMBA.

The main tasks of this service unit are the injection of ES cells into blastocysts [also tetraploid] and 8-cell, and DNA into the pronucleus of fertilized mouse eggs. The service also provides for the transfer of 'clean' embryos into our Animal House, freezing of embryos for the preservation of specified mouse strains, and teaching basic embryological techniques to the staff of IMP and IMBA.

Many different ES cell clones and DNA/BAC constructs are being injected every year. The activities of the department are supervised by an Animal User Committee, which meets on a regular basis to set priorities and coordinate tasks. Currently it is chaired by Meinrad Busslinger.

Figure 1: *Injection of embryonic stem cells into mouse blastocyst.*

Figure 2: *Mouse blastocysts.*

MOLECULAR BIOLOGY SERVICE

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Harald Scheuch / Head of facility

Zuzana Dzupinkova / Sanger Sequencing
Kristina Marinova Uzunova / Protein Expression Specialist
Robert Heinen / Molecular Biology Specialist
Ivan Botto / Technical Assistant
Markus Hohl / Technical Assistant
Caroline Schuster / Technical Assistant
Sabina Maria Kula / Technical Assistant
Elisa Hahn / Trainee
Anna Haydn /Freelancer

The facility offers a variety of standard services and high-throughput methods for scientific experimentation for IMP, IMBA and GMI scientists. We also provide expertise in training and implementing molecular biology techniques.

Production of monoclonal antibodies, preparation of competent cells of various E-coli strains, Sanger DNA sequencing, a routine mycoplasma testing service for tissue culture cells, and robotics for high throughput liquid handling, still are the most prominent tasks performed at our department.

In 2013, we have established two new services, a "Speed Congenics" service and the expression of proteins.

"SPEED CONGENICS" SERVICE

Molecular Biology Service has started to offer a sequence length polymorphisms (SSLP)-based analysis of the genetic background of mice. The screening is achieved by using a set of approximately 90 markers evenly dispersed in a distance of 20 cM (≈ 20 - 40 Mbases) over the 19 autosomal chromosomes.
The information generated by this method is used for selecting only mice with the highest percentage of the genome of the receiver strain for further breeding.
The elimination of the donor genome can be achieved efficiently. Thus, a congenic strain can be developed within 4-5 backcross generations instead of 10 generations by using a traditional breeding strategy that is based on statistical assumptions. The time savings can also be translated to significant cost-savings for a research program, including mouse breeding and maintenance costs and accelerated phenotype analysis and data gathering.

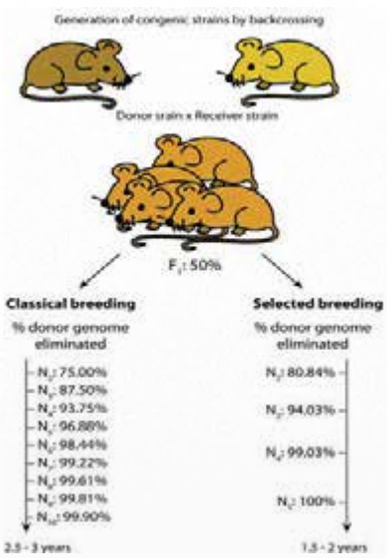


Figure: *A comparison between classical breeding for backcrossing and selected breeding (Speed Congenics).*

PROTEIN EXPRESSION

The use of recombinant proteins in daily scientific research has increased greatly in the last years. We have established *E.coli* expression systems for production of proteins like enzymes used for molecular biology experiments or growth factors for mammalian tissue culture to be used by our scientists. Since many of the proteins expressed in E.coli are found in inclusion bodies and thus require an additional re-folding step which lowers the protein yield and activity we are testing alternative expression sytems. In order to establish a more efficient method for producing growth factors that can also be used for other proteins we have started to explore *Pichia pastoris* as a eukaryotic expression system.

MAX PERUTZ LIBRARY

library@imp.ac.at

Karlo Pavlovic / Librarian

The Max Perutz Library is a specialized reference library located at the Campus Vienna Biocenter. Its mission is to develop and maintain collections and services that support research at the various institutes.

The main task of the library is to provide comprehensive scientific literature pertaining to the areas of research pursued at the institutes. The Max Perutz Library holds approximately 3000 titles in print. These are partly available in the library's premises itself, being labeled and shelved according to a specific classification system. A part of the titles are shelved in the group laboratories and offices. Increasingly, online books are being licensed in order to provide access for many readers simultaneously and from every workstation. Those 300 individually selected online books along with the print books can be searched for systematically in the online catalog, where each title is described in respect of its availability and accessibility. The most heavily used kind of literature resource are the licensed online journals.

Several bibliographic and full-text databases can be searched for relevant literature on a given topic. This also applies to literature on methods and protocols, including Springer Protocols, Current Protocols or the Journal of Visualized Experiments. A document delivery option is offered for the literature not held by the library, which is delivered within some hours for online content and one week for printed matter.

Study environment

The reading room serves as a quiet and well-equipped place for reading, writing or just relaxing. Six study desks and a cozy lounge as well as two public computers, wireless LAN and a printing facility are provided.

Teaching

The library offers special individually oriented training on literature search tools such as catalogs and bibliographic databases for all library users. This ranges from a comprehensive hands-on course on Pubmed searching, to a specific consultation concerning a single challenge in retrieving literature. Assistance is also provided for a variety of client- and web-based literature management tools, such as Endnote or Mendeley.

