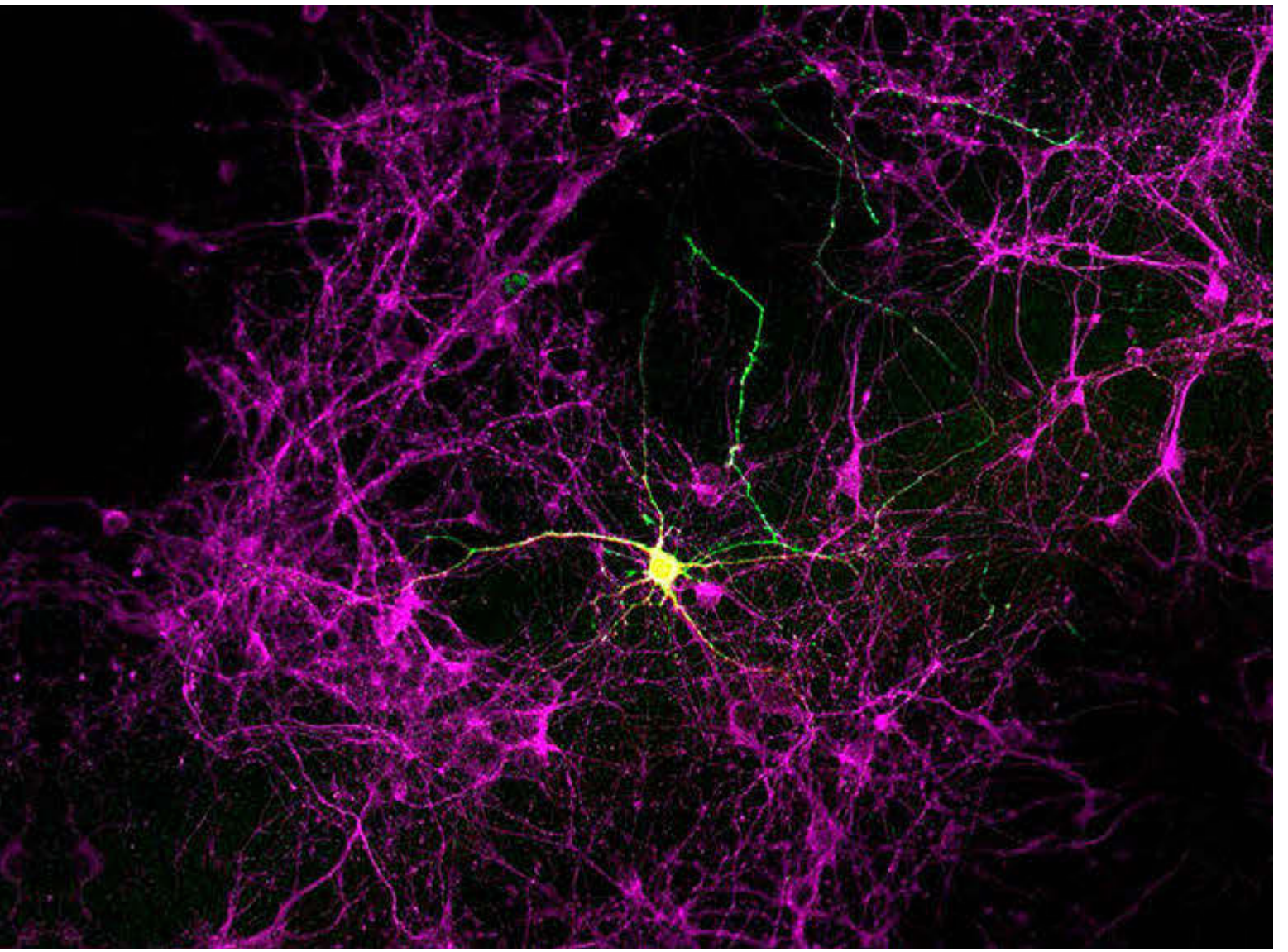
RESEARCH INSTITUTE OF MOLECULAR PATHOLOGY

VIENNA BIOCENTER

2012



Boehringer Ingelheim

IMP
Research Institute of Molecular Pathology

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2012 has been an eventful year for the IMP. The year has been bracketed by new scientific appointments and (planned) departures. And in between there has been much to celebrate - scientific successes, personal milestones, and the exciting perspective of a new home for the institute. Let's take things in more-or-less chronological order.

As the year began, we were in the midst of a search for new group leaders, which was quickly concluded with the recruitment of Luisa Cochella and Rushad Pavri. Luisa, an Argentinian, trained in RNA biochemistry at John Hopkins and developmental genetics at Columbia. She will combine both of these interests in her future work at the IMP, exploring the mechanisms that confer spatial and temporal specificity of miRNA function in a multicellular organism, using *C. elegans* as a model. Rushad, from India, worked on histone biochemistry in New Jersey and mouse immunology at the Rockefeller. At the IMP, he will investigate the transcriptional mechanisms that generate antibody diversity and how they can lead to genome instability. Rushad takes up his position at the end of 2012; Luisa at the start of 2013. We are delighted to welcome them both as new group leaders and wish them all the best as they begin this exciting new chapter in their scientific and personal lives.

With one search over, another began, now for the much-anticipated Coordinator of Scientific Training, a new position we recently created to oversee all scientific training activities at the VBC, including the summer school, PhD programme, and workshops and courses for scientists at all levels. This search was successfully concluded with the appointment of Ines Cristósomo. Ines was previously the Advanced Training Coordinator at the Instituto de Medicina Molecular, Lisbon, Portugal and also Executive Coordinator of the doctoral programme at the Lisbon Academic Medical Centre. We're all looking forward to her arrival in Vienna early in the new year, and wish her likewise success and enjoyment as she takes on the many challenges and opportunities in this important new position.

The summer and autumn gave us much to celebrate. Wulf Haubensak was awarded a Starting Grant from the European Research Council (ERC), bringing the IMP's total to 7 ERC grants. And Dave Keays and Alex Stark were recognized by the European Molecular Biology Organization as Young Investigators. Moreover, both Dave and Alex fared well as they came up for evaluation at the Recess this year. For Alex, this resulted in the extension of his group leader position for the additional 3 years; for Dave, the promotion from IMP Fellow to Group Leader.

A few of us also passed major personal milestones this year, including 50th birthdays for Jan-Michael Peters and myself (B.J.D.), and Meinrad Busslinger's 60th. Both Jan and Meinrad celebrated by organizing scientific symposia that brought together friends, colleagues and former students and postdocs. These meetings highlighted the tremendous contributions both Jan and Meinrad have made in their respective fields and to the careers of those they have trained.

Importantly, the foundation was also laid this year for a major milestone for the IMP itself. In September Boehringer Ingelheim approved our proposal to construct a new laboratory building for the IMP at the VBC and released the funds for the acquisition of the construction site and the technical planning. The building is foreseen to be located adjacent to the Academy building, allowing us to maintain the direct physical connection to the Academy building which is essential for the close scientific interaction and the shared service facilities. The construction of the building is currently scheduled to begin in autumn 2014, with the relocation of the IMP then planned for summer/fall 2016. This decision is obviously a major landmark in the development of the IMP and reflects the strong commitment of Boehringer Ingelheim and the shareholder families to fundamental research in the life sciences and to the special research culture and creativity at the IMP. We are extremely grateful for the continued commitment and generosity of our shareholder, and are convinced that we will merit this confidence by maintaining the highest international standards in scientific research.

Further notable developments on the campus this year were the continued growth of the campus support facilities (CSF) and the child care facility. The CSF started to offer two new services. The Protein Characterization facility provides high quality protein production in eukaryotic expression systems as well as biophysical characterization of proteins. The pre-clinical phenotyping facility is offering expertise and equipment for metabolic, neurophysiological, behavioural, imaging and histological analysis in mice. These new facilities will further strengthen the first class scientific core infrastructure we are able to offer scientists at the IMP. The Campus Child Care Facility, although just 3 years old, had already outgrown its initial space and was expanded significantly in late summer. With these new premises, child care for about 100 children from 0-6 years is now provided in immediate vicinity of the IMP.

Finally, as the year draws to a close, so too does our partnership at the helm of the IMP. With the end of the year, I (B.J.D.) step down as Scientific Director, ahead of a move to the Janelia Farm Research Campus of the Howard Hughes Medical Institute in the U.S.A. in 2013. Boehringer Ingelheim has initiated the search for a successor. In the interim, Jan-Michael Peters, Deputy Scientific Director since 2011, takes on the role of Acting Scientific Director to join H.I. as the new leadership duo.

It will be difficult to leave the IMP and Vienna after what will finally be a total 15 years at the campus, 5 as an group leader at the IMP, 3 as IMBA's first senior scientist, and 7 as Scientific Director of the IMP. It has been a thrill to witness and help shape the astonishing development of the campus over this period. The IMP has continued to rise as a leading international biomedical research center. It's preeminent position within the Austrian and Viennese scientific community has also encouraged significant public investment in science at the site, including the establishment of IMBA, GMI, and the CSF. These private and public sponsors have provided an exceptional framework, but it is above all the people that make this such a special place to work: bold and creative scientists, committed administrative and support staff, and talented and highly motivated students. I am particularly proud of the scientific recruitments we have made over the past 7 years, the new opportunities we have created for young scientists, the strengthening of the scientific and social infrastructure, and securing the new building for the IMP. For me (B.J.D.) it is time to move on to new adventures. And with a new building and a new directorship on the horizon, it is time for the IMP too to begin an exciting new phase in its development. There is a lot to look forward to in the coming years.

Barry J. Dickson and Harald Isemann

THE RELUCTANT HERETIC

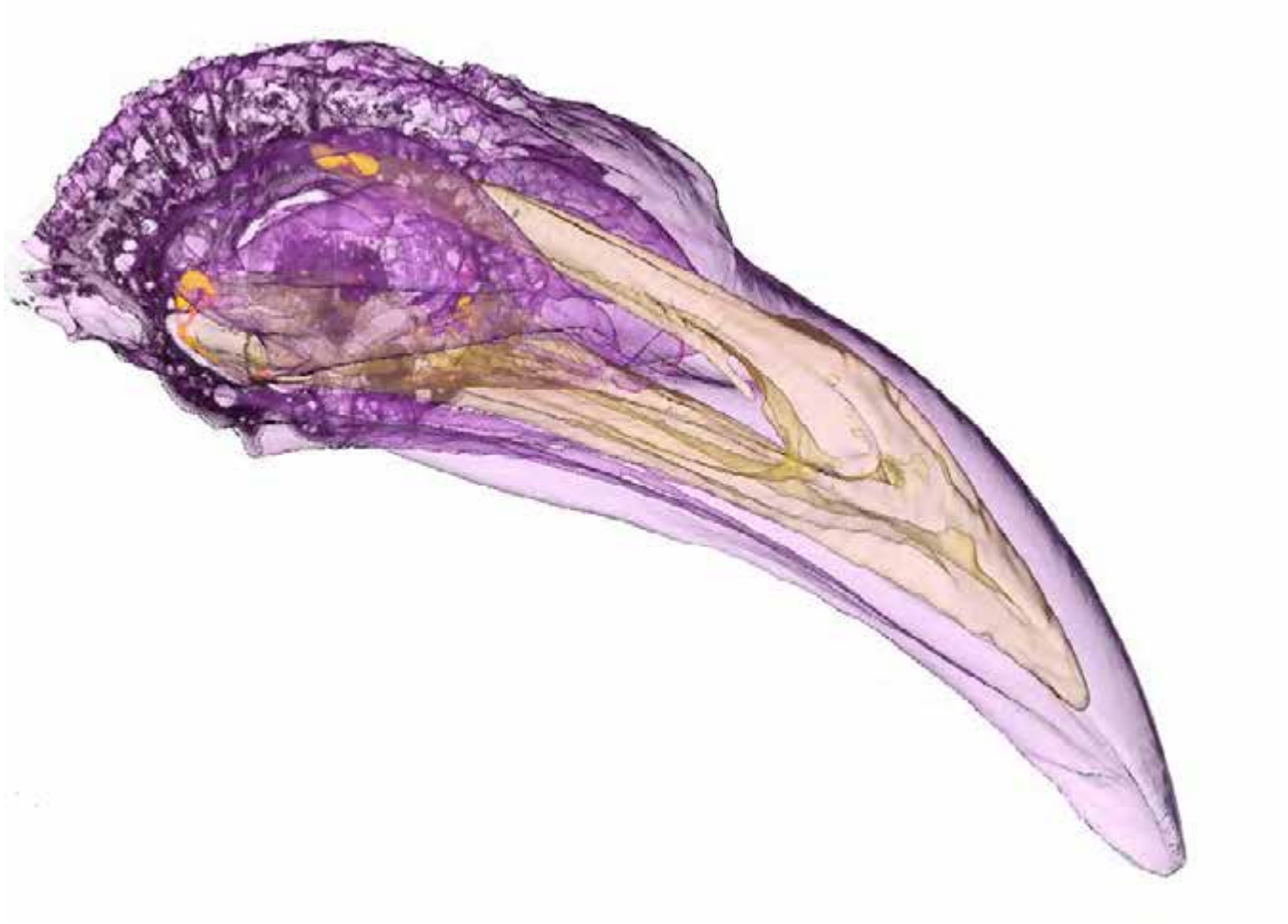
In former centuries heretics were generally put to death by any one of a wide variety of imaginative means. If they were exceptionally fortunate, they were simply exiled or subjected to lifelong house arrest. A few of them, such as Galileo, were even ultimately rehabilitated, although generally not until they were long dead. In short, the lot of a heretic is rarely a happy one and so heresy is not a popular career choice, especially for a young scientist who has only recently embarked on independent research.

David Keays certainly never intended it and challenging the accepted dogma was far from his mind when he came to the IMP. During his post-doctoral research at the University of Oxford he had heard Gero Miesenböck lecture about optogenetic tools, including neurons that could be individually activated by pulses of light. Many neurons are hard to access and Miesenböck speculated that in the future it might be possible to activate single neurons not by the cumbersome use of lasers but with magnetic fields, enabling their involvement in certain behaviours to be tested under almost natural conditions. David thought this might be possible by coupling existing magnetic receptors to neurons and presented a research programme along these lines when he applied for a fellowship at the IMP.

The best studied animals with magnetic receptors are pigeons. It is widely assumed that all birds are able to detect magnetic fields and previous work had indicated that iron-containing neurons in pigeons' upper beaks act as magnetic receptors. David's initial approach was to attempt to replicate these findings and identify the receptors before proceeding to a molecular and genetic analysis. Unfortunately, though, the diploma student working on the project, Christoph Treiber, could not repeat the previous studies. Working with thin sections of pigeons' beaks – a technician in David's group, Marion Salzer, prepared about 2400 10µm sections from each of approximately 200 pigeons – Christoph was able to stain cells with Prussian Blue, indicating that they contained iron, but the stained cells were definitely not neurons: they did not stain with any of three antibodies known to bind to neuronal structures. (In fact, a few cells, below 1%, did stain with Prussian Blue and with neuronal markers but these are probably an artefact of the sectioning, whereby a Prussian Blue-positive cell is superimposed on a neuron.) The iron-containing cells contained nuclei – previous work had stated that they did not but Christoph noticed that they stained with nuclear fast red. Furthermore, under the electron microscope they could be seen to carry long filopodia that sometimes appeared to engulf neighbouring cells. This suggested that the cells might actually be macrophages, which are widespread in pigeons and unlikely to be involved in sensing magnetic fields. Indeed, the cells were found to stain positive for major histocompatibility (MHC) markers, confirming that they are some kind of antigen-presenting cell rather than neurons.

The finding ran directly counter to the widespread belief that the iron-containing cells in the pigeon's upper beak were neurons that respond to magnetic fields. The implications of the discovery were enormous: all of the recent studies on magnetoreception in birds had been based on a misconception. David first presented the work at the April 2011 conference of the Royal Institute of Navigation. He was new to the field and knowing only a couple of people at the meeting had been allocated a ten-minute slot at the tail end of the programme. By the time he came to speak, there had been a number of presentations based on the idea that the iron-containing cells in birds' beaks were neurons that responded to magnetic fields. Predictably, David's presentation was not particularly well received and many of those in the audience decided that the interloper was clearly wrong and quite possibly mad. Some, however, were supportive and encouraged David to publish his work immediately.

More in hope than expectation, David sent a manuscript to *Nature* and waited for the reviews. Only one of the four referees was at all critical, suggesting that the group had simply missed the magnetoreceptors, or that perhaps the pigeons they analysed didn't have them. The Keays lab decided that the best way to respond to the criticism was to repeat the staining on pigeons from another source. *Nature* had allowed one month for the revision, so Christoph flew back from Oxford, Marion returned from London and the two of them began sectioning and staining more pigeons' beaks. As David recollects, "nobody slept much for a month but we could replicate our histological findings in the second cohort and we managed to resubmit our manuscript on time." This time all the referees were positive and the paper was published (Christoph Daniel Treiber, Marion Claudia Salzer, Johannes Riegler, Nathaniel Edelman, Cristina Sugar, Martin Breuss, Paul Pichler, Herve Cadiou, Martin Saunders, Mark Lythgoe, Jeremy Shaw and David Anthony Keays, *Clusters of iron-rich cells in the upper beak of pigeons are macrophages not magnetosensitive neurons*, Nature **484**, 367-370).



The group was completely unprepared for the reaction. Christoph was perplexed because "after all, despite looking at about half a million sections we hadn't actually managed to find the magnetoreceptor." Nevertheless, the results were widely covered in the popular press and attracted considerable attention from within the scientific community. Not surprisingly, the groups that had been most active in promoting the idea that the iron-containing cells were neurons were keen to denounce the new results, which the main authors of the original paper described as "garbage" in a comment in the *Frankfurter Allgemeine Zeitung*. Further opposition came from behavioural scientists who had spent the past decade inactivating the supposed magnetoreceptors in pigeons' beaks and examining the effects on the birds' behaviour. As David succinctly puts it, "the interpretation of all such experiments needs revisiting in the light of our discovery that the cells in the beak are not magnetoreceptive neurons."

To attempt to convince the sceptics, David and his colleagues visited the group of Roswitha and Wolfgang Wiltschko at the University of Frankfurt, where much of the behavioural research had been performed. Gerta and Günther Fleissner had undertaken their original description of the iron-containing cells at the same university and David hoped to be able to examine their data. He took many of the slides showing that the iron-containing cells in pigeons' beaks were not neurons. In fact, the Prussian Blue-stained slides prepared in Frankfurt looked identical to those of the IMP group but the key slides containing the neuronal stains were unavailable: they were apparently in a locked cupboard to which nobody had the key.

Although the Fleissner group has still not publicly accepted David's findings, many of their previous adherents have, including Henrik Mouritsen, who wrote a supportive piece in the *Süddeutsche Zeitung* as well as a News & Views comment in *Nature* in which he generously concluded that the onus of proof had shifted to those who still believe that the iron-containing cells in the pigeons' beaks are magnetic sensors. David himself senses that the community is gradually coming around to the new way of thinking.

Marion Salzer

Christoph Treiber

David freely accepts that his work has essentially "put research in the field back by about ten years. We thought we knew what the magnetic sensors were but we now accept that we were mistaken." He thus feels that he is in some way responsible for advancing it again, ideally by finding the true magnetic receptor. The IMP represents the ideal environment for challenging work of this kind thanks to its willingness to support exploratory, high-risk research and its excellent core facilities (David and Christoph have made heavy use of the electronic microscopy service) and due to the generous budgets it allocates to its fellows: David doubts he would have been able to afford the slides he used had he been working elsewhere. He has recently been promoted to a position of Junior Group Leader, giving him a further eight years not only to find the magnetic receptor but to characterize it and implement it in neurons that can be magnetically activated. The reluctant heretic may one day be able to pave the way for the behavioural studies of which Miesenböck in his lecture could only dream.

WORKING TOGETHER, EVEN AT A DISTANCE

As anyone who has constructed their own house can confirm, building is a tiresome enterprise. A large number of workmen are usually involved and individual workmen must perform the tasks allocated to them not only correctly but also at the appropriate time. The entire operation is usually coordinated by a foreman, whose task it is to follow the architect's plans. Nevertheless, mistakes are frequent, many of them costly and time-consuming.

The task of building a cell is no less complex but mistakes are far less common. The initial step is the establishment of asymmetry, or polarity: something happens to a perfectly symmetrical cell to define left and right, anterior and posterior. Once this is done, the rest is conceptually straightforward: the intracellular machinery – the workmen – assemble the complicated internal structures and position them in accordance with the design plans. How symmetry breaking occurs has puzzled developmental biologists for generations but recent work in the group of Carrie Cowan at the IMP has provided important new information. The findings were published earlier this year in the journal *Current Biology* (Vol. **22**, pp. 583-589).

Carrie has long been interested in how cells interpret the information contained in their genomes – the building plans – to undertake this initial step in their construction. She traces her fascination to her own difficulties in deciphering plans or maps: like many people she struggles to assemble DIY furniture and despite rotating maps so that they are aligned with landmarks she can see, she still has difficulties working out which way to go. Her cells are much better at interpreting plans than she is. How do they determine where to place structures and do so both correctly and absolutely reproducibly?

One of the key players, the architect in the above analogy with building a house, is the cell centrosome. This has been known for well over a hundred years but some of its functions have only recently come to light. Centrosomes organize microtubules and are involved in regulating the cell cycle. They have also been implicated in the asymmetric segregation of various proteins and nucleic acids to daughter cells following cell division, which is absolutely required to enable daughter cells to have different fates (and thus for multicellular organisms to arise). Carrie's previous work had suggested that centrosomes may also signal to the cell cortex (the "foreman") to establish cell polarity. The prevailing belief was that the process involved a type of signalling cascade: the centrosome would approach the cell cortex and modify some component (e.g. by phosphorylation), setting in train a series of interactions that ultimately define polarity. But nobody had determined how the centrosome initially located the cell cortex nor defined the nature of the signal it sends to the cortex. Carrie elected to tackle these issues, using the nematode worm *Caenorhabditis elegans* as a model system. The worm has proven extremely popular with biologists because its development is so tightly controlled. All embryos develop identically, at least at the gross scale, making it easy to see when something changes. However, the high degree of reproducibility had made it difficult to understand precisely how the centrosome functions: changes have no discernible effect on cell polarity.

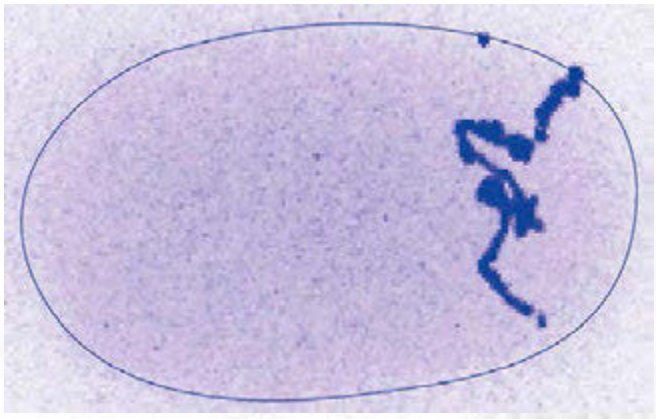
Carrie wanted to observe what happens in living one-cell *C. elegans* embryos before the establishment of asymmetry, i.e. in the first half an hour after fertilization, using a fluorescent marker to follow the position of the centrosome. The approach was not unique but Carrie had two significant advantages over her competitors. First, she was working at the IMP, which houses a latest generation spinning-disk confocal microscope that enables her to take images extremely rapidly. "In our previous work we were able to record a frame every 15-20 seconds, whereas with the new set-up we could take three frames a second. This turned out to be crucial for visualizing a process that happens in less than two minutes." Her second advantage arose when she recruited the PhD student Dominika Bienkowska to her group. Dominika is one of that rare breed of biologists with a real affinity for analysing quantitative data. As Carrie recalls, "When she came to interview she said her favourite course at university had been physical chemistry. How many people could claim that?" Dominika also came with prior experience of working with *C. elegans* embryos. It seemed like a perfect match.