Users

The core user group consists of affiliates of the Research Institute of Molecular Pathology (IMP), the Institute of Molecular Biotechnology (IMBA), and the Gregor Mendel Institute of Molecular Plant Biology (GMI). External users from the Max F. Perutz Laboratories (MFPL), the FH Campus Vienna and other readers from the Vienna Biocenter are welcome to visit the library premises.

CAMPUS SCIENCE SUPPORT FACILITIES

The Campus Science Support Facilities GmbH (CSF) was established in 2011 at the Campus Vienna Biocenter to provide top scientific infrastructure operated and constantly further developed by highly qualified experts. The CSF supports IMP, IMBA and other institutions and companies situated on the Campus at the forefront of science. Besides scientific infrastructure, the CSF also offers social infrastructure such as the Campus Child Care Facility. The CSF is located directly on the Vienna Biocenter Campus and is a non-profit organisation funded by a 10-year grant of the Austrian Ministry of Science and Research and the City of Vienna.

For more information visit the CSF website: www.csf.ac.at

Preclinical Phenotyping (PPF)

Phenotypic screening is becoming increasingly important in the complex analysis of genetically manipulated mice. Thus, the primary objective of the Preclinical Phenotyping Facility (PPF) at CSF is to provide centralized support to investigators at the Campus Vienna Biocenter (and off-campus academic/Industrial companies), developing and studying genetically modified animal models relevant to human diseases.

We have designed a broad range of standardized tests for a high-throughput phenotypic screen in whole mouse including Metabolism, Neuro-Physiological and a range of Behavioral tests. In addition to the comprehensive panel of phenotyping tests, our facility provides a range of other services such as consultation, tailoring protocols to the need of the investigators, guidance, training, assistance to a complete performance and analysis of the experiments.

Next Generation Sequencing (NGS)

The NGS Facility provides cutting edge next generation DNA sequencing technology. We offer advice and guidance of sequencing projects as well as a set of selected bioinformatics tools. All common sequencing applications are supported, the development of novel methods and protocols encouraged.

Bioinformatics & Scientific Computing (BioComp)

The Bioinformatics & Scientific Computing (BioComp) facility provides unique expertise and services in the areas of bioinformatics and scientific computing to academic and industrial researchers.

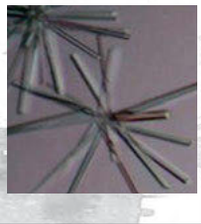
Our services include:

- Developing and applying advanced data analysis tools for the analysis of high-throughput data
- Providing innovative software solutions for biological experiments including image and video processing and hardware-related programming
- Delivering specific data management and processing tools to translate biological results into new insights
- Offering trainings and consultations in the area of bioinformatics, statistics and programming

Structural Biology (SBF)/ Protein Technologies Facility

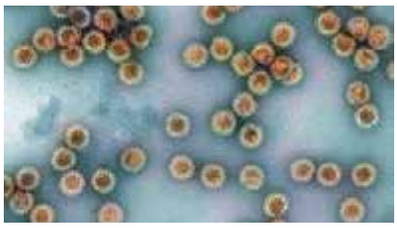
The mission of the Structural Biology Facility (SBF) is to further research in protein biochemistry and structural biology by overcoming two major bottlenecks in these fields – protein production and purification. We provide services encompassing: molecular cloning; protein production in prokaryotic and eukaryotic expression systems; protein purification, including purification of antigens for antibody generation; biophysical characterization of proteins. We are currently working towards expanding our molecular cloning services to include preparation of constructs for genome editing/engineering projects as well as expression of fluorescently tagged proteins in a variety of systems.

In 2014 the name of the SBF will be changed to the Protein Technologies Facility (ProTech) in order to more accurately reflect our service portfolio.



Electron Microscopy Facility (EM)

The Electron Microscopy Facility offers a variety of preparation techniques for biological samples of diverse origin as a service: the techniques range from conventional preparation methods to cryo-preparation techniques for phenotyping the ultrastructure of tissues and cells. Furthermore, the EM Facility provides the processing of purified molecules for conventional and cryo-electron microscopy as well as sample preparation for scanning electron microscopy. Additionally, the facility offers trained users access to a comprehensive set of equipment for sample preparation and visualization of biological specimens at nanometer resolution, including the newly acquired FEI 200 kV transmission EM for tomography of resin embedded specimens and high resolution EM.



Vienna Drosophila RNAi Center (VDRC)

The Vienna Drosophila RNAi Center is a bioresource center maintaining and further developing one of the largest collections of transgenic RNAi lines, together with Gal4 driver lines, for conditional in vivo gene function studies. Currently, over 40,000 Drosophila lines are available to researchers world-wide. To date VDRC has shipped >800,000 lines to >2,000 registered users. We will further develop and expand our resources according to emerging new technologies and community needs.

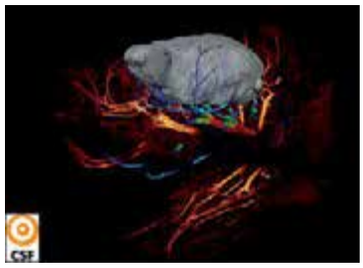
VDRC
VIENNA DROSOPHILA RNAi CENTER

Plant Growth & Phenotyping (PG&P)

The PGP Facility operates 22 high quality, state-of-the-art and highly specialized plant growth chambers. Several chambers are capable of providing non-standard environmental conditions like for instance low temperature (frost), high temperature, different light intensities, light spectra and gas conditions (e.g. CO_2) allowing precise environmental simulation across different climate zones. In addition, the PGP offers an automated high throughput plant screening service. Parallel to basic plant research, the PGP facility will focus on the production of plant-derived biopharmaceuticals (molecular pharming) in the future. Within spare capacities all services will also be available for external customers.