Dominika began by investigating how centrosomes locate the cell cortex and how they move towards it to initiate polarity establishment. She examined a number of *C. elegans* mutants in which it was known that the centrosome did not approach the cortex and was puzzled to find that the cells nevertheless managed to establish polarity. This observation was highly surprising and Dominika and Carrie initially found it difficult to accept. If the centrosome did not come into close proximity to the cortex, how could it possibly signal to it to initiate the polarization process? Dominika was naturally concerned that she was doing something wrong and simply not seeing the result that she and Carrie expected. But her data were clean and of high quality and eventually she and Carrie were persuaded that the centrosome did not need to approach the cortex. Instead, the centrosome moves through the cell apparently at random and at the appropriate time it signals to the closest cortex. Dominika noticed that polarization took place more rapidly when the centrosome happened to be very close to the cortex. Asymmetry could still arise when the centrosome was about 10 µm from the closest cortex, although distances of up to 5 µm were more usual. Remarkably, the final result was the same no matter where in the cell the centrosome was located or how far it was from the closest cortex. In the house-building analogy, it appears as though the architect does not actually have to meet the foreman. The two of them can work together at a distance, although the work proceeds faster if they are within normal speaking range. And the resulting house is the same no matter where the foundation stone is laid.

The signalling process is thus far more flexible than previously supposed. Carrie has no difficulty in rationalizing this finding, stating "It makes a lot of sense. We have to remember that the worms normally live in a highly variable environment. The temperature of soil can fluctuate, the oxygen concentration changes and any number of pathogens may be present. It seems as if the system to establish asymmetry and to start forming a worm from a fertilized egg is extremely robust: not much can go wrong after fertilization."

Nevertheless, the nature of the centrosome-cortex signal remains puzzling. An interesting observation is that depleting the so-called "Aurora A kinase" from embryos enables them to establish asymmetry in the absence of centrosomes and even permits multiple polarities to arise simultaneously in a single cell. In other words, centrosomes are not always required for the initiation of polarization, so it is conceivable that they do not act by directly stimulating the cell cortex. Perhaps the signal from the centrosomes is not positive but negative, locally inhibiting or inactivating the Aurora A kinase and thus facilitating the start of polarization. According to this notion, the entire developmental process is self-organizing and the centrosome's function at the start is to prevent it from running out of control. Our previous conception of the very first stage of cell development may need to be completely rethought. In the analogy, the centrosome would no longer be the architect but the planning authorities, impeding the construction of inappropriate buildings. The actual building work would be the sole responsibility of the foreman and the workmen, who would operate in a reliable and self-correcting manner.

Carrie recognizes that her latest findings will change the way people view the establishment of asymmetry. Her results show that her previous ideas were incorrect but she is not too concerned to have been wrong. "As scientists we should be naturally happy to be able to show anything in a convincing way. Whether our previous model was right or wrong doesn't really matter: we now understand better how the process of symmetry breaking is initiated and can focus our efforts on studying the mechanism involved."



Legends:
1. Dominika on a worm-hunt in Ischia, Italy.
2. The path of a single centrosome during polarity establishment. Images were acquired at 3 frames per second and then projected into a single image. The embryo outline is indicated by the blue line.

MEINRAD BUSSLINGER

Stem cell commitment in hematopoiesis

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Acquired immunity to 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 STAT5, E2A, EBF1 and Pax5. STAT5, a downstream mediator of IL-7 signaling, fulfills a permissive role by controlling cell survival during early B cell development. E2A and EBF1 function as B cell specification factors and Pax5 as the B cell commitment factor that restricts the developmental potential of hematopoietic progenitor cells to the B cell pathway. Pax5 controls B cell identity throughout B lymphopoiesis, as conditional Pax5 loss enables mature B cells from peripheral lymphoid organs to dedifferentiate to early uncommitted progenitors in the bone marrow, which subsequently develop into functional T cells. As E2A, EBF1 and Pax5 are required throughout B lymphopoiesis, we currently investigate the role of these transcription factors in the generation and function of different mature B cell types, which are essential for humoral immune responses to foreign pathogens (Figure 1).

T cell development

Signaling through the Notch1 receptor is essential for initiating the development of T cells in the thymus. Early T cell specification also depends on other transcription factors, such as GATA3 and E2A. However, little is known about target genes that mediate the effects of these transcriptional regulators in early T cell development. We are therefore elucidating the molecular functions of these 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 and mature B cells by genome-wide mapping of DNase I hypersensitive sites, transcription start sites and chromatin modifications, in order to delineate active enhancers and promoters (Figure 2). By ChIP sequencing, we identify the binding sites of the different transcription factors at these regulatory elements (Figure 2). 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.

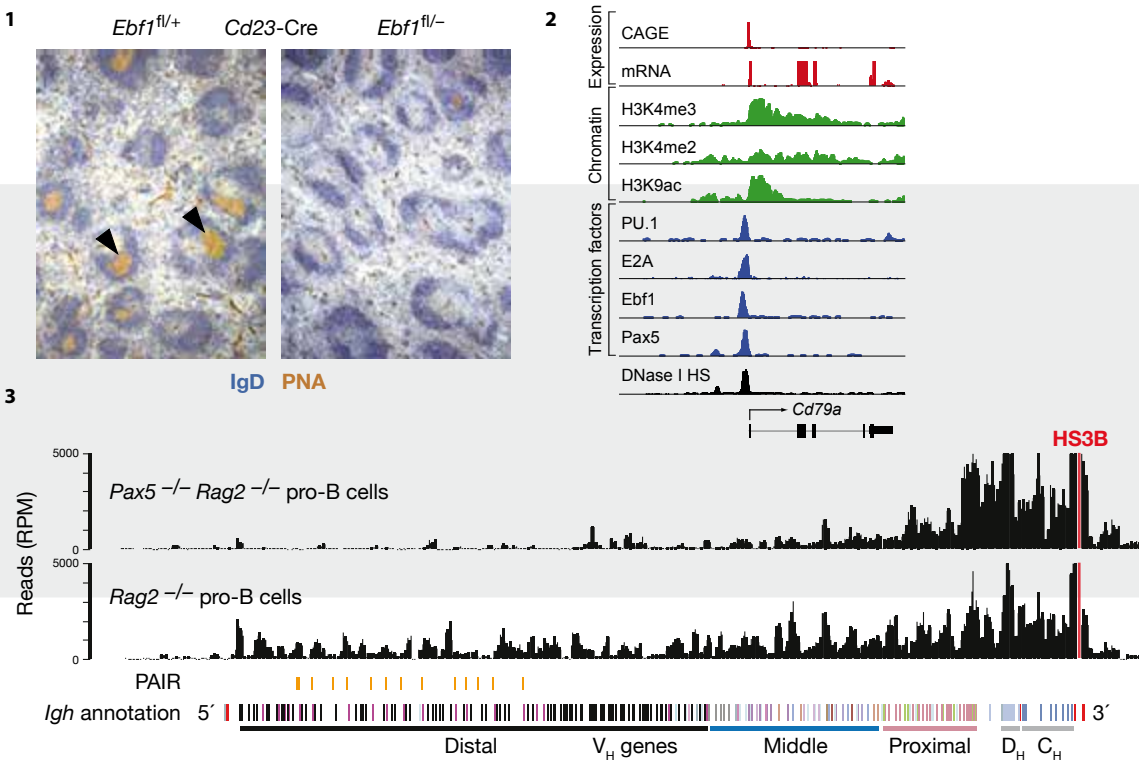

Figure 1: ***Critical role of EBF1 in germinal center (GC) B cell development.*** *The Cd23-Cre line deletes the floxed (fl) Ebf1 allele in mature B cells of control Cd23-Cre Ebf1$^{fl/+}$ and experimental Cd23-Cre Ebf1$^{fl/-}$ mice. PNA^{+} GC B cells (arrowhead) are present in control mice, but absent in experimental mice 14 days after immunization with sheep red blood cells, as shown by immunostaining of spleen sections.*

Figure 2: ***Genome-wide identification of transcription factor target genes.*** *The Cd79α gene is shown as a representative example to indicate the different parameters that were determined by global genomic analyses. Transcriptional start sites were mapped by the Cap analysis gene expression (CAGE) assay, gene expression profiles by mRNA sequencing, DNase I hypersensitive (HS) sites by deep sequencing, and active histone modifications (H3K4me2, H3K4me3, H3K9ac) and transcription factor-binding sites (for PU.1, E2A, EBF1 and Pax5) by ChIP sequencing in pro-B cells.*

Figure 3: ***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 functional rearrangement of the *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, which facilitates synapse formation and V-(D)J recombination. Our previous work demonstrated an 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 high-resolution mapping of chromatin loops, we have recently shown that the extended *Igh* locus in Pax5-deficient progenitors consists of several local interaction domains (Figure 3). In committed pro-B cells, these local interaction domains engage in long-range interactions across the entire *Igh* locus and thereby coalesce into one large chromatin-folding unit (Figure 3). We currently elucidate the function of *cis*-regulatory elements and *trans*-acting factors involved in this process.

FURTHER READING:

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Molecular mechanisms of protein quality control

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The misfolding and aggregation of protein molecules is a major threat to all living organisms. Cells have therefore evolved a sophisticated network of molecular chaperones and proteases to prevent protein aggregation (Fig. 1A). In addition, protein quality control and regulatory proteolysis are important mechanisms in the defense line of several bacterial pathogens. My group is performing a structure-function analysis of prokaryotic and eukaryotic factors that combat folding stress and, in parallel, ensure controlled digestion of specific target proteins. A better understanding of protein quality control might disclose novel strategies to counteract protein folding diseases and bacterial pathogenicity.

Housekeeping HtrA proteases - Guards of the extracytosolic compartment

A combination of chaperone and protease function in a single protein could provide a direct and rapid response to protein folding problems. The heat shock protein DegP (HtrA) can switch between these dual functionalities in a temperature-dependent manner (Fig. 1B), and thus offers unique possibilities to investigate how cells distinguish between proteins that can be refolded and "hopeless" cases that need to be degraded.

DegP from *E. coli* is a central component of the protein-quality-control system in the bacterial envelope that is involved in eliminating misfolded proteins and in OMP (outer membrane protein) biogenesis. To investigate the molecular basis of these dual activities, we characterized different DegP/substrate complexes. Binding of misfolded proteins transformed the resting DegP hexamer into large, catalytically active 12- and 24-meric multimers. Structural analysis of these particles revealed that DegP assembles a huge protein packaging device (Fig. 2), whose central compartment is adaptable to the size and concentration of substrate. Moreover, the inner cavity serves antagonistic functions. While encapsulation of folded OMP protomers is protective and might permit safe transit through the periplasm, misfolded proteins are eliminated in the molecular reaction chamber. Oligomer re-assembly and concomitant activation upon substrate binding may also be critical in regulating other HtrA proteases that promote diverse biological functions. Our current data provide excellent leads to address the molecular mechanisms of human DegP homologues, homologues from pathogenic bacteria, and functionally related PDZ proteases that exert housekeeping functions in mitochondria, chloroplasts and the extracellular space. Moreover, the identified activation mechanism should be helpful to obtain structural data concerning protease-substrate or protease-inhibitor complexes, which are indispensable for understanding how aberrant proteins are partitioned between refolding and degradation pathways.

The CtsR/McsB stress response

All cells have evolved highly efficient signaling pathways that sense the presence of damaged proteins and transmit the signal "folding stress" to dedicated transcription factors, which then adjust the expression of the protein-quality-control factors. One of the most intensely studied stress-response pathways is the bacterial heat-shock system. In *B. subtilis*, the transcription factor CtsR is a major component of this system that represses the *clpC* heat shock operon, the *clpE* and *clpP* genes, by binding specifically to a 7-nucleotide direct repeat sequence located upstream of the transcriptional start sites (Fig.3A). Stress-induced transcription of the *clp* genes depends on the inactivation of CtsR by McsB. To delineate the molecular basis of CtsR and McsB in the bacterial stress response, we screened the respective proteins from various Gram-positive bacteria for

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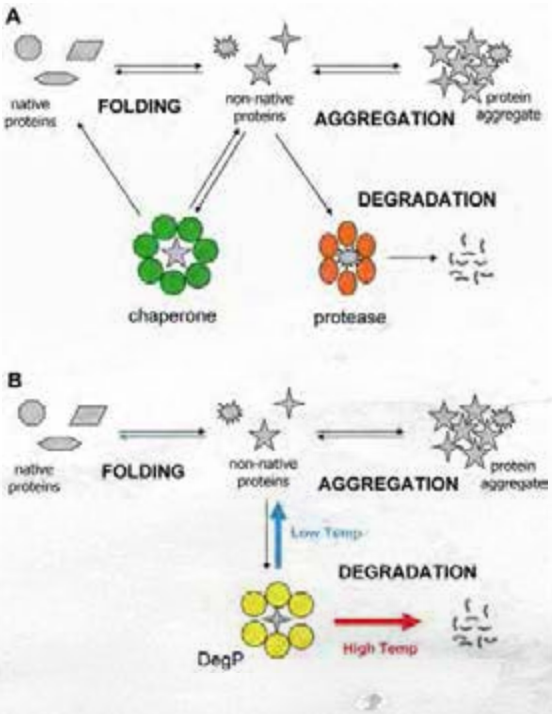

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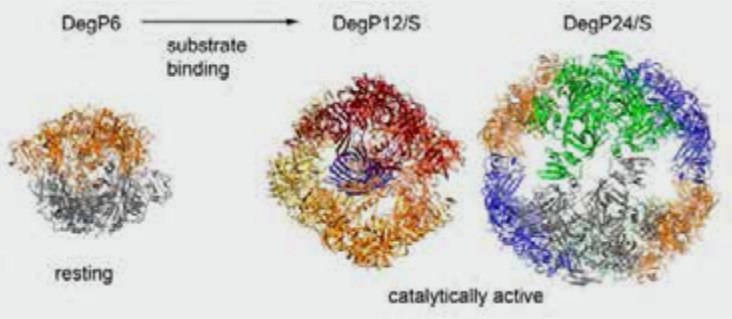

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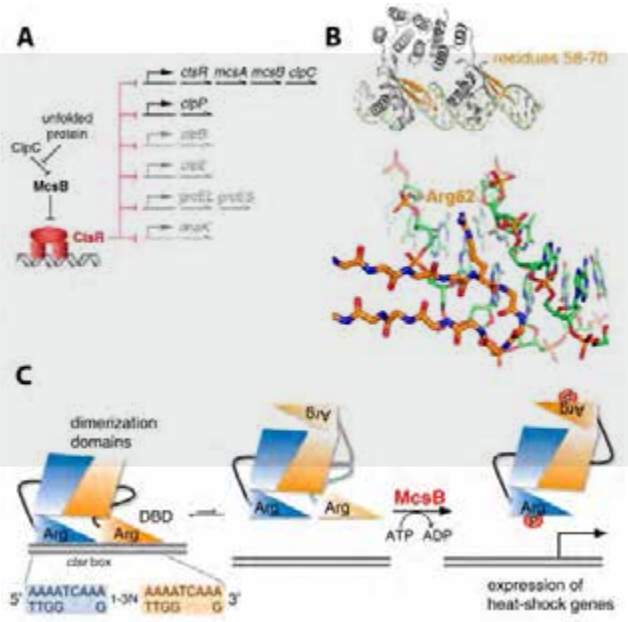

Figure 1: *Protein Quality Control (A) The scheme illustrates the different fates of misfolded non-native proteins. According to Gottesman and co-workers' "kinetic partitioning model", aggregate formation is counteracted by the action of proteases and chaperones. (B) DegP combines the antagonistic activities of a protease and a chaperone, and thus guarantees a rapid cellular response to protein folding stress. The switch in activity is regulated by temperature. At low temperatures (<25°C) the protein acts as a chaperone, whereas at higher temperatures the protease function is dominant.*

Figure 2: *Structural analysis of DegP Ribbon presentation of the resting DegP hexamer (DegP6) that is transformed upon substrate binding into the catalytically active DegP12- and DegP24-multimers. The constituting trimers are colored differently and, for DegP12, the encapsulated OMP substrate is shown in blue.*

Figure 3: *The CtsR/McsB stress-response system (A) The CtsR regulon of Gram-positive bacteria. (B) Position of the phospho-peptide (orange) which was identified by mass spectrometry is highlighted in the CtsR2/DNA structure. The binding mode of Arg62 (that also represents the main phosphorylation site) at the floor of the DNA minor groove (green) is shown in detail. (C) CtsR binds as a dimer to the highly conserved ctsr box, thereby inhibiting transcription of downstream stress genes. The phosphorylated CtsR repressor is unable to bind to DNA, thereby permitting expression of heat-shock genes. "Arg" marks critical arginine residues in the DBD, in particular Arg62, phosphorylated by McsB.*

recombinant production, and succeeded in reconstituting the *Bacillus stearothermophilus* CtsR/McsB system *in vitro*.

The crystal structure of the CtsR repressor in complex with DNA revealed how partial asymmetry in a dimeric transcription factor allows high affinity binding to tandem DNA repeats. Moreover, biochemical characterization of McsB highlighted a novel protein kinase activity. McsB specifically phosphorylates arginine residues in the DNA-binding domain of CtsR, like for example Arg62 of the beta-wing (Fig. 3B), thereby impairing its function as a repressor of stress response genes. Identification of the CtsR/McsB arginine phospho-switch (Fig. 3C) creates new perspectives to enhance our understanding of prokaryotic and eukaryotic transcriptional regulation.

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CARRIE COWAN

Symmetry breaking during cell polarization

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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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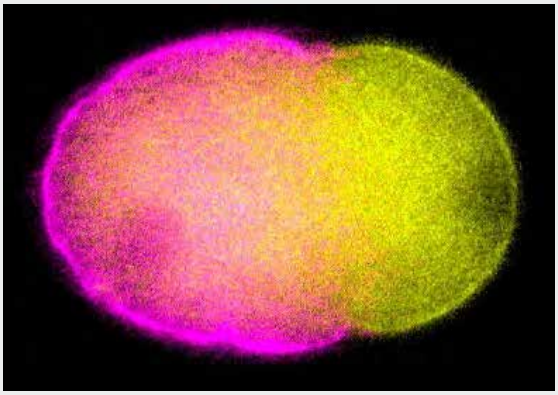

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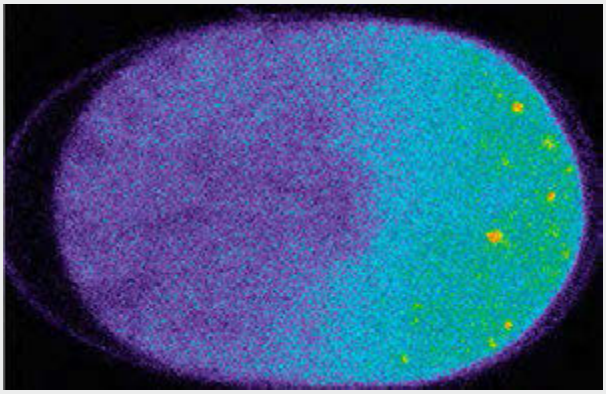

Figure 1: *Centrosome position (line) relative to the cortex (dots) during polarity establishment. The color scale indicates elapsed time (blue: -500 s, red: 300 s).*

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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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.

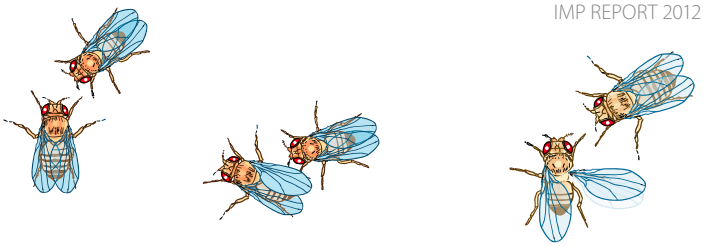
A major goal for neuroscience is to understand how information processing in neural circuits guides animal behaviour. At any given moment, the brain receives a rich set of sensory inputs, from both external and internal sources. This information must be integrated, interpreted in the light of knowledge 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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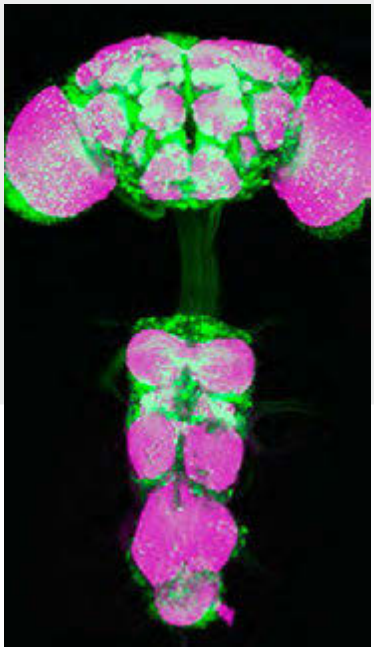

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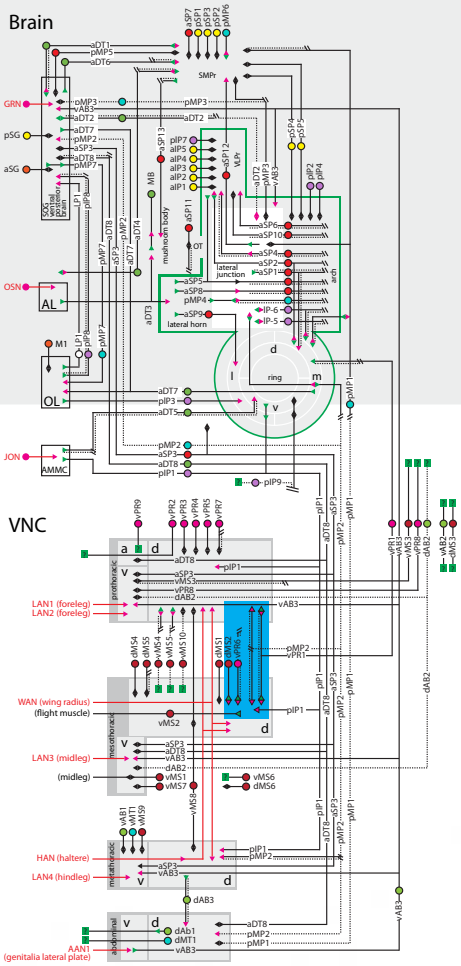

Figure 1: ***fru⁺ neurons in the CNS, shown in green.*** *Synapses are stained in magenta. This is a confocal image of the brain and ventral nerve cord of a fru^{GAL4} UAS-GFP male.*

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.

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CHRISTINE HARTMANN

Functions of Wnt Signaling in Formation and Patterning of the Vertebrate Skeleton

Christine Hartmann / Group Leader

In February 2012, Christine Hartmann left the IMP and took up a position as Professor at the University of Münster. She is Head of the Department of Bone and Skeletal Research at the Institute of Experimental Musculo-Skeletal Medicine.

The skeleton is essential for vertebrates; it supports the body, provides the mechanical framework for physical movements, and protects internal organs. During embryonic development the sites where the future skeletal elements (bones) are formed and their size as well as the positions of articulations (joints) are determined. Most skeletal elements are formed as cartilaginous templates and need to be remodeled into bony tissue to strengthen the skeleton - this process is referred to as endochondral ossification. We use mouse as a model organism to gain insight how different aspects of skeletogenesis are regulated by Wnt-signaling pathways during embryonic and postnatal development. In addition, we use mouse embryonic stem cells to dissect the different functions of β-catenin, a core component of the canonical Wnt-pathway, in signaling and cell-adhesion.

Regulation of cell lineage differentiation

The different cell types of the appendicular skeleton, chondrocytes, osteoblasts and the cells contributing to the future synovial joints are of mesenchymal origin. Over the past years we demonstrated that the canonical Wnt/β-catenin pathway plays very important roles for the differentiation of the different skeletal lineages in the mouse (see Figure 1). In the absence of functional canonical β-catenin signaling, osteoblast precursors, the osteo-chondroprogenitor cells, differentiate into chondrocytes (Hill et al., 2005). Furthermore, we have shown that the canonical Wnt/β-catenin pathway is required to suppress the chondrogenic potential of cells in the joint interzone (Spaeter et al., 2006). This suggests that differentiation along the chondrocyte lineage may be the default and that increased levels of β-catenin are required to enable the differentiation along the other two lineages.

Differentiation and Maturation of Chondrocytes

The various skeletal elements of the vertebrate skeleton differ in size and shape, but little is known about the molecular mechanisms controlling these two features. Nevertheless, perturbations in the chondrocyte maturation process lead to changes in the size of skeletal elements. Studying knock-out animals for Wnt9a we uncovered a very specific requirement for this Wnt-ligand during long-bone development: Wnt9a via β-catenin controls the expression of the central regulator of chondrocyte maturation, Indian hedgehog, in prehypertrophic chondrocytes in a spatio-temporal manner (Spaeter et al., 2006). Thus, providing a mechanism to fine-tune the size of the future skeletal element. Currently, we are interested in the regulation of hypertrophic chondrocyte maturation and of their removal and turnover into trabecular bone and study amongst others the role of β-catenin in this process.
As we have previously uncovered a potential role for Calcium/Calmodulin dependent kinase II (CaMKII) in the maturation process of chondrocytes in the chick (Taschner et al., 2008) (Figure 2), we are currently analyzing whether this kinase plays a similar role in the mouse using transgenic approaches.

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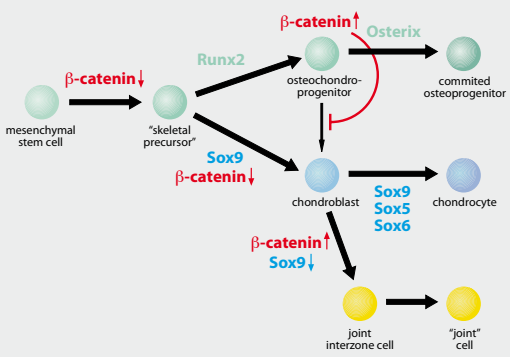

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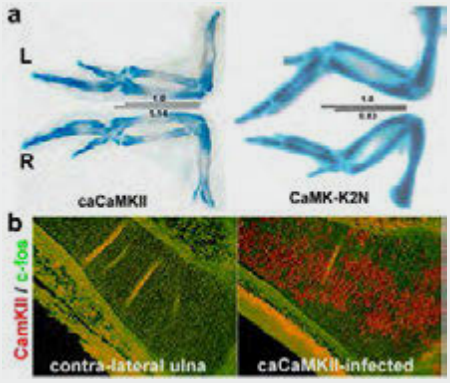

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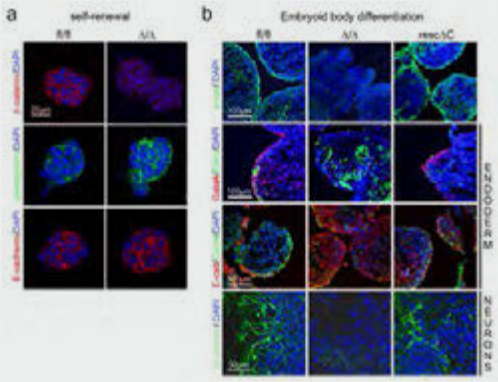

Figure 1: *β-catenin levels need to be modulated during skeletal lineage differentiation: only mesenchymal cells expressing low levels of β-catenin can differentiate into skeletal precursor which will give rise to osteoblasts, chondrocytes, and cells of the joint. Within the osteoblast and joint lineage, β-catenin levels need to be increased to enable the differentiation into osteoblasts and cells of the joint, respectively. While within the chondrocyte lineage β-catenin levels need to be maintained at a low level.*

Figure 2: *a) Expression of an constitutively active form of CaMKII (caCaMKII) results in lengthening of the skeletal elements in the infected right (R) chicken limb, while expression of the inhibitor CaMK-K2N results in shortening of the infected R limb. b) Lengthening of the skeletal element is associated with the down-regulation of c-fos expression (green) in the caCaMKII infected regions (red).*

Figure 3: *a) Staining for β-catenin, plakoglobin und E-cadherin in β-catenin fl/fl and deficient Δ/Δ ESCs under self-renewal conditions. b) Staining for β-catenin, the endodermal markers Gata4 and Cxcr4, as well as the neuronal marker β3-tubulin in embryoid bodies derived from β-catenin fl/fl, Δ/Δ ESCs and Δ/Δ ESCs carrying the transcriptional inactive variant of β-catenin (rescΔC.)*

Synovial joint development

Wnt9a was identified as a major player for the induction of synovial joint development in chick studies (Hartmann and Tabin, 2001). In the mouse, Wnt9a is expressed in the early joint interzone and, after the formation of the synovial joint, in the articular cartilage and joint capsule cells. Loss of Wnt9a results in ectopic cartilage nodule formation in the humeral-radial joint. In humans this phenotype is known as synovial chondroid metaplasia. Two other Wnt-genes are also expressed in the joint regions, Wnt4 and Wnt16. Wnt4;Wnt9a double-mutant mice show synovial chondroid metaplasia in two additional joints and fusions of carpal and tarsal elements (Später et al., 2006a, 2006b), but not complete fusion of all joints. In conclusion, the embryonic phenotypes point to a role for Wnts maintaining joint integrity.

β-catenin – co-transcriptional activity versus cell adhesion function

We used mouse embryonic stem cells (mESCs) to distinguish between the requirements of β-catenin in functioning as a transcriptional co-activator and as a component of cell adherens junctions. mESCs deficient for β-catenin function show no self-renewing defects under standard conditions (LIF & Serum) and only minor cell adhesion defects (Figure 3a). However, these cells fail to differentiate into derivatives of all three germ layers and show massive cell adhesion defects during differentiation. Rescue experiments using a Tcf/Lef-signaling defective, but cell adhesion competent variant of β-catenin revealed a requirement for the cell adhesion function of β-catenin for the derivation of neurons (an ectodermal derivative) and for the definitive endoderm, while rescuing cell-adhesion did not influence mesoderm formation (Figure 3b) (Lyashenko et al., 2011). Hence, the function of β-catenin in cell-adhesion is probably playing are marginal role compared to its function as a transcriptional co-activator in the formation of mesoderm. We are currently developing tools that may allow us to distinguish between these two roles during embryonic development.

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; reward-related emotions induce the converse. Emotions are a central part of our mental self and linked to a variety of psychological conditions. How are emotions wired in the brain? To investigate their underlying neural basis, we apply molecular genetic, pharmacogenetic, and optogenetic methods 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.

Understanding how various emotions emerge from the neurocircuitry of the brain and how these emotional states are modulated by genes and pharmacology is a complex issue. One straightforward approach is to investigate, in exemplary fashion, how basic emotions are processed in selected key elements of the brain emotion system. Numerous studies established the limbic system as the central hub of emotion processing (LeDoux, 2000). It integrates sensory information, encodes emotional states, and instructs other brain centers to regulate physiology and behavior. However, the limbic system consists of many distinct and highly interconnected neuronal populations. Resolving how emotions are processed in this network at the level of single neural circuits is a major challenge. To resolve this problem, we combine genetic manipulation of brain circuitry to map circuit anatomy and function (Luo et al., 2008) with electrophysiological recordings (Du et al., 2009) for probing circuit interactions.

Circuit mechanics of emotions

We screen for limbic microcircuits that could serve as emotion hubs. Pharmacogenetics, optogenetics and viral tracing revealed a local inhibitory circuit of two antagonistic neuronal populations in the lateral central amygdala (CEl), which gates amygdala output to control conditioned fear (Haubensak et al., 2010). Results from combined pharmacogenetics and *in vivo* electrophysiological recordings suggest that antagonistic neuronal populations operate like a seesaw alternating between two states: a) in the absence of a conditioned stimulus (CS) so-called CEl-$_{off}$ neurons, identified by the expression of PKCδ (Fig. 1, left), are active, inhibiting their counterpart CEl-$_{on}$ neurons and amygdala output; b) in the presence of the CS, CEl-$_{on}$ neurons are active, inhibiting CEl-$_{off}$ neurons which disinhibit amygdala output and fear signals to the brain stem (Fig. 1, middle). We are currently testing this circuit mechanism and its modulation by emotional learning and midbrain dopamine, using neural modeling, optogenetic perturbations (Fig. 1, right), electrophysiology, and behavior. These experiments will put forward a mechanistic framework for understanding how emotions are reflected in activity states of neural circuits, and how these circuit dynamics are shaped by experience. Perhaps not surprisingly, these microcircuits do not operate in isolation but in cooperation with other brain structures. Interestingly, anatomical circuit mapping and optogenetics revealed that CE circuits are modulated by prefrontal inputs. It is tempting to speculate that this is one mechanism underlying the top-down control of emotions by higher cognitive processes in psychology.
We are currently complementing our research on fear in the amygdala by stepping back to a more general question: how are emotions differentially encoded in the brain? For instance, what makes fear different from reward states? In principle, they could emerge from dedicated emotional sub-systems, each specific for either fear or reward, or they could both be represented by alternative states of the same system. We are addressing this by tracking where limbic pathways for fear and reward diverge and converge.

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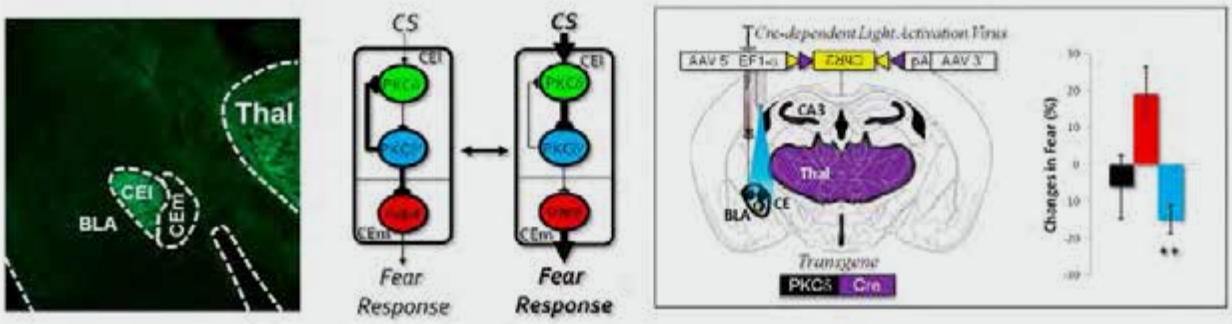

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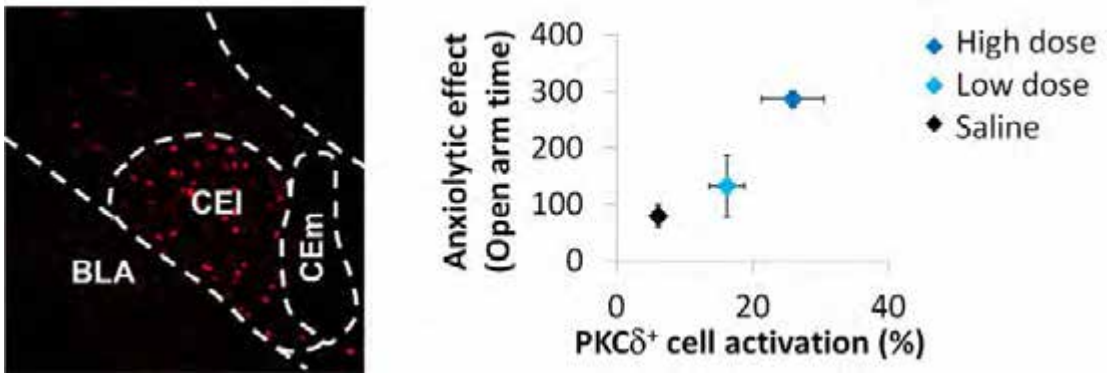

Figure 1: *Emotional gating in the central amygala (CE). Left, PKCδ identifies a subpopulation of neurons in CE. Middle, Neural circuit model of information flow through the CE. Right, Combinatorial viral/transgenic optogenetic manipulation of CE circuits. Light-induced changes in fear upon activating CEm output (red; middle) or CEl PKCδ⁺ neurons (blue; middle). BLA, basolateral amygala; CEl/m, lateral/medial central amygdala; Thal, thalamus.*

Figure 2: *Psychopharmacology of CE circuitry. A, Drug-induced neural activity and c-fos expression in CEl (left) correlates with anxiolytic effects (right). ChR2, channelrhodopsin; BLA, basolateral amygala; CEl/m, lateral/medial central amygdala.*

Genetic and pharmacological modulation of emotional states

While the molecular mechanics by which genes and drugs control neural activity at the cellular level have been worked out in great detail, the circuit mechanics by which this translates into behavior changes have not yet been resolved. The circuits identified above provide an ideal access to study this problem. We will investigate gene effects (anxiolytic and anxiogenic genes and polymorphisms) and the effects of drugs (anxiolytics, addictive drugs) on the activity of the emotion network identified above, and explore how these changes in activity modulate emotional states and behavior. Indeed, preliminary results suggest that CE circuits are targets of psychoactive drugs, which change the balance of neural activity in this network (Fig. 2).

Taken together, we hope that our research will disclose general principles of the network organization of emotions and provide a framework for understanding the genetics and pharmacology of emotions in health as well as in disease (such as anxiety disorders or addiction).

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KATRIN HEINZE

Nanoscale Functional Imaging of Cell Membranes

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Discoveries in bioscience are stimulated by the development of new scientific tools. The interactions of molecules with nanostructures provide an important prerequisite for such a tool when it comes to quantification of molecular scale phenomena in biology, or affordable ultra-sensitive diagnostics and sensing.
At our laboratory, we focus on understanding and tweaking mutual interactions of common fluorescent molecules via plasmonic and metamaterial structures with the aim of developing low-invasive fluorescence techniques beyond the usual spatial and temporal resolution limits.

Understanding interactions of complex molecules with nanostructures

Interactions of molecules with nanostructures are widely used in fundamental, applied, and medical research. Applications in life sciences range from high resolution studies of complex molecular dynamics and improved DNA/RNA sequencing technologies to the common pregnancy test and `naked-eye' ultra-sensitive HIV detection platforms.
Many advances in the field of metamaterials and nanostructures may be attributed to the advances in our ability to fabricate and accurately characterize almost arbitrary sub-wavelength-scale metal-dielectric structures, and thus tailor electromagnetic interactions between specific molecules and nanostructures for a given purpose. Nevertheless, modeling such interactions is still challenging. While promising the technology is at a relatively early stage.

We recently developed a theoretical description for a long-standing controversy regarding the interpretation of the fluorescence lifetimes of molecules in certain inhomogeneous environments (see Figure 1). Particularly near plasmonic structures, where often a certain distribution of lifetimes (so-called stretched exponential decay) is observed, previous justification of this phenomenon has purely been based on its empirical merits. Our results allow for correctly modeling time-resolved as well as steady-state measurements of an ensemble of fluorophores interacting with a nanostructure.
We have also worked towards realistic models for resonant energy transfer between molecules near nanostructures (see Figure 2). Recent experimental data from our laboratory are found to be consistent with such theoretical treatment. The implications are of direct significance for ultra-fast time-resolved and spectroscopic measurements employing such structures.

Plasmonic rulers: When material science meets biology

A drawback of many "superresolution" techniques is the difficulty to obtain nanometer resolution in axial (z) direction. This is compounded by the fact that the axial diffraction limit is already larger than that it is in lateral (xy) direction. We have countered this problem by designing a nanostructure that permits localization of fluorophores by means of their spectral fingerprint, and thus their separation from the structure to nanoscale precision. The position of emitters from the structure can be calculated on-the-fly by measuring the spectrum over several discrete wavelengths ranges (see Figure 3a). We have shown that, for several common dyes, this provides improvements in axial resolution by a factor of ~30 (Δz = 10-20 nm at visible wavelengths).
The underlying principle of the technique involves the translation of spatial information into spectral information by designing nanostructures with optimized dispersion properties. Since the spectral signal is no longer subject to the constraints of the classical diffraction limit, one is able to achieve superresolution without resorting to other time consuming

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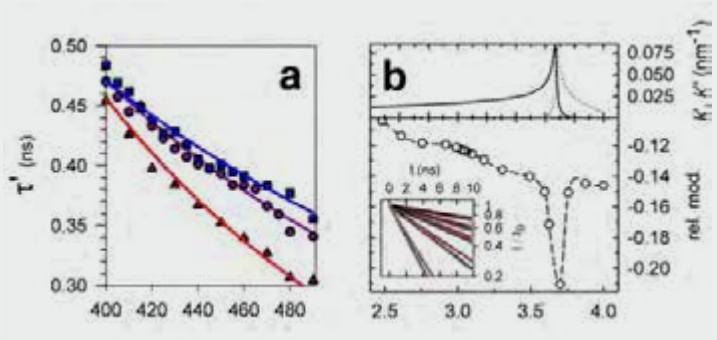

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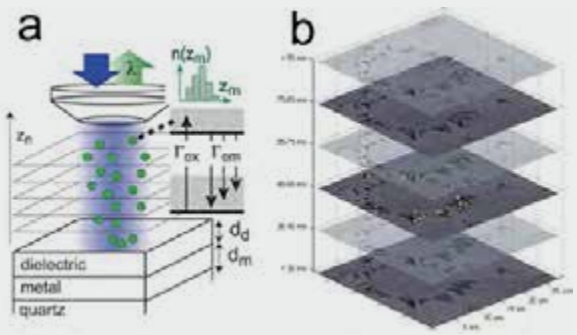

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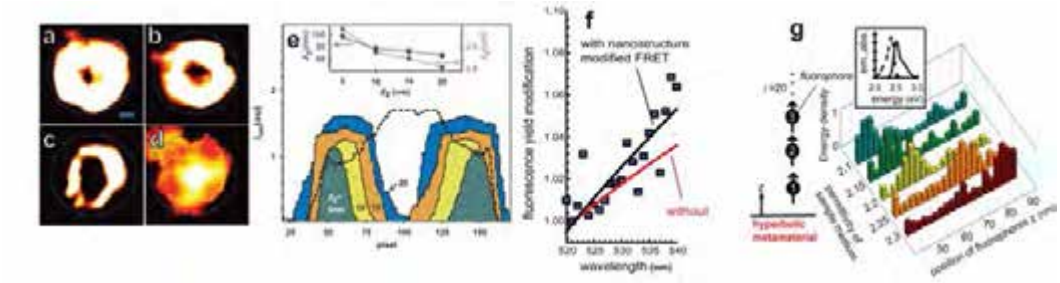

Figure 1: *Measured fluorescence lifetime (a) of a fluorophore (Alexa350) obtained from stretched exponential fitting, as a function of wavelength above two different metal-dielectric structures (blue and purple) and a hyperbolic metamaterial (red). Frequency dependence (b) [circles] of the deviation of the measured decay rate from a theoretical model employing only a single effective lifetime of a monolayer of the dye above a metal film. A clear dip at the surface plasmon polariton resonance frequency (the dispersion of which is plotted immediately above) is seen, as predicted in our model. The inset shows decay curves at different frequencies. Red: experimental data fitted with stretched exponential model, black: theoretical calculation assuming a 2-level single exponential model.*

Figure 2: *Images of polystyrene beads coated with a FRET pair on four different metal dielectric-coated substrates. Maximal FRET efficiency is at different distances from the interface, depending on the exact design of the metal-dielectric films (a-d). Averaged lateral cross-sections (e) of images such as those in panels a-d for metal-dielectric structures differing in the thickness of a high permittivity dielectric (z_2). The inset shows variation of the distance of maximum values from the center of the bead (r_p), and the corresponding axial distance from the interface as a function of the parameter z_2.(f) Modification to the fluorescence yield of two fluorescein monolayers separated by lipid bilayers near a metal-dielectric structure. The black and red line shows the predicted modification when resonant energy transfer via the metal-dielectric structure is included in, and excluded from the calculations, respectively. Note the high sensitivity of nanostructure-modified FRET (g). The plot shows the results of simulation for average re-distribution of energy in a long chain (20 molecules) of randomly excited homo-FRET pairs (spectrum shown in inset) aligned perpendicular to a nearby hyperbolic metamaterial as a function of the permittivity of the medium they are immersed in.*

Figure 3: *Schematic diagram of the setup with a fluorescent sample on a Si_3N_4/Ag-coated substrate, where excitation and λ-resolved detection are achieved through the same objective lens (a). The detected fluorescence at λ is expressed as the sum of measurable decay rates at discrete axial distances (z_n, n = 1, 2, 3...). Reconstruction (b) of the distance of paxilin-GFP from substrate in migrating paxillin-GFP-transfected B16 fibroblasts. Cells were cultured on a quartz slide with optimal Ag/Si_3N_4 coating.*

excitation and detection approaches such as mechanical scanning, stochastic activation/deactivation, blinking, etc.. Unlike other spectroscopic rulers employing distance-dependent resonances of nanostructures, our technique exhibits two important features for applications in molecular life-sciences: First, it was found to be fully bio-compatible; second, it employs common fluorophores. Recently, the approach proved successful for studying adhesion dynamics in fibroblasts (see Figure 3b), and we expect further extensive applications in the future.

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The Molecular Basis of Migration

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One of the most remarkable aspects of life, whether it be a single cell or a multicellular organism, is the ability to move. This is even more astounding when one considers that this movement is frequently equipped with directionality. Whether it be a migratory bird that crosses continents, or a cell that inches forward a micrometer at a time, both have a drive, a direction and a destination. What molecular mechanisms underlie this migration? The Keays lab is investigating two very different forms of migration: (1) the migration of neurons; and (2) the migration of animals mediated by magnetic information.

Tubulins in Neuronal Migration and Disease

Neuronal migration underlies the organisation 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 as it determines the destination of a given neuron as well as the circuit in which it operates. Moreover, a host of neurodevelopmental diseases are known to be linked to defective neuronal migration. An example is lissencephaly, a disease that is characterised by a "smooth brain", epilepsy and mental retardation.