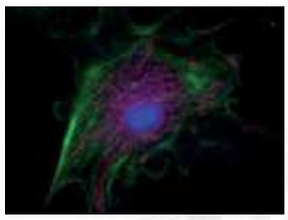
Preclinical Imaging Facility

The Preclinical Imaging Facility offers ultrahigh field magnetic resonance imaging at 15.2 T for advanced anatomical and functional characterization of transgenic (mouse) models, both for whole animal screening and for focusing on specfic organs. Special expertise and support is provided in qualitative and quantitative image analysis.



Advanced Microscopy (AM)

The Advanced Microscopy facility provides access to cutting edge light microscopy techniques as well as the development of tailored light microscopy solutions. We also provide training and consulting on all our available microscopes, as well as sample preparation techniques and, in general, optical imaging techniques. Currently available techniques include 3D Structured Illumination Microscopy (SIM), time-domain Fluorescence Lifetime Imaging Microscopy (FLIM), Fluorescence Correlation Spectroscopy (FCS), and Fluorescence Anisotropy Microscopy. Through the continuous construction of "user friendly" microscopes that have already been determined to be cutting-edge, robust, and relevant to active research areas at the VBC, we give users access to the "technologies of tomorrow" – today (i.e. often before commercial solutions exist). Instrument development is typically in close collaboration with several research groups and financially partly also supported by them via the "Instrument Credit Point (ICP) Policy" (full details on CSF website). In this way the developed instruments and user interfaces are such that they are best suited to the desired applications.



Advanced Microscopy Facility

Publications Research Groups

BUSSLINGER GROUP

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HAUBENSAK GROUP

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Seminar Speakers

JANUARY

15.01.13 Scott Emr
Cornell University
Protein quality control at the plasma membrane – Essential role for an arrestin - ubiquitin-ligase adaptor network

16.01.13 Thomas U. Mayer
University of Konstanz
XErp1/Emi2, a novel mitotic inhibitor of the APC/C

23.01.13 Thomas Walzthöni
Institute of Molecular Systems Biology, ETH Zurich
Structural characterization of macromolecular protein assemblies by protein cross-linking and mass spectrometry

24.01.13 Oliver Rando
University of Massachusetts Medical School
Epigenetic inheritance from yeast to mouse

30.01.13 Dan Bumbarger
MPI for Developmental Biology
Comparing Nematode Connectomes

31.01.13 Yves Barral
ETH Zurich
Nuclear dynamics and ageing: what can yeast tell us about it?

FEBRUARY

06.02.13 Alan Colman
Institute of Medical Biology, Singapore
iPSC modelling of human disease: the good and bad side of the female X chromosome

06.02.13 Itzhak Fried
UCLA Department of Neurosurgery
Single neuron recordings in the human brain

14.02.13 Anne Bertolotti
MRC Laboratory of Molecular Biology
Misfolded proteins: Prion-like propagation and potential cure

18.02.13 Florian Breitwieser
CeMM
isobar: Quantitative Analysis of Protein and PTM iTRAQ/TMT Data

21.02.13 Laurence Pelletier
Samuel Lunenfeld Research Institute
Order in the chaos: a structured-illumination glimpse of centrosome biogenesis

28.02.13 Dirk Schuebeler
FMI Basel
Genetics and epigenetics: How DNA sequence guides the epigenome

MARCH

06.03.13 Ning Zheng
Howard Hughes Medical Institute, University of Washington
Targeting Ubiquitin Ligases - Hormones and Metabolites

07.03.13 Mei Zhen
Samuel Lunenfeld Research Institute
The Genetic and Circuit Regulation of C. elegans Rhythmic Locomotion

14.03.13 Steven Reppert
University of Massachusetts Medical School
Monarch butterfly migration: from behavior to genes

19.03.13 Roger Pocock
BRIC, University of Copenhagen
Control of neuronal fate and migration choices in C. elegans

28.03.13 Elmar Schiebel
ZMBH Heidelberg
The role of epidermal growth factor in regulating the timing of spindle assembly

APRIL

03.04.13 Larry Abbott
Columbia University College of Physicians and Surgeons
Order and Disorder: A Variety of Circuit Motifs in Olfaction

04.04.13 James Berger
University of California, Berkeley
Molecular mechanisms for initiating replication: running rings (and spirals) around DNA

11.04.13 James Ferrell
Stanford University School of Medicine
Bistability and trigger waves in mitosis

16.04.13 William R. Schafer
MRC Laboratory of Molecular Biology
Sensory molecules and mechanisms in C. elegans

18.04.13 Daniel Zilberman
University of California, Berkeley
Evolution and function of DNA methylation in the context of chromatin