We have shown that mutations in an 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 neuronal migration is further evidenced by our finding that mutations in a beta tubulin gene (*TUBB2B*) (Jalgin et al, 2009), cause another rare neurdevelopmental disorder known as asymmetric polymicrogyria. Most recently, *TUBA1A* and *TUBB2B* have also been implicated in autism spectrum disorders (Neale et al., 2012; Pinto et al., 2010). 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 (Figure 2). We are employing ENU mutagenesis, as well as transgenic methods to create 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.

Circuits, Cells and Molecules in Magnetoreception

Many species on the planet, whether they be birds, fish or insects rely on the earths 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 called magnetite (Fe_3O_4) transduce local magnetic information into a neuronal impulse.

This hypothesis originates from the discovery of magnetotactic bacteria. These aquatic bacteria use the Earth's magnetic field to direct swimming towards growth-favouring regions in natural waters. It has been shown that magnetotatic bacteria possess organelles called magnetosomes. Magnetosomes consist of membrane-enclosed magnetite crystals that twist into alignment with the Earth's magnetic field – thereby directing bacterial movement. The theory of magnetite based magnetoreception has been supported by the discovery of magnetite in a range of other organisms that detect and respond to magnetic fields;

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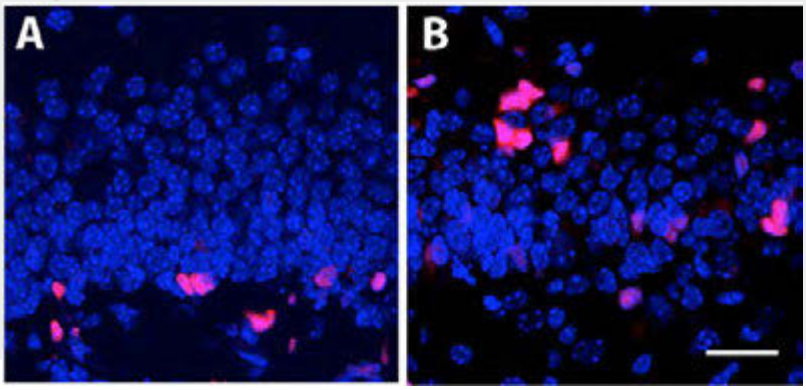

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Figure 1: *The Jenna mutant mouse. This mouse harbours a S140G mutation in the Tuba1a gene which results in defect neuronal migration during development. As a consequence the Jenna mouse is characterised by abnormal lamination of the hippocampus that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

Figure 2: *The developing hippocampus in the Jenna mutant mouse. TBR2 staining of dentate progenitor cells at birth in wild type mice (A) and the Jenna mutant mouse (B). This experiment revealed a disorganised subgranular zone in mutant animals, a result of abnormal migration in utereo.*

Figure 3: *Nathaniel Edelman operating the magnetoscope, which is able to identify cells based on their intrinsic magnetic moment.*

most notably birds, fish and bees. Previous studies have asserted that pigeons employ a magnetite containing sensory apparatus located at six specific loci in the subepidermis of the beak. We have shown that this dogma is false, and in reality clusters of iron-rich cells in the beak of pigeons are macrophages not magnetosensitive neurons (Treiber, Salzer et al. 2012). So where are the magnetosensory cells in avian species?

To answer this question we have build a microscope, the "magnetoscope", that produces a rotating magnetic field around a sample of interest. Following the dissection and trypsination of tissue, cells with their own magnetic moment can be identified as they produce a spinning behaviour. Employing the magnetoscope we have identified a range of morphologically distinct types of cells with magnetic properties. To ascertain the identity of these cells we have developed a panel of qPCR markers for single cell transcript analysis. This molecular characterisation is being complemented by an assessment of the sub-cellular architecture employing single cell transmission electron microscopy, and functional analysis employing calcium imaging. Our ultimate objective is to identify the molecules nature employs to construct a magnetoreceptor.

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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.

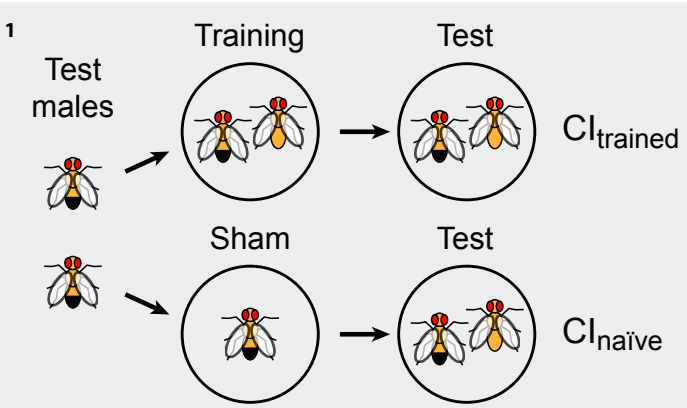
The learning paradigm we have chosen to investigate is courtship conditioning in *Drosophila*. This is naturally occurring form of learning in which males learn to choose appropriate females as courtship objects. Naïve *Drosophila* males court both mated and virgin females, yet only virgins will be receptive 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.

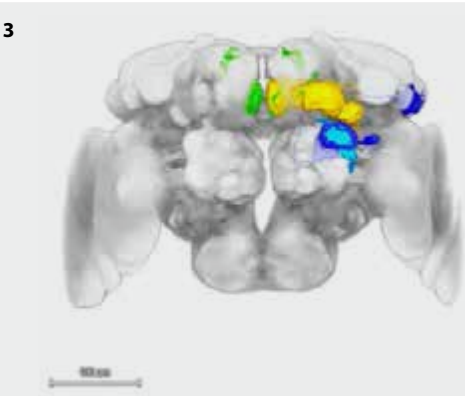
Molecular mechanisms of courtship memory

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].





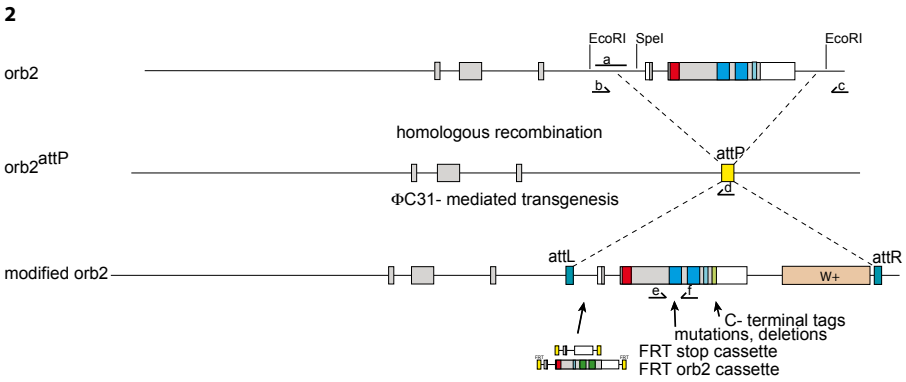


Figure 1: *Courtship conditioning. When tested with unreceptive mated females, males previously exposed to mated females court less than naïve (sham-trained) males (CI = courtship index; $CI_{trained} < CI_{naïve}$)*

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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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 as *Yersinia, Shigella, Pseudomonas, enteropathogenic/ enterohemorrhagic E. coli (EPEC/EHEC)* and *Salmonella* in animals and *Erwinia, Ralstonia* and *Xanthomonas* in plants employ type III secretion systems (T3S systems) for the infection process and are required for persistence inside the host. Human diseases in which type III secretion (T3S) is involved range from mild, such as diarrhea, to deadly, such as bubonic plague. T3S systems are multi-component macromolecular machineries that are usually encoded on specific pathogenicity islands (Figure 1). Their function is to inject proteinacous toxins, referred to as "effectors", into the host cell upon intimate contact. 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 make the host accessible to bacterial infection.

The Injectisome

The T3S system in Gram-negative bacteria has evolved to a complex molecular machine that achieves protein translocation across three membranes – the inner and outer membrane of the bacterial cell and the plasma membrane of the eukaryotic host cell. It consists of many components, its most prominent one being the needle complex, a large hetero-oligomeric membrane protein complex with a molecular weight of about 3.5 megadalton (Figure 2). The name stems from the needle-like protrusion visible in electron micrographs of whole bacterial cells. These protrusions are protein filaments that engage with the host cell and are believed to serve as a conduit for the secretion substrate. The needle filament is linked to the membrane-embedded basal body, which in Gram-negative bacteria spans the inner and outer membrane (about 30x30nm) including the periplasmic space. The basal body has a cylindrical shape, defining a central space within which the inner rod and the socket/cup are localized. The inner rod presumably connects the socket/cup with the needle filament and may help to stably anchor the filament into the basal body. Recently, our lab was the first to provide an experimentally validated map of the topology of the proteins within the complex (Schraidt et al 2010). We subsequently determined the structure of this large organelle to sub-nanometer resolution by cryo EM and single particle analysis (Schraidt & Marlovits, 2011). The structure serves as a model to further understand the structural determinants required for protein translocation across several membranes and thus bacterial infection, and may also be used to design small molecules that interfere with the assembly pathway.

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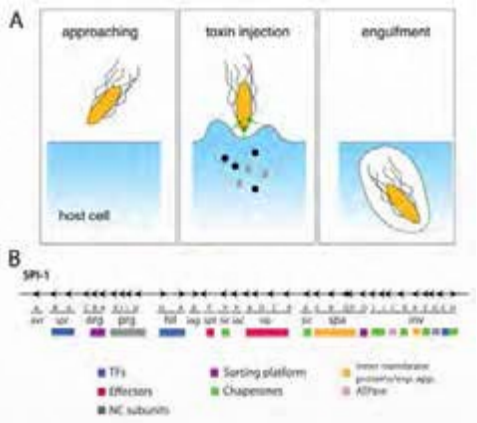

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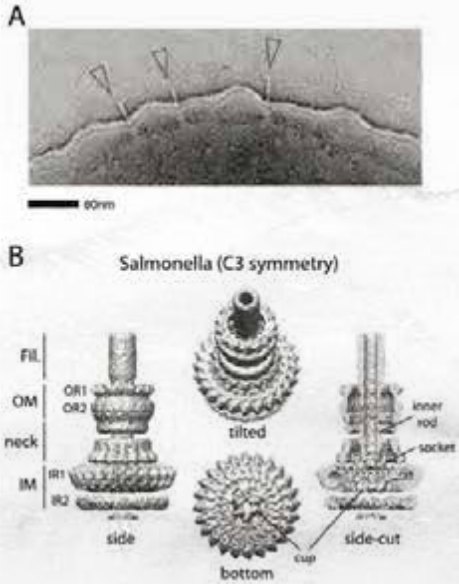

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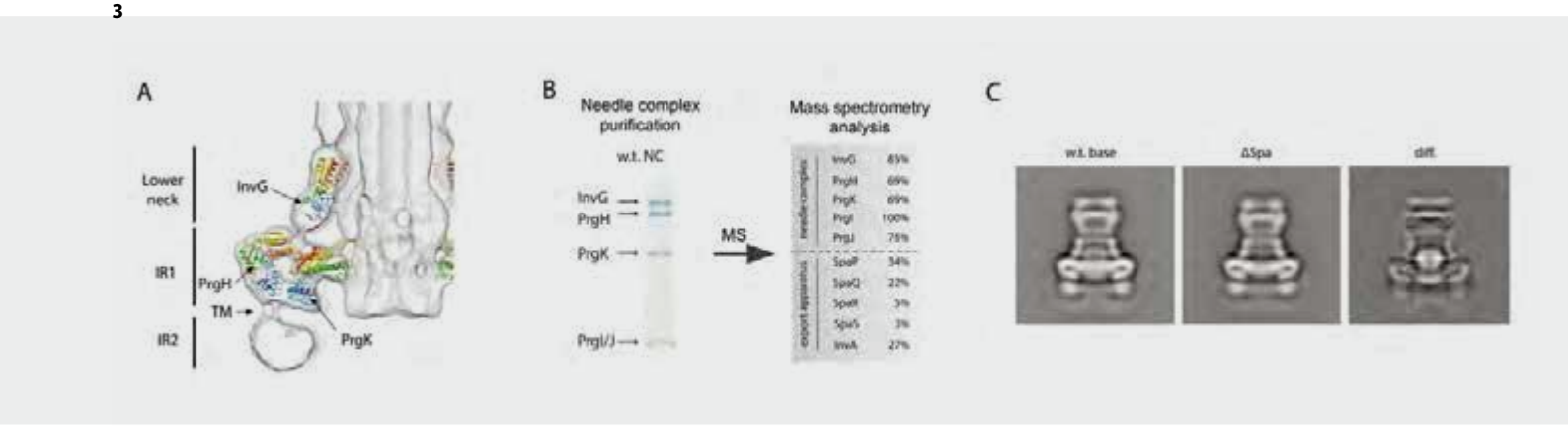

Figure 1: *Infection pathway and gene organization of pathogenicity island encoding for the Type-3 secretion system form Salmonella*

Figure 2: *Needle complexes of the Type-3 secretion system visualized by electron microscopy and after three-dimensional reconstruction by single particle analysis (OR outer ring, IR inner ring)*

Figure 3: *The export apparatus is located centrally. (A) Three dimensional reconstruction of the needle complex to sub-nanometer resolution and docking of atomic structures of individual protein domains reveal the presence of additional proteins located centrally. (B) Export apparatus proteins are found in isolated wild-type complexes by mass spectrometry. (C) Formation of the socket/cup is dependent on the presence of the export apparatus proteins (SpaPQRS, InvA). Single particle analysis of w.t. and ΔSpa bases reveal strong differences within cup and socket region.*

Controlled Steps during Assembly

The needle complex from *Salmonella* (SPI-1) is composed of multiple copies of approximately ten proteins (PrgH/K/I/J, InvG, SpaP/Q/R/S, InvA). A system at this level of complexity requires defined and controlled steps during the assembly. It is initially dependent on the cellular sec-machinery, in particular during the early ring-forming events of assembly. The export apparatus, a group of essential and conserved inner membrane proteins in T3S systems, plays a critical role during the initial phase of the NC assembly. It generates sub-complexes that may serve as nucleation points for the subsequent concentric ring organization of the two inner membrane rings (Wagner et al., 2010) (Figure 3) and is thus localized in the center of assembled needle complexes. In the past year, we have set out to understand the role of the individual members of the export apparatus using structural and biochemical approaches. We have learned that the very early steps of needle complex assembly require only three export apparatus proteins, all of which are essential to arrive at functional complexes. Due to its central position within complexes, we speculate that the export apparatus proteins may also play a role during protein transport. Thus, in the future we will address how substrates engage with the needle complex.
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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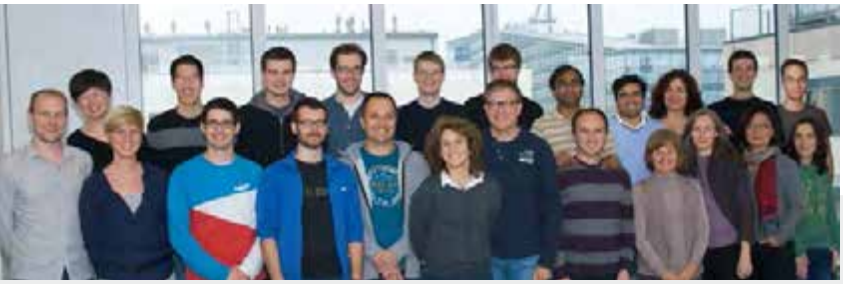

JAN-MICHAEL PETERS

Mitosis and chromosome biology

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[1] until February, [2] since February, [3] since March, [4] since April, [5] until May, [6] since June, [7] since July, [8] since September

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, enabling division of one cell into two genetically identical daugther 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. It is well established that the resulting cohesion is essential for chromosome segregation and for DNA damage repair, but how cohesion is established and maintained for many hours, or in the case of mammalian oocytes even for years, is poorly understood. We discovered recently that cohesin is converted into a 'cohesive' form that binds to DNA very stably by the protein Sororin, which associates with cohesin during DNA replication. Our data indicate that Sororin stabilizes cohesin on DNA by antagonizing a protein that can dissociate cohesin from DNA, called Wapl.

How does cohesin control chromatin structure and gene regulation?

Although cohesin is best known for its role in mediating cohesion, we and others discovered that cohesin has also important roles in gene regulation. We suspect that these functions are the reason why cohesin binds to chromatin already before cohesion is established and why cohesin associates with DNA even in postmitotic cells, which will never establish cohesion. We found that cohesin co-localizes in mammalian genomes with the transcriptional insulator protein CTCF and showed that cohesin is required for gene regulation at the imprinted *H19-IGF2* locus. Gene expression at this locus is believed to be controlled by formation of a chromatin loop which forms between CTCF sites specifically on the maternal allele. Our recent work indicates that cohesin is required for this chromatin interaction. Our future aims are to test if cohesin has a general role in forming chromatin loops, and to understand the mechanistic basis of this function.

How is sister chromatid cohesion dissolved during mitosis?

Sister chromatid separation in anaphase depends on the removal of cohesin from chromosomes. We discovered a number of years ago that this process depends on two mechanisms in vertebrate cells, the dissociation of cohesin from chromosome arms in prophase and the proteolytic cleavage of cohesin at centromeres in metaphase. The prophase pathway depends on the cohesin associated protein Wapl, whereas the metaphase pathway is mediated by the protease separase. Although the prophase pathway has been identified many years ago, its function and importance for chromosome segregation are still unknown. We have therefore generated a conditional Wapl "knockout" mouse to study the role of the prophase pathway *in vivo*.

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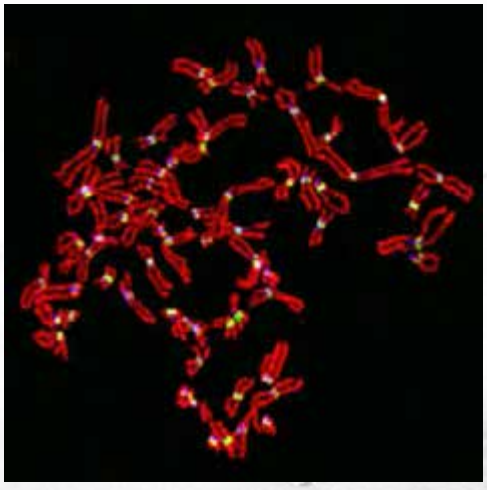

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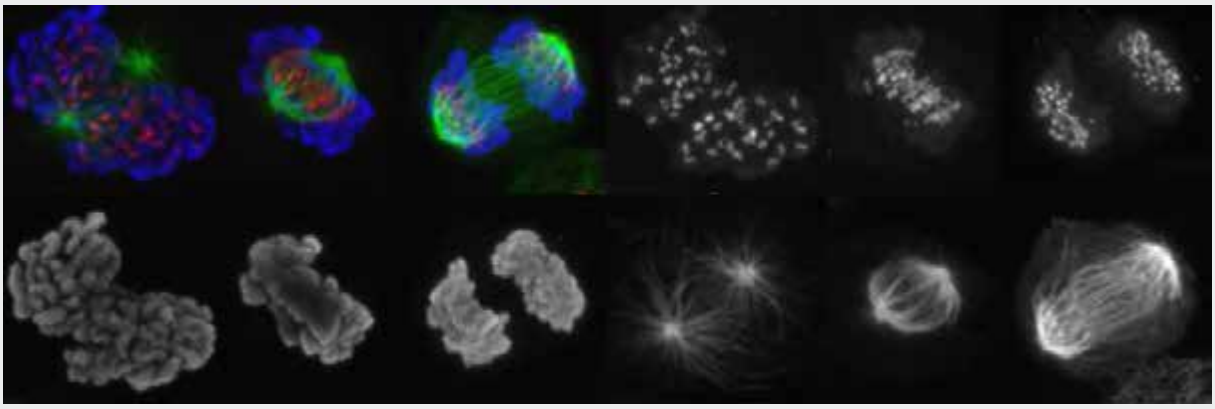

Figure 1: *Human mitotic chromosomes stained for condensin (red), cohesin (blue) and the centromere-specific histone Cenp-A (green). Courtsey of Peter Lenart.*

Figure 2: *Structure of human APC/C in different states. 'Inhibited', bound to the mitotic checkpoint complex (red); 'primed', bound to the coactivtor Cdh1 (red); 'active', bound to substrate (blue) and Cdh1. For details, see Herzog et al., Science 323, 1477-1481, and Buschhorn and Petzold et al., Nat. Struct. Mol. Biol. 18, 6-13, 2011.*

Figure 3: *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).*

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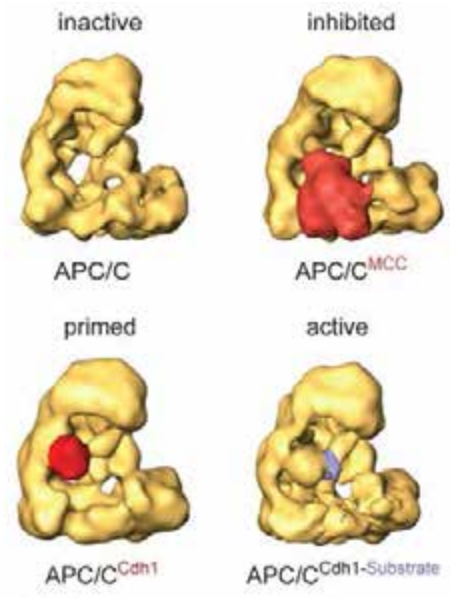

How does the APC/C initiate anaphase?

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 the 26S proteasome allows 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 crucial importance of the APC/C, it is poorly understood how this complex is inhibited by the SAC, how this 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 complicated process is far from complete. From 2004 to 2009, the MitoCheck consortium, funded by the European Union, has therefore developed and applied genomic and proteomic approaches to study mitosis. The consortium has used RNA interference screens to identify proteins required for mitosis in human cells, tagging of genes in bacterial artifical chromosomes (BACs) to enable the intracellular localization and affinity purification of these proteins, and mass spectrometry to identify protein complexes and mitosis-specific phosphorylation sites on these. This work has identified about 100 human protein complexes, many of which had previously not or only incompletely been characterized. Importantly, the approaches developed by MitoCheck will generally be applicable to high throughput analyses of other processes in mammalian cells. In a new project funded by the European Union, called 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 is focused on the development, function, and plasticity of neuronal circuits. Specifically, we would like to know how memory is stored over long periods of time. 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 multiple memory traces are coordinated, we are currently applying *in vivo* imaging techniques to the auditory cortex of mice. The auditory cortex mediates processing of sounds and is involved in the formation of memories of sounds.

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. We are currently investigating the impact of auditory learning paradigms on the dynamics of a given set of synapses in the auditory cortex.

In vivo imaging not only permits analysis of synaptic connections, but also 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 surprisingly strongly constrained into very few response modes. Interestingly, these discrete activity modes can 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. In the future we will investigate the circuit mechanisms that lead to the generation of sounds representations in discrete activity modes, and to what extent auditory learning paradigms cause changes in these neuronal representations of memorized sounds.

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 thus far.

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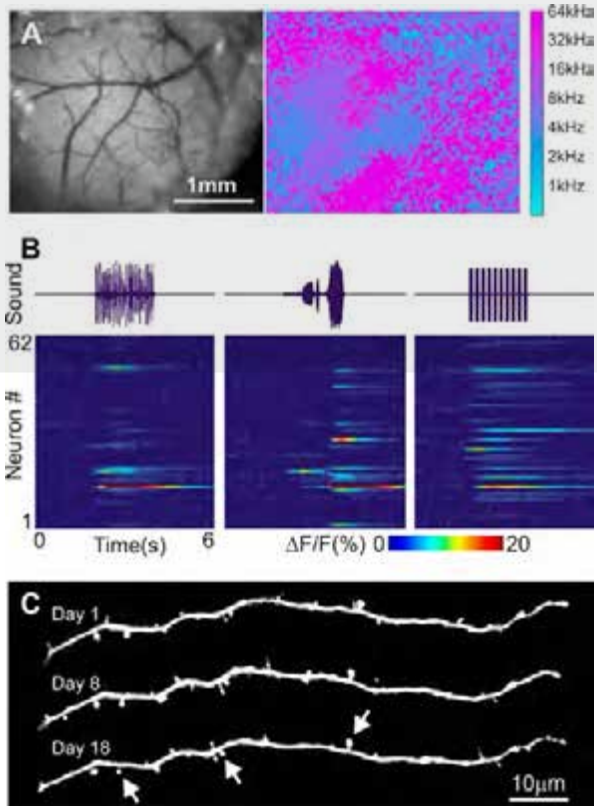

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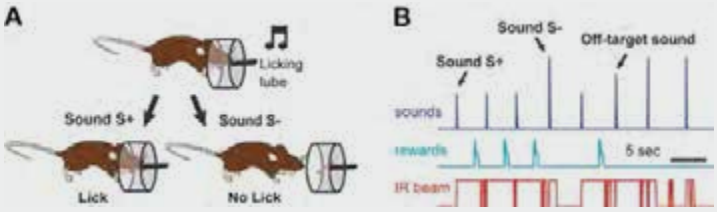

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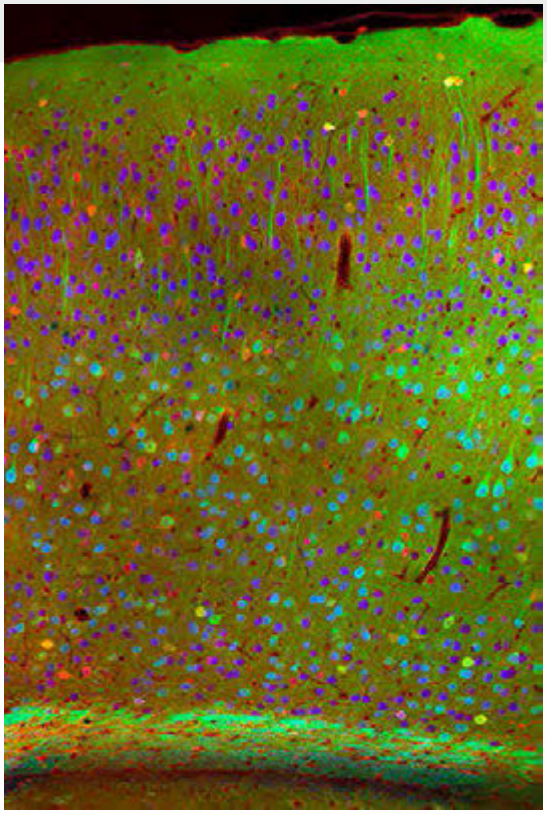

Figure 1: ***Imaging the auditory cortex.*** *(A) Mapping tonotopic organization of the auditory cortex using intrinsic imaging. (B) Using calcium imaging to monitor sound-evoked responses in multiple neurons simultaneously. Average population activity of 62 neurons in response to three different sounds. (C) Two-photon images of an individual neuronal process with synaptic contacts (so called spines, indicated by arrows) taken on different days. The connectivity of the neuron can be monitored by following the appearance, disappearance, and size of synaptic spines.*

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: ***Genetic approaches to photolabel individual neurons.*** *Expression of photoactivatable GFP (PA-GFP) permits photolabeling of neurons after functional characterization using calcium imaging. The figure shows a coronal section of the auditory cortex taken from a transgenic mouse expressing PA-GFP immunostained for a neuronal marker (NeuN, blue), inhibitory neurons (GABA, red), and PA-GFP (green).*

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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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1

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 (e.g. in reporter gene assays), but the exact requirements for enhancer function, i.e. a regulatory code, remains unknown and 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, i.e. in various tissues or at different stages of development. Typically, they bind to and 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*, i.e. the combinations of partner motifs that determine binding in each context.
For example, transcription factor binding during embryonic mesoderm and muscle development is highly stage dependent (Zinzen et al., 2009), and we find that many motifs are differentially distributed between binding sites at different stages (Figure 2) and that this differential distribution is predictive of stage-specific binding. We 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 are also determining the context-specific targets of the circadian clock factors (Meireles-Filho et al., in preparation) and homeobox (Hox) transcription factors.

In vivo and in vitro enhancer screens

Collections of enhancers that function similarly across cell-types would be an invaluable resource to study the sequence basis of enhancer activity. The Vienna-Tiles (VT) library established in collaboration with the Dickson lab and the VDRC consists of currently about 8000 transcriptional reporter constructs integrated at a single defined genomic position in transgenic *Drosophila* lines, each carrying a distinct ~2kb long candidate DNA fragment. We are determining the temporal and spatial enhancer activity of these fragments in transgenic *Drosophila* embryos by *in situ* hybridization against the reporter transcript. Among the first 4000 assayed and manually annotated enhancer candidates, we find an activity-rate of ~50% with diverse activity patterns throughout embryogenesis (Figure 1). Interestingly, the activity rate increases from ab out 10% in early to about 35% in late embryos, reflecting the increasing complexity of the embryo with increasingly many distinct tissues and cell-types. In a pilot study, we have found that enhancers additively contribute to the overall expression pattern of a gene. Groups of enhancers with similar activity can be predicted based on transcription factor occupancy or the enhancers´ DNA sequences using machine-learning approaches. We also found that highly-occupied target (HOT) regions function as transcriptional enhancers with diverse activity patterns (Kvon & Stampfel et al., 2012). We have also established a high-throughput screen based on next-generation sequencing to quantitatively assess enhancer activity in specific cell types (STARR-seq; Arnold et al., in revision). Its application to the *Drosophila* genome identified thousands of cell-type specific enhancers across a broad continuum of strengths, linking differential gene expression to differences in enhancer activity and drawing the first genome-wide

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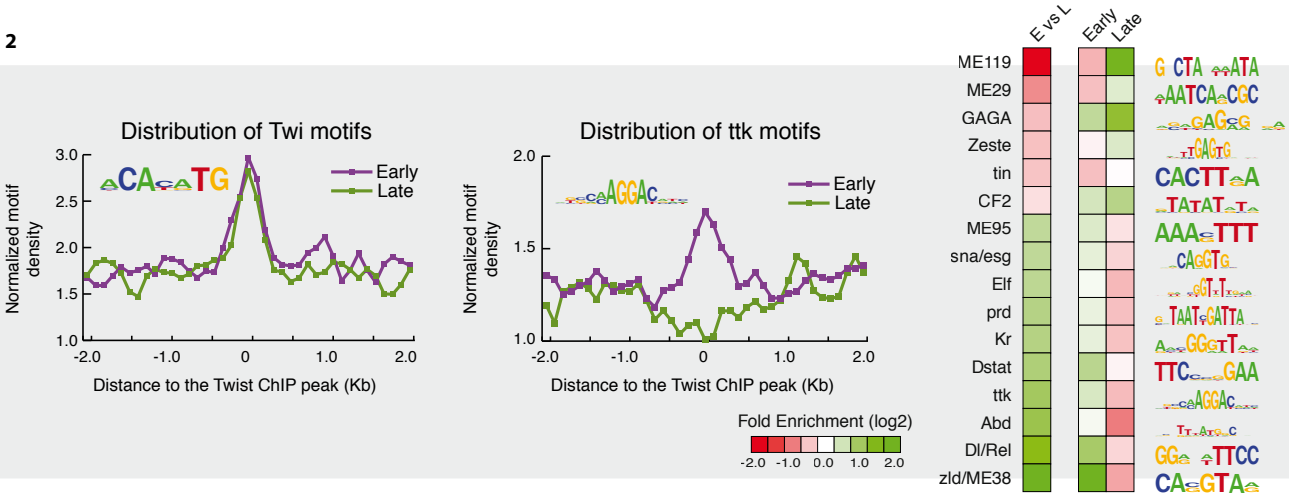

Figure 1: *Enhancer screen in transgenic Drosophila embryos. Shown is a sample of embryos that stain positively in our enhancer assay and display diverse activity patterns. Embryos are at different stages and shown in lateral orientation, anterior to the left. The inset shows a magnification of a blastoderm embryo with an enhancer activity signal in the dorsal ectoderm.*

Figure 2: *Motifs of Twist partner transcription factors (but not of Twist itself) are differentially enriched near Twist binding sites in the early (E) or late (L) Drosophila embryo. This differential enrichment is predictive for stage-specific binding of Twist (Yáñez-Cuna et al., 2012).*

Figure 3: *Conserved Twist binding at a functional enhancer in the tinman locus. D. melanogaster and 5 other Drosophila species at increasing phylogenetic distances show highly significant ChIP-Seq tag enrichment, indicative of positionally conserved binding (He & Bardet et al., 2011; Bardet et al., 2012).*

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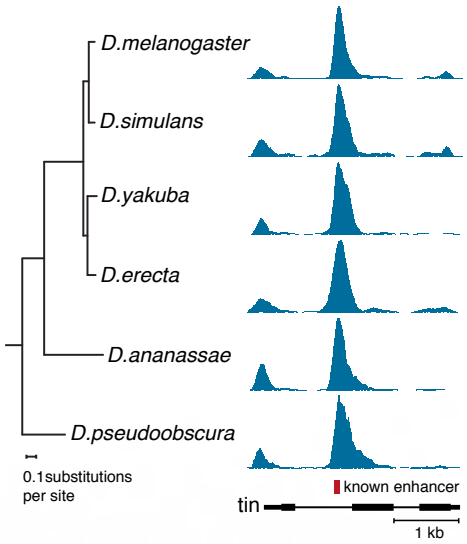

quantitative enhancer map in any eukaryotic cell. We are applying STARR-seq in several projects in both *Drosophila* and human and will analyze the identified enhancer sequences using bioinformatics and machine-learning tools.

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 (Figure 1) and to compare enhancer activity patterns with gene expression patterns obtained from BDGP (Tomancak et al., 2007). We have established a collaboration with the Christoph Lampert group (IST Austria) on image analysis. Clustering genes and enhancers by their spatio-temporal co-expression and intersecting transcription factor expression patterns will enable us to suggest regulatory interactions and integrate these data with sequence analyses.

Comparative genomics and the evolution of transcriptional regulation

Functional elements in a genome are typically under evolutionary selection to maintain their functions in related organisms. In collaboration with the Zeitlinger group (Stowers Institute), we study *in vivo* transcription factor binding sites in 6 *Drosophila* species at various evolutionary distances from *D. melanogaster* (Figure 3; He & Bardet et al., 2011). We find that transcription factor binding is highly conserved in species as distant from *D. melanogaster* as platypus or chicken from human. Conserved binding correlates with sequence motifs for Twist and its partners, permitting the de novo discovery of their combinatorial binding.

Comparative genomics and related bioinformatics approaches will permit us to integrate our data and knowledge to predict developmental enhancers, regulatory targets for transcription factors, and the expression patterns of genes.

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), and are collaborating with many experimental groups on campus and abroad.

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ANDREW STRAW

The neural basis of locomotory visual guidance in *Drosophila*

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The brain of a fly is capable of steering the animal through a complex environment at high relative speeds, avoiding stationary obstacles and moving predators. Because it is relatively easy to study how flies do this at several levels, from the behavioral to the cellular, fly vision has long been recognized as an ideal system to address a fundamental question in neuroscience -- how does the distributed activity of neurons orchestrate animal-environment interactions to result in successful coordinated behavior? We work on this basic question using techniques including automated realtime fly tracking, virtual reality displays, molecular genetic tools, and neuroanatomy.

A powerful toolkit to study neural basis of visual behavior

To enable our experiments, we use and develop virtual reality systems. In one such apparatus, freely flying flies are tracked in realtime by a multi-camera computer vision system. This unique technology allows rapid testing of physically unmanipulated and unrestrained flies to repeated presentations of arbitrary visual stimuli projected on the arena walls and floor. This device is used to perform experiments that allow the fly to exhibit a large portion of its natural behavioral repertoire while simultaneously measuring and limiting the effect of behavioral variability. Thousands of digitized 3D flight trajectories are collected in virtual environments with the capability of experimentally triggered events such as object disappearance.

Such behavioral experiments are combined with targeted genetic manipulation of the nervous system and analyzed to reveal the magnitude and reliability of effects. The activity of individually identified neurons is perturbed via transgenic expression of exogenous ion channels or altered synaptic machinery, and using statistical tests from the field of machine learning, the effects of these genetic manipulations on flight control are quantified. Ultimately, these techniques to control stimulus conditions and measure behavioral responses in detail allow us to show the contribution of individual neurons to behavior.

Mapping the visual circuits of the fly brain

Neurons in the fly visual system, especially the lobula plate tangential cells, have been used for decades as a model to study cellular neurophysiology including the basis of visual motion detection and spike timing precision, the behavioral role of most of these cells remains unclear. To link our knowledge of cellular properties with natural behavior, we are performing genetic ablations on small numbers of neurons and measuring behavioral deficits in response to precisely defined visual stimuli. To attain greater cellular specificity than allowed by the standard Drosophila GAL4-UAS system, we are making use of recent intersectional techniques such as split-GAL4 and alternative binary expression systems such as LexA. Flies with such manipulations are tested behaviorally in our virtual reality free flight arena, and the affected neurons are identified using immunostaining and confocal microscopy. The aim of this work is to establish a causative link between identified cells and their function in visual guidance.

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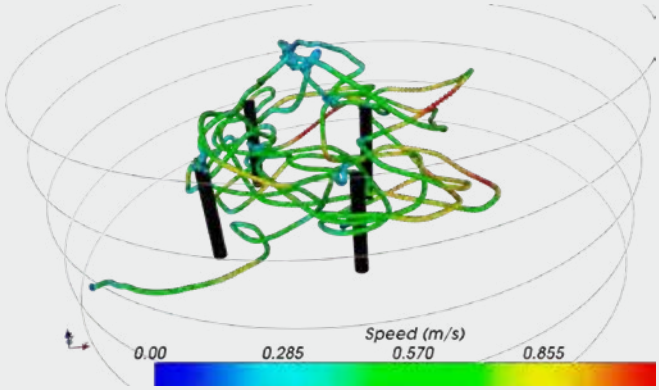

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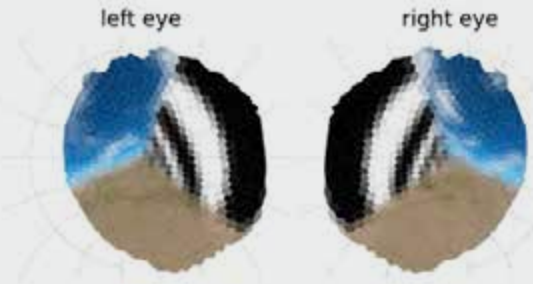

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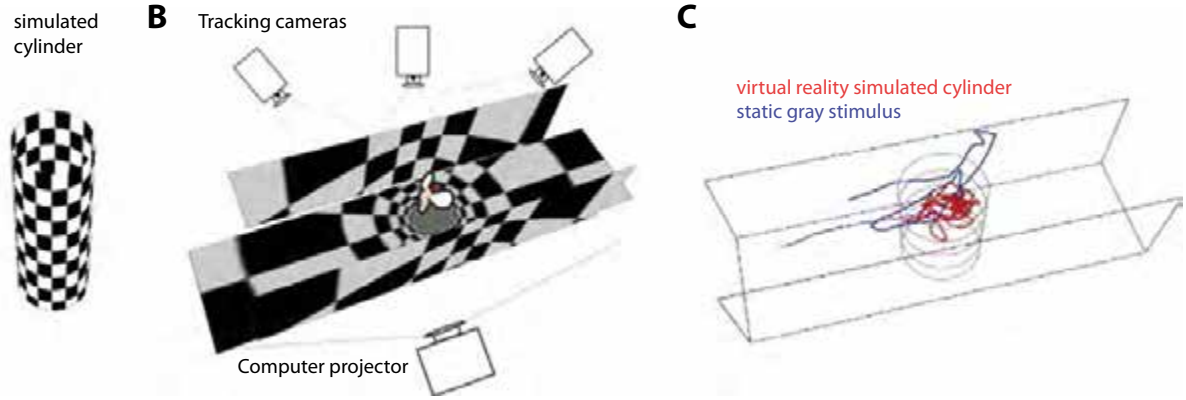

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Figure 1: *Top view of a 3D fly trajectory captured as a fly explored its environment, a 2 meter diameter cylinder and four vertical posts.*

Figure 2: *A simulated fly's eye view of a virtual world consisting of a black-and-white pattern on a desert landscape with a partly cloudy blue sky.*

Figure 3: *To perform virtual reality simulations, a simulated environment, in this case a cylinder made of a checkerboard wallpaper (A), is projected onto the walls and floor of a flight arena (B) such that, from the perspective of a freely flying fly, it is within this virtual object. The simulation is compelling enough to confine the flies within this virtual cylinder (C).*

Figure 4: *A virtual reality arena for tethered flight allows for high resolution measurements and stimulation.*

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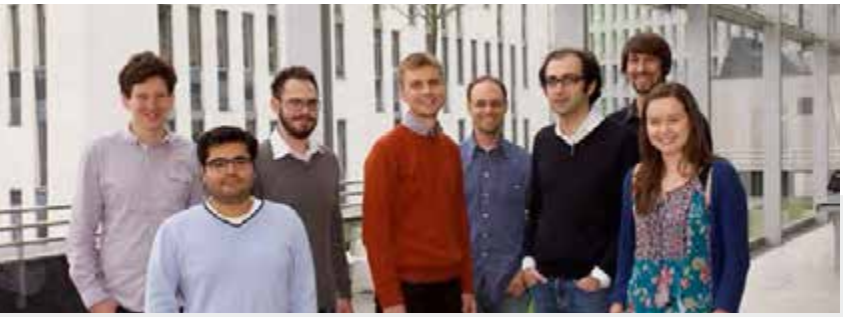

ALIPASHA VAZIRI

Multi-scale understanding of biological function

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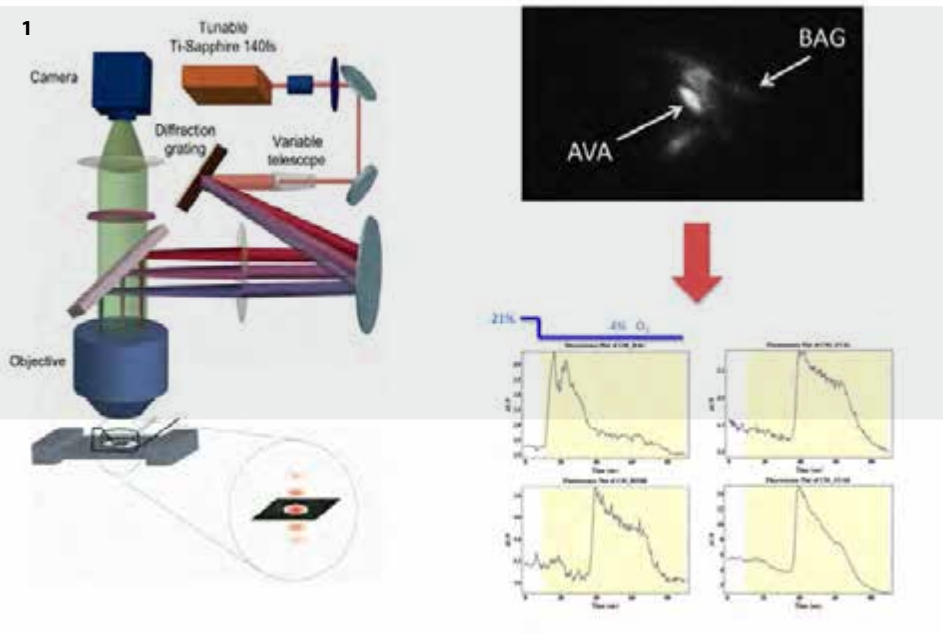
We aim to understand biological function on a multi-level scale. This includes the fundamental principles of protein and bio-molecular dynamics in nanoscale and dynamic interactions in cellular networks, such as neuronal networks, giving rise to macroscopic function and behavior at the organism level. The success of this endeavor will critically depend on the development of new techniques and approaches, which are one of the main fields of our research.

High-speed volumetric functional imaging of neuronal population activity:

The ability to stimulate and record neuronal activity in a fast, non-invasive, targeted and cell-type-specific fashion from large numbers of cells is essential to investigate a wide range of issues in fundamental neuroscience. Conventional two-photon scanning microscopy (TPM) provides the necessary spatial resolution, but is limited in terms of image acquisition speeds. We recently built a tool for high-speed functional volume imaging that uses a so-called "light sculpting" technique and overcomes some of the limitations of TPM. Our method is capable of following the activity of large neural populations with high spatiotemporal resolution. This method of 3D imaging was achieved with most recent state-of-the-art scientific imaging cameras, piezo-actuators, and ultrafast laser systems. We combined our technique with calcium reporters such as G-CaMP and voltage sensors in various model organisms. Ultimately this will permit correlation of behavior with the corresponding activity map of neuronal circuitry. Using the model system *C. elegans*, we aim to explain the role of sensory interneurons in oxygen sensing. We also plan to extend our techniques to larger neuron populations and combine them with optogenetic stimulation techniques using actuators such as channelrhodopsin (ChR2). We will investigate how sensory information is represented in neuronal networks, and study the mechanisms that underlie learning and storage of information in these networks.

Quantum coherence-assisted ion transport and selectivity in ion channels - We are interested in understanding the molecular mechanism responsible for high ion selectivity coupled with high transport rates in ion channels, and the potential involvement of vibrationally assisted coherent transport. Based on our recent theoretical model, we hypothesize that an interplay between coherence transport and environmental noise is responsible for extreme ion selectivity of the potassium channel and its high throughput rate. Our goal is to experimentally identify signatures of transient interactions of K^+ with binding sites of the selectivity filter during ion conduction. One of the approaches we use is to employ advanced spectroscopic techniques such as 2D infrared femtosecond spectroscopy (2DIR), which provides the necessary (picoseconds) time resolution to observe dynamic interactions of ions with carbonyls of the selectivity filter. In order to spectroscopically single out the signatures of these carbonyls out of the rest in the protein we produced $^{13}C^{18}O$ isotope-labeled amino acids. An approach based on *in vitro* amber suppression will be used to place them specifically inside the selectivity filter. In recent 2DIR spectroscopy of uniformly $^{13}C^{18}O$-isotope-labeled KcsA, we demonstrated the predicted red shift of $^{13}C^{18}O$ labels from the amide I band and observed changes due to interaction with K^+. One of the important steps of this project will be to perform 2DIR spectroscopy under non-equilibrium conditions. This will be done by K^+ uncaging and other strategies for synchronizing transport. Simultaneously, we are pursuing a complementary approach based on quantum resonances, which will be triggered and detected via a modified patch clamp setup.