25.04.13 Axel Borst
Max Planck Institute of Neurobiology
Neural Circuits for Fly Visual Course Control

MAY

02.05.13 Rene Ketting
Hubrecht Institute
Transgenerational effects of small RNAs

13.05.13 Gottfried Schatz
Biocenter, University of Basel
What it takes to succeed in science

15.05.13 Mathew Diamond
SISSA, Italy
Perception and working memory of noise-like whisker (and fingertip) vibrations

16.05.13 Joachim Wittbrodt
University of Heidelberg
Fate restriction and multipotency in retinal stem cells

23.05.13 Kristian Helin
BRIC/University of Copenhagen
Role of epigenetic regulators in stem cells and cancer

31.05.13 Matthias Groszer
INSERM & Université Paris Curie
Addicted to speak ? Foxp2 in reward circuits

JUNE

03.06.13 Liliane Schoofs
KU Leuven
Evolutionarily conserved neuropeptide signalling in Caenorhabditis elegans

05.06.13 Claude Desplan
New York University
Generating neuronal diversity in the Drosophila visual system

06.06.13 Walter Kolch
Systems Biology Ireland & Conway Institute
Dynamic protein-protein interactions coordinate signalling between the Raf and MST2/Hippo pathways

17.06.13 Matthieu Louis
Centre for Genomic Regulation (CRG), Barcelona
Sensorimotor computations during larval chemotaxis

19.06.13 Tiago Branco
MRC Laboratory of Molecular Biology
Dendritic computations in single neurons

25.06.13 Alexander Pouget
University of Geneva
Neural computation as probabilistic inference

JULY

11.07.13 Michael Orger
Centro Champalimaud
Whole brain imaging of neural circuit activity in behaving zebrafish

19.07.13 Bart Deplancke
EPFL
Systems-based, quantitative analysis of gene regulatory networks mediating fat cell differentiation

24.07.13 Evelyn Rampler
University of Natural Resources and Life Sciences
Quantitative mass spectrometric assays for yeast characterization

24.07.13 Wieland B. Huttner
Max Planck Institute of Molecular Cell Biology and Genetics
Neural stem and progenitor cells and the evolution of the cerebral cortex

25.07.13 David Glover
University of Cambridge
Plk4, not only the master regulator of centriole formation, but also. . .

AUGUST

13.08.13 Christine Buske
Mekentosj
Papers; beyond the reference manager

20.08.13 Barry Thompson
London Research Institute
Tissue growth and form: from gradients to planar polarity

21.08.13 Constanze Bonifer
University of Birmingham
Mechanistic insights into chromatin programming at the onset of hematopoiesis

26.08.13 Alexander Gottschalk
Institute of Biochemistry and Buchmann Institute for Molecular Life Sciences
Optogenetic analyses of synaptic transmission and neuronal networks in Caenorhabditis elegans

SEPTEMBER

02.09.13 Ross Dickins
Walter and Eliza Hall Institute
Mechanisms of leukemia suppression by Pax5 and Ikaros

04.09.13 Alcino Silva
Brain Research Institute, UCLA
Molecular, cellular and circuit mechanisms of memory allocation

05.09.13 Mark Hochstrasser
Yale University
The ubiquitin-proteasome system: Tales from the beginning to the end

10.09.13 Supriya Srinivasan
The Scripps Research Institute
Neuroendocrine Circuits of Energy Regulation in C. elegans

12.09.13 Sue Hadjur
Cancer Institute, University College London
Cohesin is a key determinant of interphase chromosome domain structure

12.09.13 Frank Buchholz
MPI of Molecular Cell Biology and Genetics, Dresden
From high-throughput screens to biomedical knowledge

16.09.13 Natalie de Souza
Nature Methods
Behind the scenes at Nature Methods

19.09.13 Jan Löwe
MRC Laboratory of Molecular Biology
The Bacterial Cytoskeleton

20.09.13 Patricija van Oosten-Hawle
Northwestern University
Transcellular chaperone signaling regulates organismal proteostasis in C. elegans

24.09.13 Donal O'Carroll
Embl Monterotondo
The mammalian piRNA pathway, from transposon silencing to germ line maintenance

24.09.13 Irina Kaverina
Vanderbilt University Medical Center
Golgi-derived microtubules and their functions

25.09.13 Clare Waterman
NIH Bethesda
Myosin II mediated endothelial cell branching morphogenesis in 3D by minimizing membrane curvature

26.09.13 Marian Walhout
University of Massachusetts Medical School
Integrative systems networks in the nematode C. elegans

OCTOBER

09.10.13 Jason Lieb
Princeton University
Gene regulation in space and time

10.10.13 Frank Pugh
Pennsylvania State University
Integration of Chromatin Organization and Transcription Initiation on a Genomic Scale

15.10.13 Barbara Knowles
*A*Star Institute of Medical Biology*
Towards human Embryonic Stem Cell Quality Control

15.10.13 Davor Solter
*A*Star Institute of Medical Biology*
Developmental Epigenetics

17.10.13 Cedric Blanpain
WELBIO, Interdisciplinary Research Institute (IRIBHM)
Stem cells during development, homeostasis and cancer

21.10.13 Konrad Hochedlinger
Harvard Dept. of Stem Cell and Regenerative Biology, HHMI
Mechanisms of Cellular Reprogramming

23.10.13 Stein Aerts
KU Leuven
Identification of master regulators and cis-regulatory interactions in human and Drosophila gene networks

24.10.13 Robert Schreiber
Washington University School of Medicine
Cancer Immunoediting: Basic Mechanisms and Therapeutic Implications

31.10.13 Kay Hofmann
University of Cologne
A common evolutionary basis for cell death signaling in animals, plants and fungi

NOVEMBER

06.11.13 John Gurdon
Cancer Research UK Gurdon Institute
The activity of nuclei and macromolecules introduced into embryonic cells

14.11.13 Caetano Reis e Sousa
Cancer Research UK London
A DaNGeRous talk about dendritic cells

28.11.13 Lars Jansen
Gulbenkian Institute of Science
Self-propagating chromatin: the curious case of the mammalian centromere

29.11.13 Roberto Bonasio
University of Pennsylvania
Epigenetics: from noncoding RNAs to ants

DECEMBER

03.12.13 Dick R. Nässel
Department of Zoology, Stockholm University
Regulation of Drosophila insulin producing cells and insulin-mediated effects on neuron growth

04.12.13 David Baltimore
California Institute of Technology
MicroRNA as Guardian of Hematopoietic Stem Cell Integrity

05.12.13 Pascal Genschik
IBMP CNRS Strasbourg
When protein degradation meets RNA silencing: Regulation of siRNA pathways by autophagy

05.12.13 Enrique Martinez-Perez
MRC Clinical Sciences Centre
Cohesin dynamics during meiotic prophase in C. elegans

12.12.13 Rafal Ciosk
FMI Basel
Regulation of pluripotency in animal development

12.12.13 Joanna Wysocka
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Transcriptional mechanisms of developmental plasticity

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The IMP and its Surroundings

The Research Institute of Molecular Pathology (IMP) is a basic biomedical research center in Vienna, the capital of Austria. Its major sponsor is Boehringer Ingelheim, a globally operating pharmaceutical company with its headquarters in Germany. The knowledge created at the IMP is at the disposal of Boehringer Ingelheim for the development of innovative diagnostic and therapeutic concepts.

The Campus Vienna Biocenter

Opened in 1988 close to the city center, the IMP triggered the development of its neighbourhood into a teeming biotechnology hub. Today's "Campus Vienna Biocenter" (VBC) is also home to the Max F. Perutz Laboratories (MFPL; University and Medical University of Vienna), the Institute of Molecular Biotechnology (IMBA; Austrian Academy of Sciences) and the Gregor Mendel Institute of Molecular Plant Biology (GMI; Austrian Academy of Sciences), a University of Applied Sciences, several biotech companies, a non-profit scientific society and the Vienna Open Lab.

Close ties have been knit between the IMP and IMBA: the institutes are not only linked physically but cooperate closely on all levels and share large parts of their infrastructure. More than 1400 people from 40 different nations currently work at the Campus VBC. Campus members enjoy a scientifically and socially stimulating environment and take advantage of shared facilities such as the Max Perutz Library at the IMP and the Vienna Biocenter International PhD Program. A number of events, including seminars and lectures, are open to all.

Vienna – a City of Many Facets

For those whose interests stretch beyond science, Vienna also has a lot to offer. Home to about 1.7 million people, the city is the political and cultural center of the country and its gateway to the east. Once the heart of the largest European empire, Vienna draws on a rich cultural heritage which is reflected in splendid buildings and unique art collections. But Vienna is a city of many facets. Modern architecture, splendid galleries and stylish shops are as much a part of everyday life as the famous concert halls, the big museums and the nostalgic little streets. As any European capital, Vienna also offers a vibrant nightlife, with a rich selection of restaurants, cozy bars, and trendy clubs.

Apart from Vienna's focus on art and culture, it also has a long-standing tradition in science. Founded in 1365, the University of Vienna is the oldest university in the German-speaking world and the largest in Austria. With a student population of more than 120,000, Vienna offers not only the academic but also the cultural and social infrastructure that comes with student life.

And if your brain needs to be refreshed, there is always the call of nature. A day away from the lab may take you skiing to the nearby Alps, windsurfing at Lake Neusiedl or watching rare birds in the Seewinkel. Even within the borders of Vienna, you can enjoy a hike through dense woods, go canoeing in a National Park, climb impressive limestone cliffs or stroll through rolling vineyards. In fact, more than 50% of Vienna's surface area is covered by vegetation.

Your Career at IMP

The IMP offers a dynamic scientific environment for all scientists. Research at the IMP is multidisciplinary, combining biologists, computer scientists and physicists. The combination of top researchers and outstanding support (funding conditions and infrastructure) generates an enthusiastic drive among the scientists. This environment serves as a foundation for exceptional research at the Institute.

The IMP promotes a number of educational programs in close collaboration with other research institutes at the Vienna Biocenter Campus: the Institute of Molecular Biotechnology (IMBA), the Gregor Mendel Institute of Molecular Plant Biology (GMI), and Max F. Perutz Laboratories (MFPL). For undergraduate students, the Vienna Biocenter Summer School provides a unique two month experience of cutting-edge scientific research (www.vbcsummerschool.at). The IMP is also extremely proud of its training for graduate students. Through the Vienna Biocenter PhD Program, young researchers are coached to become independent, critical and creative (more information available at www.vbcphdprogramme.at). Postdoctoral fellows are also offered a structured program which includes not only training in relevant skills but also valuable networking activities.

Irrespective of career stage or background, all researchers will experience intellectual stimulation at the IMP. The weekly VBC seminar series invites renowned international scientists to present their discoveries. Everyone is encouraged to interact with these guests. Additionally, the IMP has initiated a seminar series named after the IMP´s founding director Max Birnstiel, to highlight outstanding scientific researchers from around the world (see pages 68-71). Besides the invited speakers, the IMP hosts „Friday Seminars" at which VBC doctoral students and post-doctoral researchers present their work to the VBC community. This is flanked by a wide range of journal clubs, while informal seminars provide opportunities to delve deeper into a subject of interest.

The IMP has a well organized infrastructure on par with the best research institutes in the world, despite the Institute's relatively small size. The support facilities and services are such that they eliminate the need to worry about mundane details and allow everyone to focus on science. Researchers will find they have few or no administrative duties, thanks to a talented and helpful administrative staff.