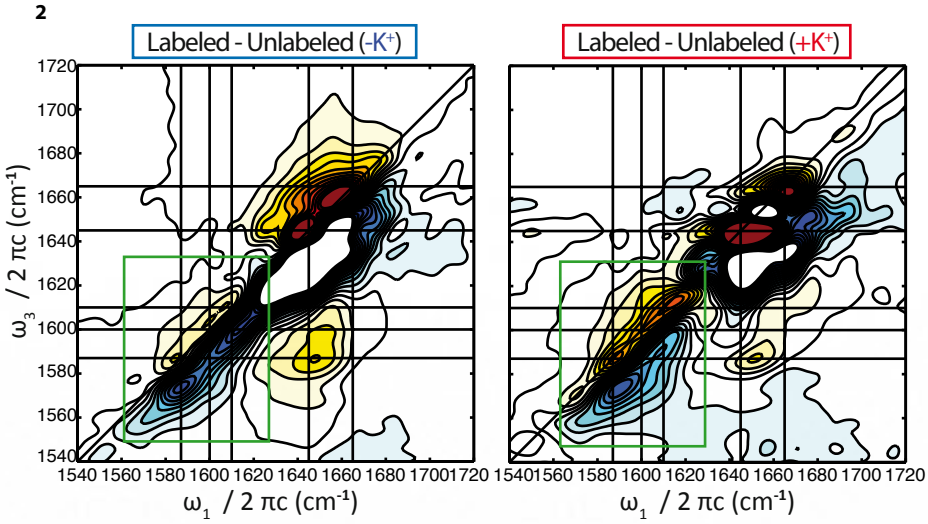


Figure 1: *Left: Schematic setup for temporal focusing microscope. Light sculpting permits imaging with a large field of view while retaining high axial resolution. This is a prerequisite for fast functional 3D imaging. Right: Typical Ca^{2+}-imaging signal (MIP) from the C. elegans interneurons. Exemplary traces of four selected neurons (BAG, AVA) are shown in the bottom panel.*

Figure 2: *Different 2DIR spectra of uniformly $^{13}C^{18}O$-isotope-labeled KcsA reveal unique signatures of K^+ binding in the red shifted region (green box), analogous to studies on K^+ binding model compounds.*

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STEFAN WESTERMANN

Molecular Mechanisms of Kinetochore Function

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Eukaryotic cells have evolved complex and extremely precise chromosome segregation mechanisms, which ensure that genetic information is passed 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.

Building a chromosome segregation machine

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 some important insights: For example, 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, to visualize the structure of the fully assembled interface and analyze how it is regulated, for example by mitotic kinases.

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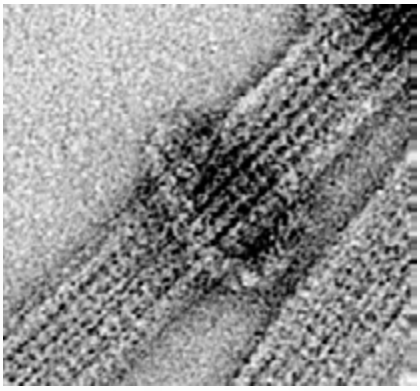

Engineering simplified chromosome segregation systems in the cell

To define functional modules within the complex kinetochore architecture we have taken a reductional approach to design simple kinetochores *in vivo*. By artificially recruiting individual kinetochore components to engineered binding sites on circular plasmids and on native yeast chromosomes we could demonstrate that the Dam1 complex is not only necessary but also sufficient to generate an interface that supports chromosome segregation (Figure 2). 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 so far remained elusive. Combining time-resolved analysis of post-translational modifications with yeast genetics should allow us to uncover general principles of regulation.

Analyzing the interaction of kinetochores with dynamic microtubules

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? Which features allow it to follow a polymerizing microtubule end, but 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

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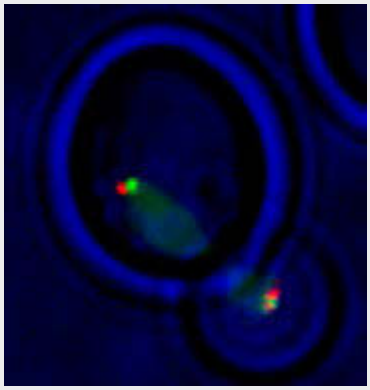

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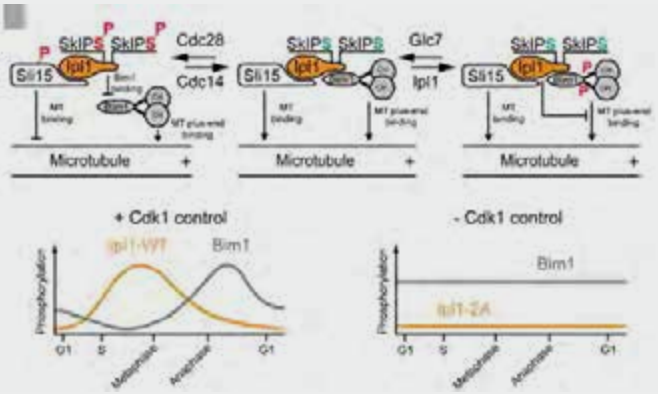

Figure 1: *The 10-protein Dam1 complex oligomerizes into a ring around the microtubule in vitro. Negative stain electron microscopy of a Dam1 ring decorating a taxol-stabilized microtubule.*

Figure 2: *Segregation of mini-chromosomes (green) during anaphase in a yeast cell. The spindle pole bodies are labeled with a red fluorescent protein (Spc42-mCherry). A brightfield image was pseudo-colored in blue to reveal the outline of the yeast cell.*

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 timely separated from Bim1 phosphorylation.*

observation of individual kinetochore complexes and microtubule-binding proteins with single-molecule sensitivity to reveal their mode of interaction with dynamic plus-ends.

Dissecting other microtubule plus-end interaction networks

While kinetochores are stable, 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, we ask how these changes in composition are established on a molecular level and what their functional consequences are. Among the proteins associated with microtubule plus ends are motor proteins with important roles in microtubule length regulation (Kip3) or kinetochore transport (Kar3). We are investigating the mechanisms by which these proteins are controlled to fulfill their functions.

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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 powerful worm genetics, quantitative behavioral assays, and functional neuronal imaging techniques to elucidate the precise neural circuits that control oxygen chemosensory behaviors.

Research activities:

An animal's decision as to how it should respond to changes in the environment is based not only on the available sensory information, but also on internal factors such as stress, sleep/wakefulness, hunger/satiety and experience. Small molecule neurotransmitters and neuropeptides in the brain modulate neural circuits according to these conditions, so that appropriate behaviors are generated. Aberrant neuromodulation is implicated in conditions such as insomnia, obesity, or anorexia. Given the complexity of most neural systems that have been studied thus far, we lack good models to investigate how neuromodulatory alterations systemically affect the activities of networks that generate behavioral outcomes. We use the simple model organism *C. elegans* as a tool to solve these questions.

Wild *C. elegans* live in soil, which is a very heterogeneous environment. As worms navigate, they are constantly challenged to evaluate their environment in order to determine the best survival tactic. The ability to locate food sources (bacteria) while avoiding pathogens, predators or other noxious conditions is far from simple. To optimize this search, nematodes have evolved a highly sophisticated repertoire of behavioral strategies. Oxygen chemotaxis is one such strategy. Local oxygen concentrations in soil range from atmospheric levels (21% O_2) to toxic hypoxia (<1% O_2), with soil bacteria creating a milieu of intermediate oxygen levels. When we present *C. elegans* with a range of oxygen concentrations in the laboratory, they show a homeostatic preference for these intermediate oxygen concentrations while avoiding both atmospheric and hypoxic conditions. Oxygen chemotaxis is regulated by various factors such as experience, nutritional status, and the genetic background of different strain isolates. It therefore serves as a tractable paradigm to study the modulation of the underlying circuits.

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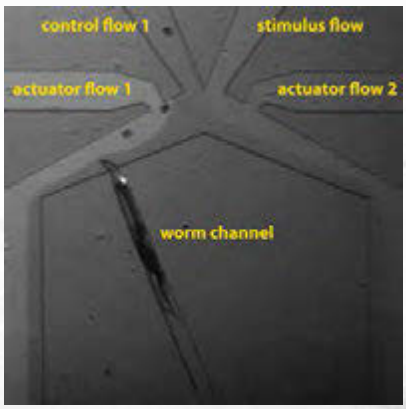

To study behavior, we are filming worm populations that experience downshifts and upshifts in environmental oxygen levels. Image processing and further computer analysis is used to quantify simple locomotion responses. To measure the neuronal activity of individual neurons, we use real-time fluorescence imaging of intracellular calcium levels. We employ microfabrication technologies to generate small microfluidic devices that allow the immobilization of worms onto microscope stages while the animals are being stimulated by chemical or gaseous stimuli (Figure 1).

Surprisingly, all behaviors observed in the assay can be explained by the action of just two sensory neuron classes in the entire worm brain: The BAG neurons cause deceleration of locomotion rate, which may persist for several minutes after oxygen concentrations drop to preferable levels. Conversely, when oxygen concentrations rise to undesirable levels, the URX neurons trigger very brief slowing responses that last for no more than a few seconds (Figure 2). Calcium imaging demonstrated that decreasing oxygen concentrations activate BAG neurons (Figure 3) while increasing oxygen concentrations activate URX neurons (Figure 3). A genetic analysis of behavioral responses and neuronal calcium signals

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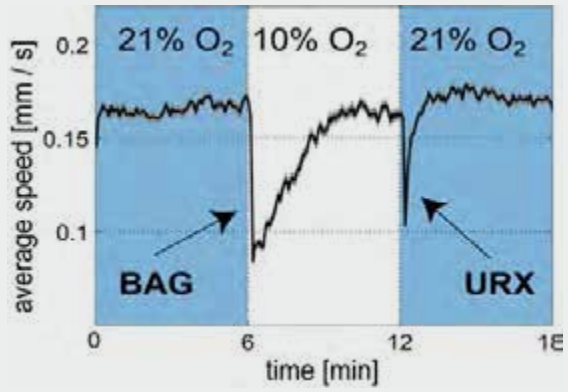

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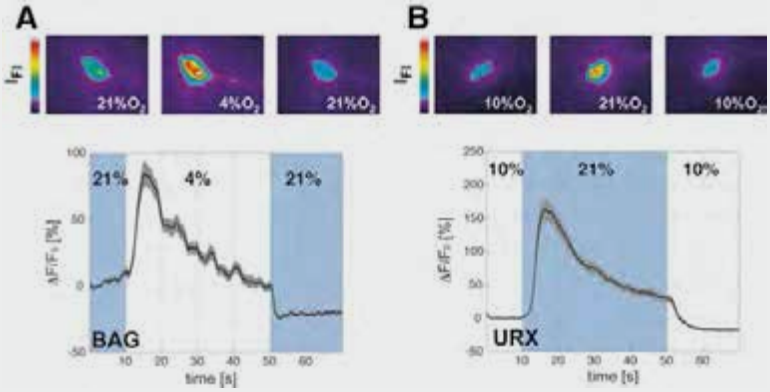

Figure 1: ***A microfluidic device for worm immobilization and stimulus delivery.*** *We designed a worm immobilization channel from an optically transparent silicon polymer. The channel is connected to a microfluidic delivery network. A worm is pushed into the immobilization channel so that its nose contacts the delivery network. Two actuator flows, which are labeled with a fluorescent dye, direct either a control flow or a stimulus flow (weak fluorescent label) to the worm's nose. The device allows for precise spatiotemporal control of chemosensory stimuli delivered to the worm's nose, while the animals can be imaged by microscopy (Chronis et. al., 2007).*

Figure 2: ***Analysis of oxygen chemotaxis behaviors.*** *Plotted is the average locomotion speed of animals exposed to changes in ambient oxygen concentrations, which shift between aversive atmospheric levels (21% O_2, blue shaded bars) and preferred intermediate levels (10% O_2). Following an oxygen downshift to 10% O_2, animals respond with sustained slowing. This behavior lasts for about 3 minutes. The response specifically requires BAG oxygen sensory neurons. Following an oxygen upshift to 21% O_2, the animals respond with transient slowing for a few seconds. This behavior specifically requires URX oxygen sensory neurons. (Zimmer et. al., 2009).*

Figure 3: ***Calcium responses in BAG and URX neurons.*** *A. The upper panels show fluorescence images of the calcium indicator G-CaMP expressed in BAG. Oxygen concentrations are indicated. The trace shows the average calcium response in BAG to O_2 downshift (21% O_2 to 4% O_2). B. The upper panels show fluorescence images of the calcium indicator G-CaMP expressed in URX. Oxygen concentrations are indicated. The trace shows the average calcium response in URX to O_2 upshift (10% O_2 to 21% O_2).*

showed that the reciprocal chemosensory properties of BAG and URX are the result of differentially expressed molecular oxygen sensors of the soluble guanylate cyclase family (Zimmer et. al. 2009).

These behavioral paradigms and imaging technologies are ideal tools to study the neuromodulation of circuits and behavior. Oxygen downshift and upshift elicit two distinct slowing responses that can be assigned to the activities of just one sensory neuron in each case. Thus, behavior can be studied in a robustly quantifiable manner at single cell resolution. Moreover, the imaging technologies we developed are tailor-made to mimic conditions under which behavioral experiments are performed. Thus, behavioral responses and neural activity can be directly correlated. BAG and URX share a small neural circuit of postsynaptic interneurons. The simplicity of this system, powerful worm genetics, and tractable behavioral and physiological assays, enable us to study the neuromodulation of circuits at all levels; i.e. at the level of networks, single cells, as well as single genes and molecules.

We are currently focusing on the following goals:

- To elucidate the precise functions of interneurons that connect to BAG and URX.
- To determine the mechanism by which neuropeptides mediate experience-dependent modulation of behavior.
- To investigate the mechanisms by which neural circuits integrate sensory information with other external and internal conditions, such as the availability of food and the nutritional status.

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JOHANNES ZUBER

Finding and probing cancer drug targets using advanced *in-vivo* RNAi

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The genetic complexity and heterogeneity of human cancers pose a daunting challenge for the development of effective targeted therapies. The advent of RNA interference (RNAi) has provided a new tool to reveal cancer-specific vulnerabilities in a systematic and unbiased way. Our lab uses an integrative approach combining genetically engineered cancer models and advanced in-vivo RNAi technologies to discover and evaluate new therapeutic targets in acute myeloid leukemia and other cancers.

Acute myeloid leukemia (AML) accounts for 80% of adult leukemias and remains the most common cause of leukemia death. Comprehensive genome analyses have identified over 100 driver mutations, which occur in complex, heterogeneous and dynamic patterns. To dissect this genetic complexity and accelerate the search for more effective therapies, our lab combines experimentally tractable AML mouse models and miR30-based Tet-on regulatable RNAi technology to systematically identify genetic dependencies through multiplexed screening and functionally evaluate identified candidate genes in established leukemia in vivo (Fig. 1).

BRD4 – a new therapeutic target in AML

One gene class that has recently emerged as promising therapeutic target space are epigenetic regulators, which modify and interpret chromatin states to control leukemia cell fate and identity. Leukemia cells are characterized by global alterations in the chromatin landscape, which are thought to provide great therapeutic opportunities, since epigenetic aberrations are reversible and controlled by a machinery that is amenable to drug modulation. In a first application of our new screening system we have recently analyzed dependencies on 243 chromatin-associated genes in an MLL-AF9;Nras^{G12D}-driven mouse model of AML (Fig. 2). As the top hit, our study identified the epigenetic reader BRD4 as a promising therapeutic target in a wide range of AML subtypes, and implicated small-molecule BRD4 inhibitors as the first pharmacologic approach to suppress the MYC oncogene in cancer. Meanwhile, several studies have confirmed the potent anti-leukemic effects of BRD4 suppression in AML, and second-generation BRD4 inhibitors are expected to enter clinical trials in 2013. Our current follow-up studies are focused on exploring mechanisms underlying sensitivity and resistance to BRD4 inhibition in different leukemia subtypes.

Systematic analysis of chromatin dependencies in AML and other cancers

Following the establishment and first successful application of our screening approach in one AML model, a major aim of our lab is to systematically explore chromatin-associated dependencies in other cancer types using a more comprehensive chromatin-focused shRNA library. To produce this key reagent, we teamed up with the Molecular Biology Service and established a deep-sequencing-based shRNA cloning pipeline, which enables the rapid production of customized shRNA libraries in a sequence-verified single clone format. In a first application, we employed this pipeline to expand our chromatin-focused shRNA library, which now covers all known chromatin-associated genes (520 genes, 2500 shRNAs). In parallel, we have established a panel of Tet-on competent leukemia models based on recurrent human mutations, which we are applying in comparative multiplexed RNAi screens to identify context-specific dependencies in different leukemia subtypes. In addition, we are expanding these screens to Tet-on competent mouse models of pancreatic

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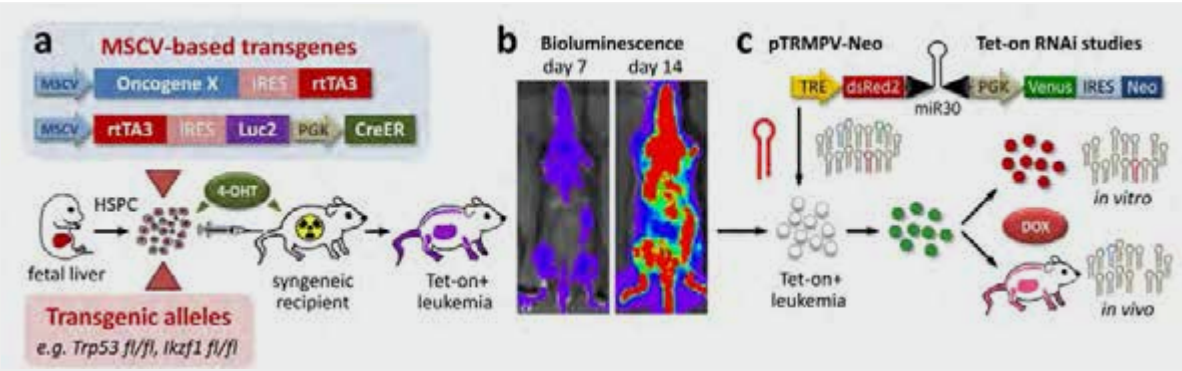

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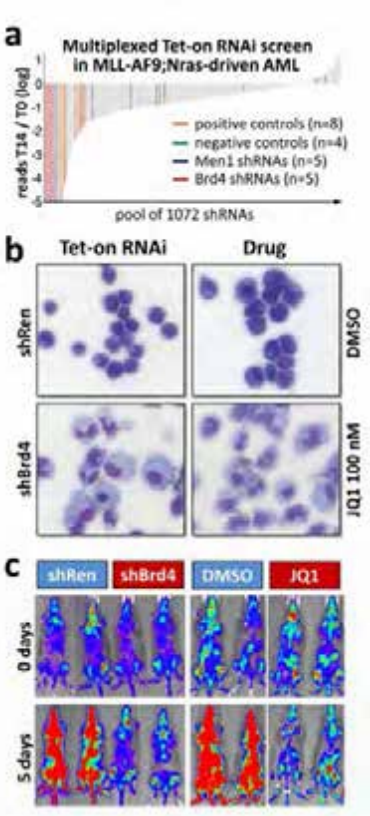

Figure 1: *Generation and use of Tet-on competent mouse models of leukemia.* ***a****, Hematopoietic stem- and progenitor cells (HSPC) of wildtype or transgenic mice are co-transduced with MSCV-based vectors co-expressing different combinations of oncogenes, rtTA3, Luc2 and CreER$_{T2}$.* ***b****, Bioluminescent imaging of HSPC transplant recipient mice at indicated time points post transplantation.* ***c****, Subsequent application of established leukemia models in Tet-on RNAi studies following transduction of pTRMPV-based shRNAs (single or pools). In multiplexed screening the abundance of individual shRNAs is analyzed using deep sequencing of shRNA guide strands; shRNAs required for proliferation and/or survival of leukemia cells in vitro (blue, green) or in vivo (red, green) are identified based on their depletion from the pool.*

Figure 2: *Multiplexed RNAi screening identifies BRD4 as a candidate target in AML.* ***a****, Depletion plot of changes in shRNA representation before (T0) and after 14 days of dox-induced shRNA expression (T14).* ***b****, Cytospins of MLL-AF9;Nras^{G12D} leukemia cells 48 hours after Brd4-shRNA induction or treatment with the BRD4 small-molecule inhibitor JQ1. In both cases, Brd4 suppression triggers terminal differentiation of leukemic blasts into macrophage-like cells, demonstrating that RNAi accurately predicts drug effects.* ***c****, Bioluminescent imaging of mice transplanted with MLL-AF9;Nras^{G12D} AML with or without five days of Tet-on RNAi- or JQ1-induced Brd4 suppression.*

ductal adenocarcinoma (PDAC), which we have developed in a collaborative project. Together, we envision that these comparative RNAi screens will generate a comprehensive functional-genetic dataset of chromatin-associated dependencies in cancer, which will greatly complement ongoing genome and epigenome profiling studies and ultimately guide the development of new targeted therapeutics in the clinic.

Exploring other therapeutic targets in leukemia

Besides our focus on chromatin regulators, we are also employing our established AML models to search for vulnerabilities in other gene classes. In a recently completed screen we have analyzed 442 "drugged genes" (for which tool compounds are available) and 506 MLL-AF9-regulated genes for their requirement in MLL-AF9;Nras^{G12D}-induced AML and mouse embryonic fibroblasts (MEFs). This study identified several druggable leukemia-specific dependencies involved in diverse cellular processes including basic metabolism, cell cycle regulation and hormone signaling, most of which have not been previously implicated as putative therapeutic targets in cancer. Another project focuses on the identification and functional characterization of key genes involved in establishing and maintaining aberrant self-renewal in myeloid progenitors, which defines a hallmark event in leukemogenesis that holds great promise for the development of targeted therapies.

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BIOOPTICS FACILITY

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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 an intense "Advanced Practical Microscopy Course" is organized on a bi-annual base including hands-on sessions as well as lectures by internal and external faculty.

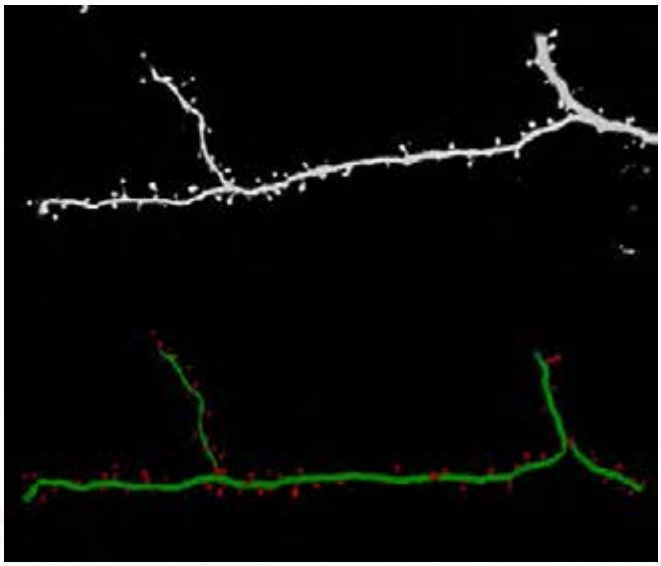

Image Processing and Analysis

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: *Samples of a transgenic mouse brain containing sparse GFP expressing neurons were immunohistochemically stained for synaptic proteins. 3D image stacks were acquired using a confocal microscope. The purpose of the image analysis was to automatically detect GFP labeled dendrites and its spines within multiple 3D volumes and extract morphological parameters from them, including volume, size, distance to dendrite, as well as the amount of labeling for the synaptic proteins. The top shows a maximum intensity projection of the original data, the bottom a 3D rendering of the classified neuron in green and the spines in red.*

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.