Not everything is science at the IMP. The Institute also hosts a variety of networking and social activities. The IMP is located in Vienna, a fantastic city with a high quality of life and international appeal. English is the official language of the institute and is widely spoken in the city. Additionally, the IMP offers excellent working conditions for its scientists and their families, and the campus has its own kindergarten.

All of the above factors contribute to the international character of the IMP and make it an exciting place for a scientific career. More information about career opportunities at the IMP is available at: www.imp.ac.at

LUISA COCHELLA: NEW IMP GROUP LEADER

Luisa Cochella joined the IMP as a group leader in January 2013. Luisa was doing graduate thesis research at Rachel Green's lab in Johns Hopkins University School of Medicine, New York. Her topic was the molecular basis of high-fidelity tRNA selection on the ribosome. Luisa is originally from Argentina and completed her undergraduate studies in molecular biology at the University of Buenos Aires. After earning her PhD in 2006, she spent six years with Oliver Hobert at Columbia University (NY), focusing on the contribution of small regulatory RNAs in the development of the nervous system and function in *C. elegans*. At the IMP Luisa's research focuses on micro-RNAs. Why they are expressed so precisely is what Luisa is trying to find out. To address this question she uses the nematode C. elegans for genetic screens. Having studied transcriptional regulation in the past, her work will now focus on additional layers of regulation, which she hopes to disclose.

MEETING "UBIQUITIN SIGNALLING AND PHARMACOLOGY"

The joint BI-IMP-IMBA meeting on 'Ubiquitin signalling and pharmacology' was held in Gumpoldskirchen from January 16 - 18. Fumiyo Ikeda (IMBA) co-organized the meeting together with Jan-Michael Peters (IMP), Guido Boehmelt (BI) and Nobert Kraut (BI). Ten external experts were invited to join the meeting. Participants presented the latest progress in the Ubiquitin research field, followed by lively scientific discussions.

MAX BIRNSTIEL LECTURE HIGHLIGHT

Itzhak Fried
UCLA Department of Neurosurgery
Single neuron recordings in the human brain
Host: Barry Dickson

01/13

02/13

03/13

RUSHAD PAVRI: NEW IMP GROUP LEADER

Rushad Pavri joined the IMP as a group leader in the beginning of 2013. Rushad is a molecular immunologist and is interested in the molecular mechanisms of antibody gene diversification.
Born in Mumbai, Rushad earned his Bachelor's and Master's degrees in India before moving to the United States for his doctoral studies. He obtained his PhD in biochemistry from the University of Medicine and Dentistry at New Jersey under the mentorship of Danny Reinberg. For the past six years Rushad has been working with Michel Nussenzweig at Rockefeller University in New York, first as a postdoc and later as a research associate. At the IMP Rushad is investigating co-transcriptional activities and cell cycle regulation. Most importantly, his group will study how these mechanisms work in vivo, using mice as model organisms.

KALEIDOSCOPE

This year's VBC Drama Club spring show "Kaleidoscope" was a joint production of the Amateur Drama Club (ADC) and members of the "MixTape Improv Group". The show presented a spectrum of the many facets of improvisational theatre and was much acclaimed by the audience.

MAX BIRNSTIEL LECTURE HIGHLIGHT

Ning Zheng
Howard Hughes Medical Institute, University of Washington
Targeting Ubiquitin Ligases - Hormones and Metabolites
Host: Doris Hellerschmied

VBC SUMMER CONCERT

In 2006, the MolBio Orchestra was formed by a group of biology students. It has since featured a number of concerts which have become a pleasant tradition on the campus. This year's VBC summer concert was held on June 12. The donations that were collected went to the charity "Kinderträume".

VBC PHD RETREAT

In June 2013, the VBC PhD students organized a joint retreat with the Friedrich Miescher Institute for Biomedical Research (Switzerland). A total of 82 students attended the three-day event, taking advantage of intense scientific exchange and networking. The students presented their projects and engaged in lively discussions. There were also career and scientific talks by four invited speakers: Bernd Pulverer (editor, EMBO Journal); Tiago Branco (group leader, LMB, Cambridge, UK); Jürg Müller (group leader, MPI, Martinsried, Germany), and Gerda Redl (patent attorney, Vienna).

MAX BIRNSTIEL LECTURE HIGHLIGHT

Larry Abbott
Columbia University College of Physicians and Surgeons
Order and Disorder: A Variety of Circuit Motifs in Olfaction
Host: Andrew Straw

MAX BIRNSTIEL LECTURE HIGHLIGHT

Claude Desplan
New York University
Generating neuronal diversity in the Drosophila visual system
Host: Barry Dickson

04/13

05/13

06/13

SCIENCE SLAM

To celebrate the 10th anniversary of its research supplement in May this year, the Austrian daily newspaper Der Standard organized a public discussion on the quality of science in Austria. To introduce the topic and break the ice among the visitors, the evening was opened by a Science Slam - a series of brief five-minute talks by selected researchers. Carrie Cowan mastered this challenge with grace and humor. Accompanied by a fluffy and stuffed *C. elegans* in bright pink, she easily won the audience's sympathy for her passion for science and quest for the secrets of cell polarity.

MAX BIRNSTIEL LECTURE HIGHLIGHT

Danny Reinberg
NYU Langone Medical Center
Molecular Epigenetics
Host: Meinrad Busslinger

FIRST IMP TREASURE HUNT

About 100 people from various departments and groups participated in the first IMP treasure hunt on June 6, 2013. After having received their first clue, eight groups set off in various directions to find a number of stations located around Vienna's first district. A variety of challenges ranging from singing a song in front of the Vienna state opera house (with a tourist or a policeman!), blind-tasting of beer near the Votiv Church, to the collection of numerous items and the completion of a questionnaire along the way guaranteed that everyone had lots of work to do, but that made it no less fun for the ambitious hunters.

DRAGON BOAT CUP

The team IMPerfectos came second in the Austrian dragon boat championship held at Vienna's Old Danube on June 14. Weekly training sessions at the Police Sports Club of Vienna had prepared the team for the contest. This year, attendance at the training was exceptionally high. In the qualifying round, the IMPerfectos reached the finishing line half a boat's length in front of the other teams. In the finals they had to compete against two teams from dragon boat sport clubs and another company team. However, they were unable to keep pace with their long-term competitor Dreamboat Elin. Nevertheless, they were very proud of their achievements, celebrated their second position happily, and accepted it with good grace.

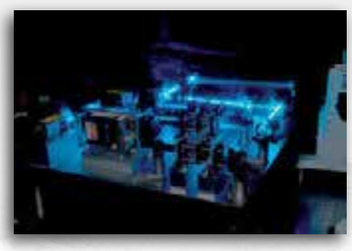
JAN-MICHAEL PETERS: NEW SCIENTIFIC DIRECTOR

Jan-Michael Peters joined the IMP in 1996 to set up his first independent research group. In 2002, he was promoted to senior scientist and in 2011 became the institute's scientific deputy director. When Barry Dickson decided to take up a position at HHMI's Janelia Farm Research Campus, Jan-Michael Peters took over the function of acting director. In June 2013, the selection committee announced its choice and nominated Jan-Michael Peters as the new scientific director of the IMP, effective from July 1st. Meinrad Busslinger succeeded him in the position of deputy director.

07/13

QUEBS WORKSHOP

The 5th International Workshop on Quantum Effects in Biological Systems was held at the IMBA lecture hall this summer. From June 29 to July 3, the leading experts of this young discipline met here to foster exchange and cooperation. As in the previous four workshops, the meeting covered the most recent advances in quantum phenomena in biological systems and chemical structures central to life. About 120 participants from all continents attended the meeting and listened to the talks of 22 invited speakers. Two poster-sessions complemented the lectures.



LATE SUMMER PRACTICAL PROTEOMICS SEMINAR

In September, more than 160 scientists came to the IMP to attend a meeting centered on technology. The seventh Late Summer Practical Proteomics Seminar focused on quantitative techniques in mass spectrometry and the analysis of post-translational modifications. The practical workshops were intended to give an introduction to bioanalytical methodologies. They covered a wide spectrum of topics ranging from phosphopeptide analysis with mass spectrometry to troubleshooting in nano-liquid chromatography. The short courses were complemented by talks from international speakers, who presented their recent work and current developments in mass spectrometry-based proteomics.

08/13

VIENNA BIOCENTER SUMMER SCHOOL

June 28 – August 30

The fourth Edition of the Vienna Biocenter Summer School was again a huge success. 22 students of 16 different nationalities joined at the campus for a two-month internship, which proved to be an unforgettable experience for one and all.

Each summer fellow worked on an independent research project under the close supervision of a graduate student. Twice a week the students would gather around to listen to a 45-minute talk, and had the opportunity to ask questions and interact with our junior and senior faculty.
Throughout July and August the students worked hard, experienced life as researchers, but also had fun. The Summer School was concluded with a symposium at which the fellows presented their projects to the VBC scientific community.

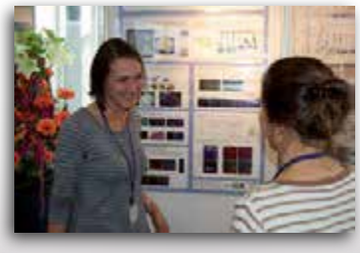
MAX BIRNSTIEL LECTURE HIGHLIGHT

Alcino Silva
Brain Research Institute, UCLA
Molecular, cellular and circuit mechanisms of memory allocation
Host: Wulf Haubensak

09/13

ROMEO & JULIET

On August 28 and 29, the Vienna Biocenter Amateur Dramatic Club performed its (partly) open-air summer spectacular - Shakespeare's "Romeo & Juliet". In this very special production, the action was transferred from Verona to Vienna, with the Montagues and the Capulets re-imagined as two warring tribes of Austrian soccer fans, while local government vainly tries to keep the peace.



IMP RECESS

From October 2-4, IMP scientists met with members of the scientific advisory board (SAB) to present their work and discuss their research. The SAB, consisting of internationally renowned scientists, was once more impressed by the scientific performance and high standards of the research presented on the occasion. The IMP would like to thank all SAB members for their tremendous support.
IMP SAB members: page 63 in this booklet.

10/13

POSTDOC RETREAT

The postdoc retreat in September brought together 50 postdocs from all four institutions on the Campus VBC. In addition, the Research Center for Molecular Medicine – CeMM – and the Institute of Science and Technology (IST) Austria were represented. The retreat took place in Brno, Czech Republic, and was organized by the Vienna Area Postdoc Organization.