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.

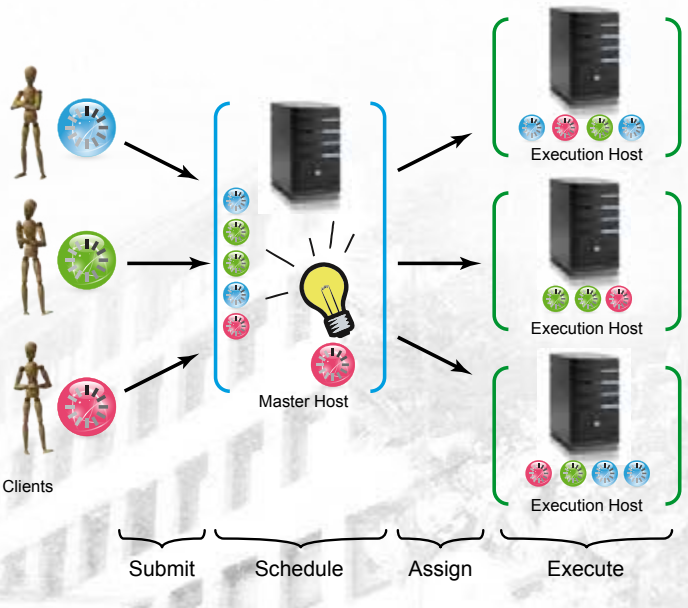

Figure: *The IMP/IMBA high-performance computing (HPC) cluster. Users may submit jobs for dedicated clients to the Sun Gridengine (SGE) master, which is responsible for running the jobs on the cluster nodes.*

PROTEIN CHEMISTRY

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Karl Mechtler / Head of Facility

Elisabeth Roitinger / Deputy Lab Manager
Christoph Jüschke [1] / Postdoc
Thomas Köcher / Postdoc
Nikolai Mischerikow / Postdoc
Peter Pichler/ Postdoc
Johannes Stadlmann / Postdoc
Werner Straube / Postdoc
Gerhard Dürnberger / Bioinformatician
Debora Broch-Trentini [2] / Phd Student
Frederico Dusberger / Master Student
Thomas Taus / Master Student
Johannes Fuchs / Technical Assistant
Otto Hudecz / Technical Assistant
Richard Imre / Technical Assistant
Gabriela Krssakova / Technical Assistant
Mathias Madalinski / Technical Assistant
Michael Mazanek / Technical Assistant
Susanne Opravil [3] / Technical Assistant
Michael Schutzbier / Technical Assistant
Ines Steinmacher / Technical Assistant
Anna Elisa Gattinger / Trainee
Dominik Mayer / Trainee

[1] *in cooperation with Knoblich lab*
[2] *in cooperation with Clausen lab*
[3] *in cooperation with Westermann lab*

Stoichiometry of Protein Complexes

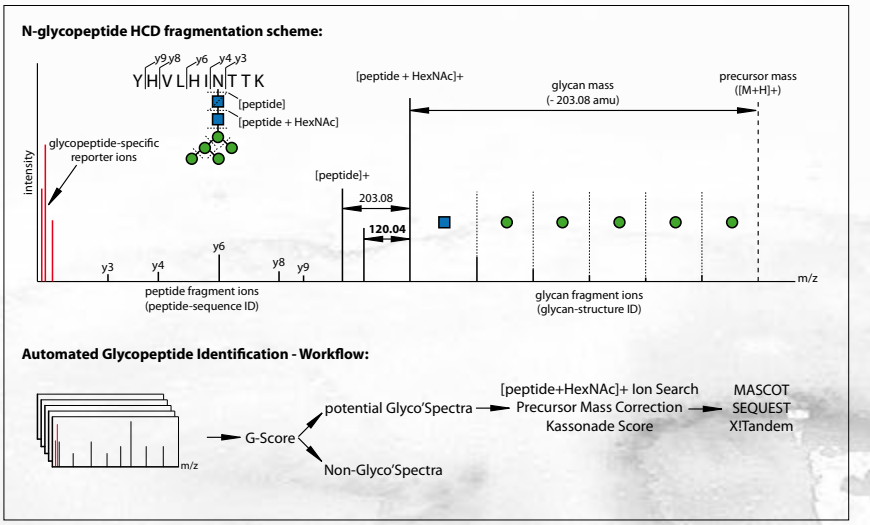
Quantitative mass spectrometry using stable isotope labeling has been extensively used in biology to quantify relative changes in proteomes. In conjunction with internal standard peptides or proteins, quantitative mass spectrometry can be used to determine absolute quantities of selected proteins in biological matrices. We have established a spectroscopic method for the production of LC-MS-compatible internal standard peptides that permits their accurate quantification in a fast and highly parallelized fashion. In collaboration with the Peters and the Westermann groups, we will apply this technique to determine cellular copy numbers of a range of mitotic proteins and determine the stoichiometries of protein complexes involved in mitosis.

Localization of post-translational modifications

Post-translational modifications (PTM) extend the functional range of a protein by attaching different chemical groups. In addition to the identification of a protein, localization of its modifications is an important and challenging task in proteomics. We developed an algorithm which permits localization of *any PTM*. It can be used for all types of peptide fragmentation modes. The algorithm assigns to each possible modification site its modification probability. This, we believe, will provide a better understanding of the functional value of a protein.

Glycoproteomics of mouse embryonic stem cells:

Glycosylation of proteins by the covalent attachment of carbohydrate structures onto amino-acid side chains is an abundant, yet functionally less characterized group of post-translational modifications. Due to the structural complexity and unfavorable fragmentation behavior of glycopeptides, current mass-spectrometric (MS) approaches to glycoprotein identification typically remove the carbohydrate portions prior to analysis. As a result, these commonly employed MS-based glycoproteomic strategies do not contain information on glycan structures. In order to provide high-confidence glycoprotein identifications as well as site-specific glycan structure information from complex biological samples, we developed a new glycoproteomic workflow. For automated identification of glycopeptides from such large scale high-mass accuracy MS/MS data sets we developed novel software tools, including species-specific glycan structure databases and spectral interpretation algorithms.



In collaboration with the Penninger group we apply this approach to ricin-resistant mouse embryonic stem cell lines and aim to identify cell surface proteins potentially mediating ricin binding and uptake by these cells.

Figure: *Glycoproteomic workflow. Based on the fragmentation behavior of glycopeptides in HCD, our software tools automatically identify and process MS/MS spectra of glycopeptides from large-scale datasets and provide both, peptide sequence identification using standard MS/MS Ion Search Engines (i.e. MASCOT, X!Tandem, SEQUEST) as well as glycan structure information.*

HISTOLOGY

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The Histology Service Department offers state-of-the art sectioning, histology and immuno-histochemistry services to all IMBA and IMP scientists. In addition, we offer training and support for researchers in new technologies.

Vukoslav Komnenovic / Head of Facility

Mihaela Zeba[1] / Technical Assistant

[1]*on maternity leave*

Histology Services

The histology services include the embedding of tissues, assistance with the sectioning of paraffin and cryo-preserved tissues, and preparation of slides for standard H&E stains, as well as specialized stainings such as PAS, Alcian blue, Cab, Gomeri, MayGruenwald-Giemsa and van Kossa stains for human, mouse, *Xenopus* and *Drosophila* studies. With these services, we are able to offer support to get quick results.

Sectioning of Paraffin and Frozen Tissues

In our group we have developed a high throughput method to cut paraffin and frozen tissues. Using this method, we could increase the quality and also the quantity of services.

Immunohistochemistry

The Histology Service Department also provides automated preparation and processing facilities for standardized immuno-histochemistry, in situ hybridization, FISH analysis, DNA microarray and tissues microarray applications. Various antibodies have been characterized for optimized in situ signaling studies. Standard antibodies such as apc, cd (several markers), l-ad, gfp, gfap, c-fos, c-jun, junB, fra1,2, jun-D, ki67, smad3, brdu, egf, egfr, H3K9me tri meth, H4K20me3 tri meth, cl. caspase3, caspase7, procatepsiK are available.
In addition, the Histology Service Department administrates legal regulatory affairs such as the record-keeping and documentation of experiments in accordance with the Austrian Histolaboratories guidelines (www.mta-labor.info).

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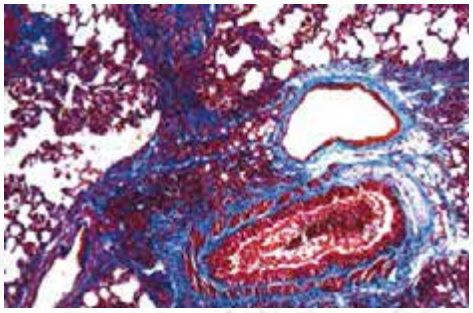

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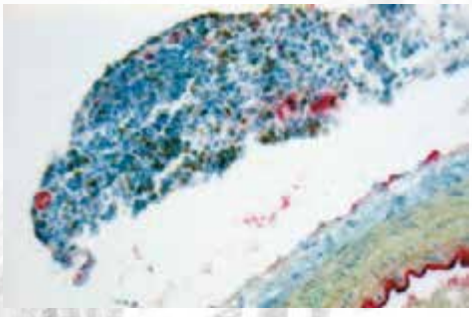

Figure 1: *Trichrom blue staining. The picture shows 4 different signals, light blue for Lung fibrosis, red for Mast cells and Neutrophils, pink for Myeloiud cells and dark blue for the Nuclei.*

Figure 2: *Double Staining with Ki67 and von Willebrand Factor. Ki67 turnes out to be brown whereas vWF shows a red signal. The counter staining was done with Hematoxylin (blue).*

1

2

COMPARATIVE MEDICINE

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TRANSGENIC SERVICE

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

Figure 2: *Mouse blastocysts.*

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.

MOLECULAR BIOLOGY SERVICE

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Sanger Sequencing Laboratory, formerly the General Service Department, is a part of newly formed Molecular Biology Service Facility. Sanger Sequencing Lab was shifted from the ground floor to the plaza floor during the summer, and joined the rest of the facility at this site.
The fly food unit and the media kitchen are now independent units.
The facility offers a variety of high-quality rapid services to IMP, IMBA and GMI scientists. DNA sequencing, preparation of competent cells strains, production of antibodies, lab automation, and various machines for molecular biology analysis are some of the tasks performed at our facility. We keep a stock of commonly used sequencing primers and competent *E. coli* strains.

Martin Radolf / Head of Facility

Harald Scheuch / Engineer
Ivan Botto / Technical Assistant
Elisa Hahn / Technical Assistant
Markus Hohl / Technical Assistant
Sabina Kula / Technical Assistant
Caroline Schuster / Technical Assistant
Zuzana Dzupinkova / Sanger Sequencing

Sanger Sequencing

The facility works with 2 DNA genetic analyzers: ABI 3730 (48 capillaries) and ABI 3730XL (96 capillaries). We sequenced approximately 140,000 samples during the first ten months of this year. The high demand was mainly due to screen projects, the fly library, and the new groups at IMP, IMBA and GMI. The facility maintains approximately 30 different strains of *E. coli* competent cells. We usually prepare DH5α competent cells for all 3three institutes, and the others on request from scientists.

Lab Automation

We recently acquired another Agilent Bravo, which enables scientists to use three Agilent Bravo96 channel liquid handling stations. The main uses of the machines are automated in situ hybridization together with the Stark group, 384 real-time PCR set up, PCR clean up for Sanger sequencing, automated plasmid preparation, generation of single-clone validated oligonucleotide-based libraries->production of custom designed shRNA in cooperation with the Zuber Lab. This robotics-supported method utilizes a DNA barcoding strategy to sequence verify complex libraries involving thousands of clones in a single deep-sequencing experiment. We first used the method to establish a single-clone validated murine shRNA library targeting all 450 known chromatin-associated genes. Following amplification and bulk cloning of 1,500 on-chip synthesized shRNA-encoding oligos, we deep-sequenced 18,000 bacterial clones and were able to retrieve 1,300 shRNAs in a validated single clone format. Following its introduction, the method is currently being used to produce additional focused shRNA libraries which, in contrast to commercially available RNAi reagents, can be highly customized and adapted to most recent design rules and optimal expression vectors. Another robotic platform we offer our users consists of two Biotek Precision XS liquid handling machines. These are small and flexible 8-channel or single-channel robots which can be used in a cell culture hood to pipette repetitive workflows. Precision XS can also be used for hit picking projects. We use the Xiril 100 for preparation of genomic DNA from single fly, and also for plasmid preparation.

Molecular Biology Services

In cooperation with the Nordborg Group from GMI we designed a 384 SNP Illumina assay that can be used as a "universal" genotyping panel for Arabidopsis. We employ Illuminas Custom VeraCode GoldenGate Genotyping kits to generate SNP-specific PCR products that are subsequently hybridized to beads. This enables us to simultaneously genotype 384 loci in a single well of a standard 96-well microplate. In 2012 we were able to analyze about 2700 samples.

This year we started to perform mycoplasma tests on a regular basis. The tests are based on the bioluminescent reaction of luciferin, oxygen and ATP in the presence of luciferase which can be analyzed with our fluorescence plate readers Synergy2 and Synergy H1. The presence of ATP is an indicator for mycoplasma contamination. We also established a PCR protocol to detect mycoplasma contamination.

MAX PERUTZ LIBRARY

library@imp.ac.at

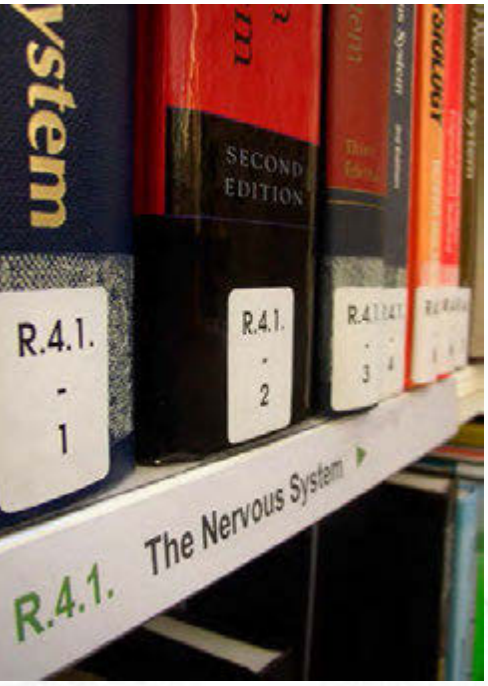
Karlo Pavlovic / Librarian

Jürgen Sallachner / 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 libra ry 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

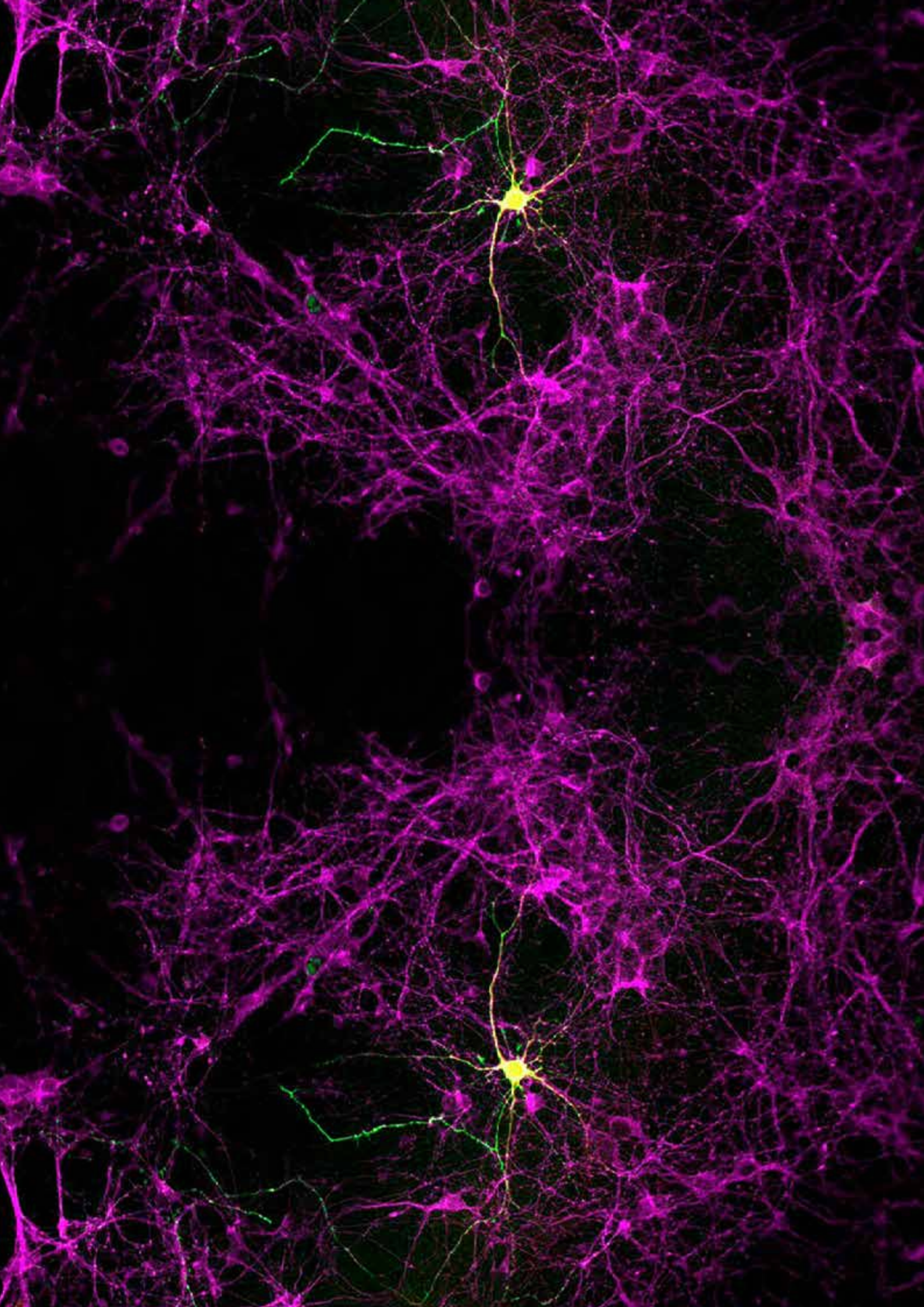
The reading room serves as a quiet and well-equipped place for reading, writing or just relaxing. Six study desks and a cozy lounge as well as two public computers, wireless LAN and a printing facility are provided.

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

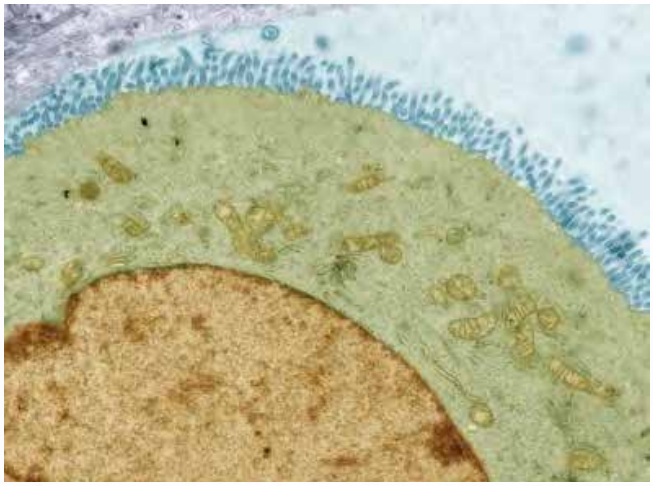
The Campus Science Support Facilities GmbH (CSF) was established in 2011 at the Campus Vienna Biocenter to provide top scientific infrastructure operated and constantly further developed by highly qualified experts. The CSF supports IMP, IMBA and other institutions and companies situated on the Campus 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

ELECTRON MICROSCOPY

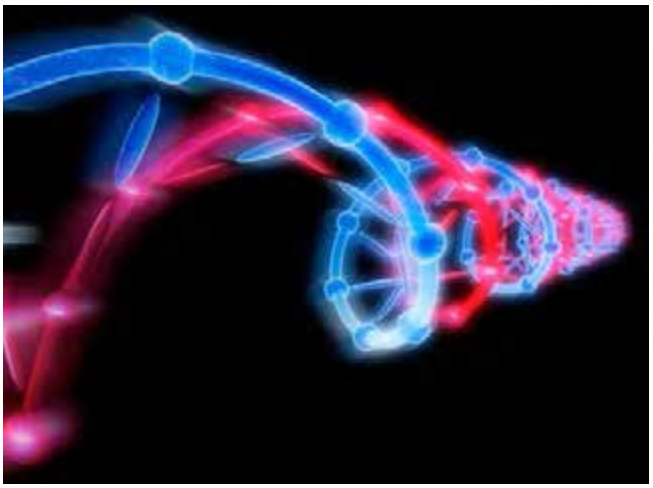
The Electron Microscopy Facility offers a variety of preparation techniques for biological samples of diverse origin. The techniques reach from conventional preparation methods to cryo-preparation techniques for phenotyping the ultrastructure of tissues and cells. Furthermore, the Electron Microscopy 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.



NEXT GENERATION SEQUENCING

The NGS Facility provides cutting edge next generation DNA sequencing technology. The facility offers 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.



SCIENTIFIC COMPUTING CORE

The scientific Computing Core will provide a scientific computing infrastructure externally to the CSF customers and internally to the other CSF facilities. This infrastructure will consist of a customer database and a service ordering/billing system. these tools will be accessible through a web portal. the facility also provides scientific software development services in the form of small projects or longer-term collaborations.

Another important task of the Scientific Computing Core is to build up a computational biology "knowledge hub" on campus. To this end training courses in biostatistics and programming are already offered to enable researchers to analyze their data more efficiently. The facility also plans to provide a platform where analysis tools written by computational biologist colleagues can be shared so that everyone on campus can benefit from their work.

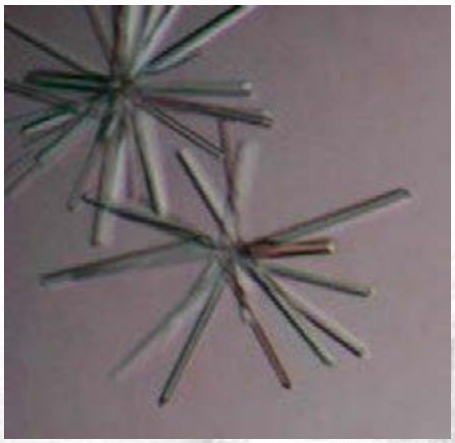
PRECLINICAL PHENOTYPING

Phenotypic screening is becoming increasingly important in the complex analysis of genetically manipulated mice. Thus, the primary objective of the Preclinical Phenotyping Facility at the 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.

The facility is offering 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, the facility also 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.

STRUCTURAL BIOLOGY

The mission of the Structural Biology Facility is to further research in protein biochemistry and structural biology by overcoming two major bottlenecks in these fields – protein production and purification. We will provide high quality protein production in eukaryotic expression systems as well as protein purification and biophysical characterization. Benefits for customers include: 1) expertise on project design and strategy, 2) access to specialized infrastructure, 3) high quality and cost effective service.



VIENNA DROSOPHILA RNAi CENTER

The Vienna Drosophila RNAi Center is a bioresource center maintaining and further developing one of the largest collections of transgenic RNAi lines for conditional in vivo gene function studies. Currently, 31,920 Drosophila lines are available to researchers world-wide. In future the VDRC will further develop and expand its resources according to emerging new technologies and community needs.

VDRC
VIENNA DROSOPHILA RNAi CENTER

Publications Research Groups

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KELEMAN GROUP

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MARLOVITS GROUP

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Uzunova, K., Dye, BT., Schutz, H., Ladurner, R., Petzold, G., Toyoda, Y., Jarvis, MA., Brown, NG., Poser, I., Novatchkova, M., Mechtler, K., Hyman, AA., Stark, H., Schulman, BA., Peters, JM. (2012). APC15 mediates CDC20 autoubiquitylation by APC/C(MCC) and disassembly of the mitotic checkpoint complex. Nat Struct Mol Biol. 19(11):1116-23

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This is a multiauthor paper; featuring U. Elling (IMBA) as shared first author; M. Novatchkova (IMP-IMBA Bioinformatics); J. M. Penninger (IMBA) as shared co-last author.

PROTEIN CHEMISTRY

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Seminar Speakers

JANUARY

11.01.12 Edvard Moser
Norwegian University of Science and Technology
Grid cells, place cells and the brain's spatial representation system

12.01.12 Alexander Rudensky
Memorial Sloan-Kettering Institute
Molecular mechanisms of differentiation and function of regulatory T cells

16.01.12 Richard Scheltema
Max Planck Institute for Biochemistry
Real-time monitoring and control of shotgun proteomics experiments

30.01.12 Helen S. Bateup
Harvard Medical School
The Tsc-mTOR pathway: an activity-dependent regulator of hippocampal synaptic plasticity and network excitability

01.02.12 Dale Dorsett
Saint Louis University School of Medicine
Cohesin, gene expression, and development

FEBRUARY

13.02.12 Titia de Lange
The Rockefeller University
How telomeres solve the end-protection problem

20.02.12 Gloria Brar
UCSF
High-Resolution View of the Yeast Meiotic Program Revealed by Ribosome Profiling

20.02.12 Elcin Unal
Massachusetts Institute of Technology
From genome partitioning to cellular rejuvenation: Unique aspects of gametogenesis

23.02.12 Keith Joung
Harvard Medical School
Genome Editing Using "Open-Source" Engineered Nucleases

29.02.12 Stefan Stricker
Wellcome Trust Centre for Stem Cell Research
Reprogramming human brain cancer cells to test the relevance of epigenetic anomalies

MARCH

01.03.12 Chris Lima
Sloan-Kettering Institute
Receptor specificity and signal transduction in ubiquitin and ubiquitin-like protein modification pathways

02.03.12 Thomas Neubert
Skirball Institute, New York University School of Medicine
Using Quantitative Mass Spectrometry (SILAC) to Study Cell Signaling in Neurons

02.03.12 Eugene Myers
Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden
Building Cellular Models with Light Microscopy

07.03.12 Matthieu Piel
Institut Curie
A cell life under confinement: effect of external constrains on cell migration and cell division

07.03.12 Nenad Ban
ETH Zurich
The structure of the eukaryotic ribosome and insights into the regulation of protein synthesis

08.03.12 John Diffley
London Research Institute
A wheel within a wheel: the chromosome replication cycle in the cell cycle

12.03.12 Gerhard Dürnberger
CeMM, Vienna
Characterization of human nucleic acid-protein interactions

15.03.12 Josh Kaplan
Harvard University, Massachusetts General Hospital
Synapse and circuit plasticity in C. elegans

21.03.12 Mark Huebener
MPI for Neurobiology
Imaging Plasticity and Sensorimotor Interactions in Mouse Visual Cortex

22.03.12 Elly Tanaka
Max Planck Institute of Molecular Cell Biology and Genetics
Cellular and molecular processes controlling spinal cord and limb regeneration

26.03.12 James Bradner
Harvard Medical School
Chemical inhibition of bromodomains in cancer therapy

APRIL

04.04.12 Eve Marder
Brandeis University
Beyond Optimality: Variability, Homeostasis and Compensation in Neuronal Networks

12.04.12 Gerry Rubin
Janelia Farm Research Campus
Studying the Drosophila Brain with Single Cell-Type Resolution

19.04.12 Steve Harrison
Harvard Medical School
Structural biology of kinetochores

25.04.12 Anne Sentenac
Institute Fresnel, CNRS
Some ideas to improve the axial &/or transverse resolution of optical microscopy

26.04.12 Michael Brecht
BCCN, Humboldt Universitaet Berlin
Rodent social touch

26.04.12 Peter Reddien
MIT Biology
The cellular and molecular basis for planarian regeneration

MAY

09.05.12 Liqun Luo
HHMI/Department of Biology
Mapping Olfaction

10.05.12 Gasper Tkacik
IST Austria
Recent progress in understanding the retinal neural code

10.05.12 Rene Medema
University Medical Center Utrecht *Phosphatases in control of checkpoint recovery*

23.05.12 Piau Siong Tan
University of Wuerzburg
New ideas for high-speed fluorescence imaging beyond the diffraction limit: Standing-waves surface plasmon resonance fluorescence microscopy

23.05.12 Doug Koshland
University of California
Shaping the genome: by form and by function

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IMCR, Zurich
The human mismatch repair interactome

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University of Tsukuba
Modeling Schizophrenia in Flies: Direct Expression of DISC1 Impairs Sleep Homeostasis and Associative Learning in Drosophila

31.05.12 Ulla Bonas
Martin-Luther-University, Halle-Wittenberg
Plant targets of bacterial pathogen effector proteins

JUNE

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Genome regulation by long noncoding RNAs

14.06.12 Thomas Schwartz
Massachusetts Institute of Technology
Taming a Monster - Toward the Structure of the Nuclear Pore Complex

18.06.12 Richard Young
Whitehead Institute and MIT
Transcriptional control of cell state

20.06.12 Andrea Hutterer
EMBO
EMBO - Funding opportunities

28.06.12 Rachel Green
HHMI, Johns Hopkins University
Quality Control on the Ribosome during Translation

29.06.12 Ansgar Büschges
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Neurobiological and Neuromuscular Mechanisms Underlying Goal Directed and Adaptive Locomotor Behaviors

JULY

05.07.12 Luis Serrano
Centre for Genomic Regulation
A quantitative systems biology study on a model bacterium

06.07.12 Stefan Leutgeb
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Neural processing in memory circuits: what information does the entorhinal cortex provide?

17.07.12 Lukas Kapitein
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From soma to synapse: sorting out polarized transport in neurons

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Looking for the engram by in vivo hippocampal imaging

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AUGUST

02.08.12 Gerald R. Crabtree
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Assessing chromatin memory in vivo and unexpected instructive roles in cellular reprogramming

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Using C. elegans to dissect mitotic mechanisms

SEPTEMBER

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Local Transcriptomes and Proteomes at Neuronal Synapses

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Organelle deterioration with age: The limits of an interconnected cellular system

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Signal processing during transcriptional regulation: from signal integration towards chromatin recognition and modification

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The mechanism of scanning and start codon recognition in translation initiation: where to begin?

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Collaboration between chromatin readers and remodelers in cancer

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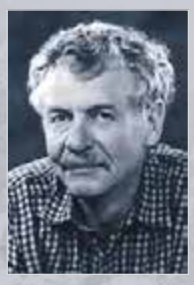

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WORKSHOP

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David KUMMERER	Deputy Head of Workshop
Vladimir KOLCAR	Workshop Technician
Michael ZIEGLER	Workshop Technician

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 by an attractive skywalk, 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 the IMP

The IMP offers a dynamic scientific environment for students and scientists at all levels of training. IMP scientists have a passion for research, which extends not only to their own work but also to work of colleagues and neighbors. Research at the IMP is multidisciplinary, combining biologists, computer scientists, and physicists, for example, in one place. Regardless of one's background, the IMP is a place to learn. The concentration of top class research at the IMP generates energy among the scientists. This intensity and excitement of discovery provide the foundations of the outstanding research at the Institute.

Researchers of all backgrounds will find intellectual stimulation at the IMP. The weekly VBC seminar series invites renowned scientists to present their discoveries; everyone is encouraged to interact with these guests. Additionally, the IMP has initiated a IMP seminar series, named after the IMP´s founding director Max Birnstiel, to highlight outstanding scientific researchers from around the world (see pages 68-71).
"Monday Seminars" provide an opportunity to find out what breakthroughs are being made within the IMP and at the VBC: doctoral students and post-doctoral researchers present their work once a year to the VBC community. Journal clubs, informal seminars, and the VBC lecture series provide opportunities to delve deeper into a subject of interest. The annual IMP recess provides an intense overview of current research from all of the IMP groups.

Vienna has a continuously expanding offering of scientific meetings and conferences. The students of the VBC International PhD program organize an annual symposium focused on an emerging field in biology, again recruiting international researchers both as speakers and as guests. Interactions with other scientific research institutions in Vienna provide opportunities to extend one's scope beyond the disciplines investigated on the VBC campus.

The IMP has a tremendous infrastructure, on par with the best research institutes in the world despite the IMP's relatively small size. The support facilities and services are substantial; they eliminate the need to worry about mundane details and allow everyone to focus on science. The IMP provides an extremely supportive environment for scientists at all levels. For instance, the grants department provides help with funding, public relations will prepare press releases, and the graphics department can advise on figures and presentations. Researchers will find they have few or no administrative duties, due to a talented and extremely helpful administrative support staff.

The excellent reputation of the IMP attracts out-standing students and post-docs from around the world. Vienna is a fantastic city, with a high quality of life and an international appeal. English is widely spoken. All of these factors contribute to the international character of the IMP and make it an exciting place to pursue scientific discovery.

More information about career opportunities at the IMP is available at: *www.imp.ac.at*

MAX BIRNSTIEL LECTURE HIGHLIGHT

Edvard Moser
Norwegian University of Science and Technology
Grid cells, place cells and the brain's spatial representation system

MAX BIRNSTIEL LECTURE HIGHLIGHT

Dale Dorsett
Saint Louis University School of Medicine
Cohesin, gene expression, and development

01/12

02/12

03/12

VBC NEW YEAR'S CONCERT

On January 18 the MolBio Orchestra together with other artists from the VBC once more created a formidable and varied program for its New Year's concert. In the first half of the concert soloists featured a wide range of compositions spanning from movie soundtracks and pop classics to folk songs from various countries. After the intermission the MolBioOrchestra performed pieces from Sibelius, Beethoven, Lehár, Strauss and Gershwin.

MAX BIRNSTIEL LECTURE HIGHLIGHT

Nenad Ban
ETH Zurich
The structure of the eukaryotic ribosome and insights into the regulation of protein synthesis

VBC SUMMER CONCERT

This years' VBC Summer Concert took place on June 5. The MolBio Orchestra was formed in 2006 by a group of molecular biology and biology students. It has featured concerts since 2010 which in the meantime have become a pleasant tradition on campus. This time the joyful concert included Offenbach, Grieg and Brahms as well as for example the Star Wars main title theme. Preceding the orchestra's performance a range of soloists featured a potpourri of Bach, Sibelius and various other pieces including Jazz, Pop or TV series' themes.

MAX BIRNSTIEL LECTURE HIGHLIGHT

Eve Marder
Brandeis University
Beyond Optimality: Variability, Homeostasis and Compensation in Neuronal Networks

MAX BIRNSTIEL LECTURE HIGHLIGHT

Liqun Luo
HHMI/Department of Biology
Mapping Olfaction

MAX BIRNSTIEL LECTURE HIGHLIGHT

Howard Y. Chang
Stanford University, HHMI
Genome regulation by long noncoding RNAs

04/12

05/12

06/12

LONG NIGHT OF RESEARCH

On April 27 IMP/IMBA took part in the Long Night of Research, Austria's biggest science event with more than 1,000 locations. The event is aimed at raising public acceptance of research and motivating younger generations toward a career in science. Guests were able to look into recent topics at various interactive stations, the more adventurous even being able to try out some simple experiments themselves.

MAX BIRNSTIEL LECTURE HIGHLIGHT

Doug Koshland
University of California
Shaping the genome: by form and by function

VBC PHD RETREAT

For this year's PhD retreat, on June 21st & 22nd, 63 VBC students, 4 IST students and 2 invited speakers headed to Schloss Krumbach, a middle-ages fortress. Topics included the results of the PhD survey and how to improve the VBC PhD program as well as the presentation of the student's individual research projects in a poster session. First of the two invited speakers was Kevin Verstrepen who talked about his studies of gene regulation in yeast and the development of his career from being a PhD student to a Harvard fellow to finally becoming a group leader back in Belgium. The second invited speaker was Andrea Hutterer, a former IMP PhD student. In her talk she told how, after doing a PhD in the Knoblich lab and a postdoc in the UK, she became the manager of the EMBO fellowships program thus providing an example of an "alternative" career.

VIENNA DRAGON BOAT CUP 2012

Dragon boating has quite some history at the IMP. Since 2007 the IMP has been represented at the Vienna Dragonboat Cup every year by the IMPerfectos boat. A dragon boat consists of 20 paddlers and one drummer. The crew are found for the boat in March, composed of people from essentially all areas within the IMP: technicians, workshop, accounting, postdocs, PhDs, diploma students and core facility members. In this year's competition the IMPerfectos reached the semi-finals after two very strong qualification races. In the semi-final they had an amazing start, were beaten only at the last second and finished in third position 0.6 seconds behind second place. In total, the IMPerfectos ended the competition ranked fifth out of 22 boats.

IMP SUMMER TRIP

This year's IMP Summer Special on July 27 took the IMP employees to the Imperial Stift Klosterneuburg for an afternoon of wine, art and food. After a welcome drink in the garden a guided tour took the visitors to the church, treasury and wine cellars of the monastery. For the kids there was an art workshop to recreate medieval times with knights, kings, queens, and dragons. Everybody enjoyed the beautiful and sunny afternoon which ended in a sumptuous BBQ-buffet.

VIENNA BIOCENTER SUMMER SCHOOL

This year saw the third VBC Summer School, 23 undergraduates from 20 different countries joining one of the labs at IMP, IMBA, GMI and MFPL for nine weeks to work on their own research projects. The results were presented at the final Summer School Symposium in which the students also competed for the VBC Summer School Prize. The whole program was accompanied by a series of lectures and many social activities – and was once again a great success.

MAX BIRNSTIEL LECTURE HIGHLIGHT

Erin Schuman
Max Planck Institute for Brain Research
Local Transcriptomes and Proteomes at Neuronal Synapses

07/12

08/12

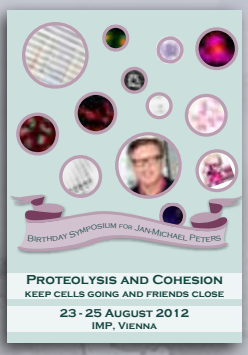
09/12

"AS YOU LIKE IT"

In 2012 the Vienna Biocenter Amateur Dramatic Club again staged its traditional annual Summer Shakespeare play on August 21-22 in the VBC courtyard. This year the group performed William Shakespeare's "As You Like It". The play follows its heroine Rosalind as she flees persecution in her uncle's court, accompanied by her cousin Celia and Touchstone the court jester, to find safety and eventually love in the Forest of Arden. More than 100 people joined the charming performance which was followed by a relaxed get-together and barbecue.

BIRTHDAY SYMPOSIUM: PROTEOLYSIS AND COHESION

On the occasion of Jan-Michael Peters' 50th birthday, several alumni from his lab had organized a scientific symposium at the IMP. It took place on August 24 and brought together a large number of experts in the field of cell biology.



POSTDOC ACTIVITIES IN 2012

The Vienna Biocenter offers a great range of further education and activities for Postdocs to optimally prepare them for the next step in their scientific career. Specific grant and paper writing courses held by external experts are scheduled at regular intervals to provide all necessary skills in these important fields. In 2012, for the second year in a row, the Postdocs at the VBC campus also organized their own Postdoc Invited Speaker Series in which the invited speakers shared their experiences getting to the junior group leader stage. The annual Postdoc Retreat in 2012 took place from 13-14 of September in the area around Hollabrunn, Lower Austria, where the VBC Postdocs enjoyed two days of science discussion in a relaxed setting.

BIRTHDAY SYMPOSIUM: IMMUNITY, EPIGENETICS AND CANCER

On October 18 and 19, a two-day conference on Immunity, Epigenetics and Cancer took place at the IMP. It had been organized to celebrate Meinrad Busslinger's 60th birthday, the speakers being former lab members and other scientific friends.

IMP RECESS

From October 3-5 IMP scientists met with members of the Scientific Advisory Board (SAB) to present their work and discuss their research. The SAB, consisting of internationally recognized scientists, was once more impressed by the scientific performance and high standards of the research presented. Next year Leslie Vosshall, Rockefeller University, USA, will join the IMP's Scientific Advisory Board. The IMP would like to thank all SAB members for their tremendous support.
IMP SAB members: page 63 in this booklet.

MAX BIRNSTIEL LECTURE HIGHLIGHT

Martin Chalfie
Columbia University
Unraveling the touch response in C. elegans

MAX BIRNSTIEL LECTURE HIGHLIGHT

Ruedi Aebersold
ETH Zuerich
Measuring Protein Networks

10/12

11/12

12/12

MAX BIRNSTIEL LECTURE HIGHLIGHT

Joe Howard
Max Planck Institute of Molecular Cell Biology and Genetics
Beat Generation: From Single Molecules to the Collective Motion of Cilia and Flagella

BIOMIMETICS

One of the highlights of every academic year at the Vienna Biocenter (VBC) is the PhD symposium organized by VBC graduate students for students and scientific fellows from all over the world. The 10th VBC International PhD Symposium was held on November 8-9, 2012, entitled "Biomimetics – Inspired by Nature".
A string of renowned speakers gave a broad overview of the interdisciplinary field of bionics, including strategies for energy generation, architectural models, nature inspired materials, robots, and bionic approaches in information technology. A highlight of the symposium was the presentation of the best dissertations of 2012 at the Campus Vienna Biocenter (Vienna Biocenter PhD Award).

Impressum

Published by

IMP-IMBA Communications Department

Project Management

Evelyn Devuyst

Layout & Design

IMP-IMBA Graphics Department

Pictures, Artwork & Credits

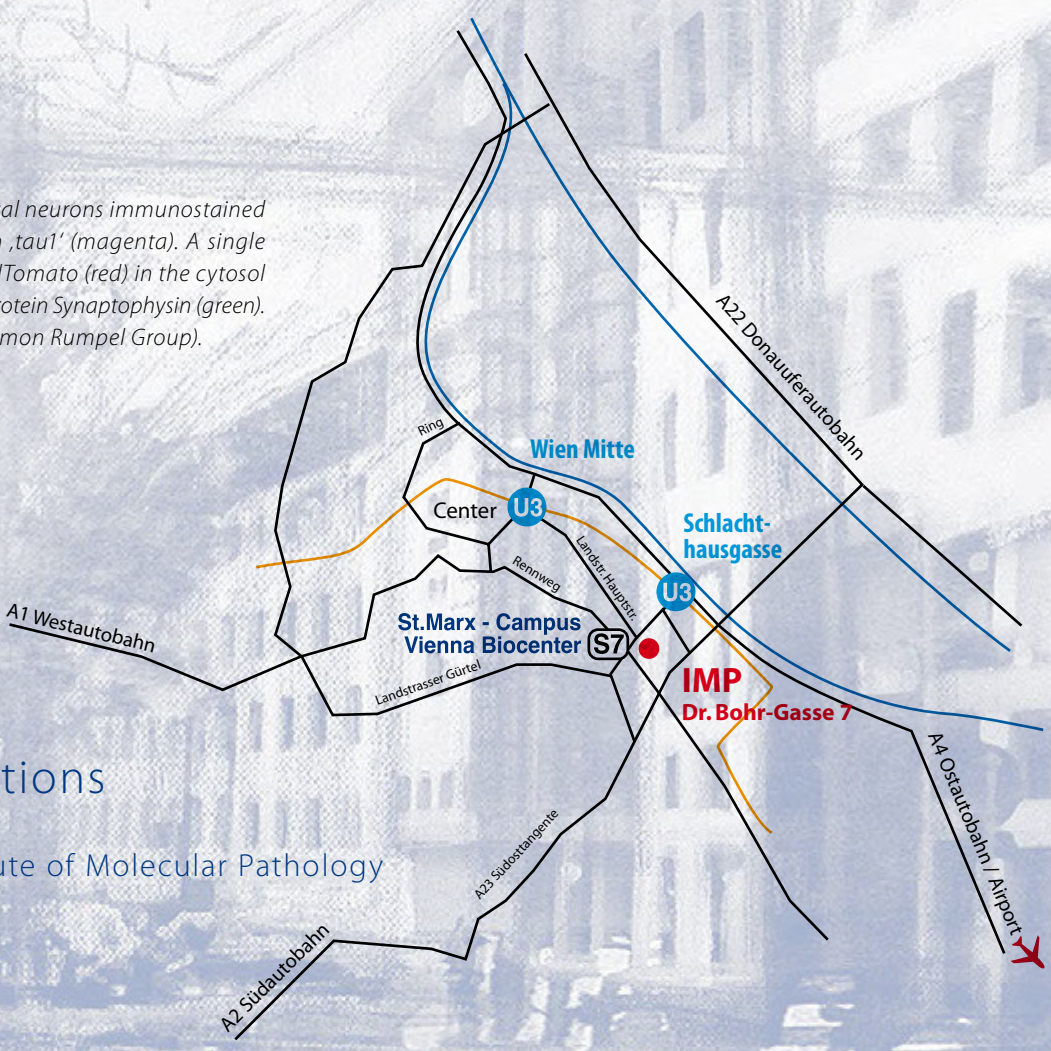
IMP/IMBA
IMP-IMBA Graphics Department
CSF (pp: 52-53)
Schaub-Walzer/PID (p: 66)
biolution GmbH (p: 67)
Lydia Scharf (pp: 68-71)
Ruben Gutzat (pp: 68-71)

Cover Illustration

Primary culture of mouse cortical neurons immunostained against axonal marker protein ‚tau1' (magenta). A single transfected neuron expresses tdTomato (red) in the cytosol and a GFP-tagged presynaptic protein Synaptophysin (green). Courtesy of Eva Wiedemann (Simon Rumpel Group).

Printed by

Ueberreuter Print GmbH
Industriestraße 1
A-2100 Korneuburg



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The IMP is a basic research institute within the Boehringer Ingelheim group of companies.
IMP is a member of the Campus Vienna Biocenter.