MAX BIRNSTIEL LECTURE HIGHLIGHT

Jason Lieb
University of North Carolina
Mechanistic links between chromatin dynamics, gene regulation, and RNA processing in development and disease
Host: Alex Stark

11/13

MAX BIRNSTIEL LECTURE HIGHLIGHT

John Gurdon
Cancer Research UK Gurdon Institute
The activity of nuclei and macromolecules introduced into embryonic cells
Host: Meinrad Busslinger

VBC PHD SYMPOSIUM

The topic of the 11th annual VBC PhD Symposium in November was "TIME - How nature sets the clock". The annual event is entirely organized by the PhD students. This year there were more than 400 registrations from within Austria and abroad. The symposium gathered young scientists and leading experts from various fields to discover the many different timekeepers of biological systems, and fuel new and unconventional ideas for the future. As Nature has clocks for a variety of time scales, a wide range of topics were explored, such as biochemical oscillations, cell cycle timing, circadian rhythms, time perception, and time-driven population dynamics.
The symposium also included an award ceremony for the best PhD theses of the preceding academic year.

CHARITY PUNCH EVENT

On November 28 – one of the first really cold nights of the season - several of the institute's service facilities teamed up to organize the second "Charity Punch Event". From a beautifully decorated hut on the IMP's parking lot, charming angels served out little snacks and steaming hot drinks with varying concentrations of ethanol that kept the customers warm. The donations that were collected in the course of the evening went to the Children's Wish Foundation, an Austrian charitable institution.

12/13

MAX BIRNSTIEL LECTURE HIGHLIGHT

David Baltimore
California Institute of Technology
How about: A MicroRNA as Guardian of Hematopoietic Stem Cell Integrity
Host: Martina Minnich & Meinrad Busslinger

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Immunohistochemical analysis of a B cell follicle from a mouse spleen. The following cell types were visualized: marginal zone B cells (red-IgM^{high}), follicular B cells (green-IgD^{high}) and T cells (blue-CD3^{+}). Courtesy of Tanja Schwickert (Busslinger group)

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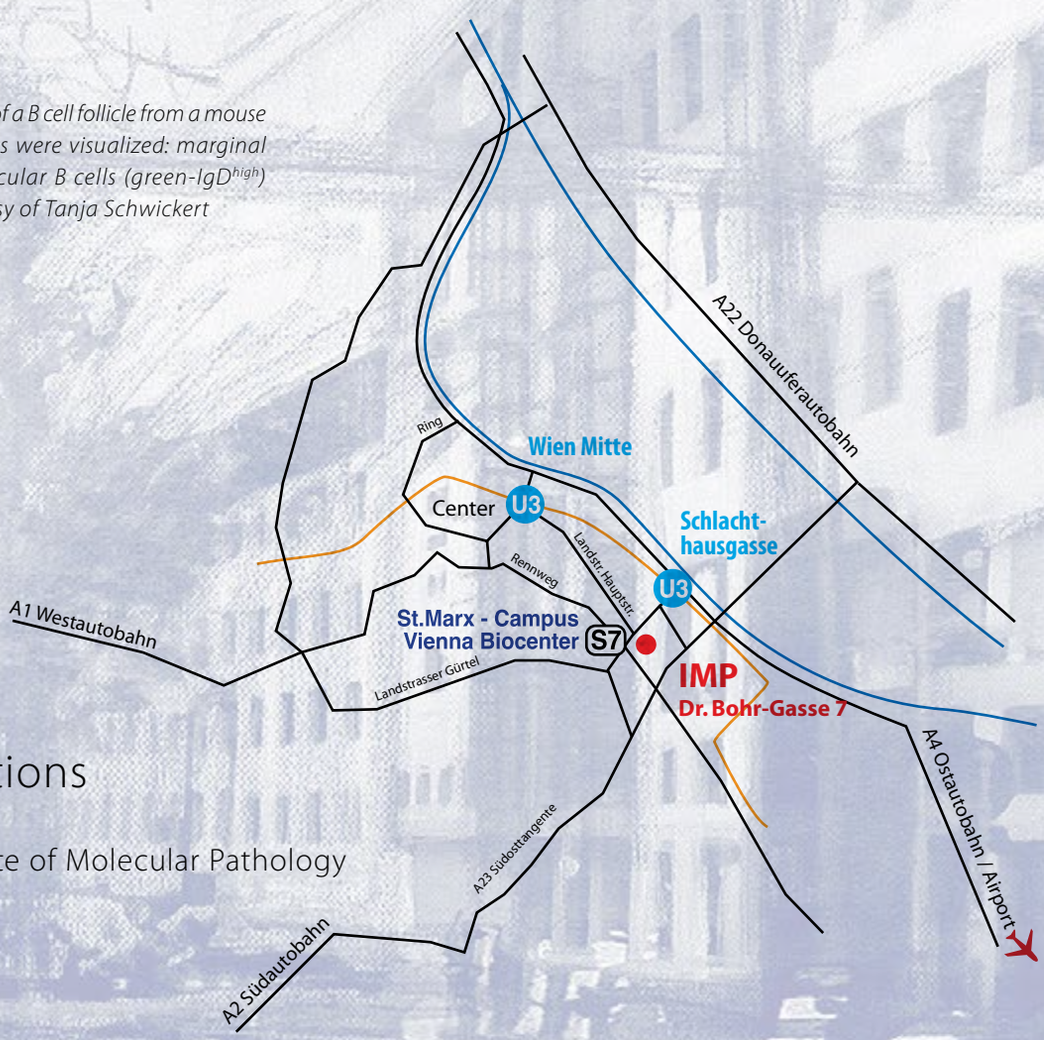

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