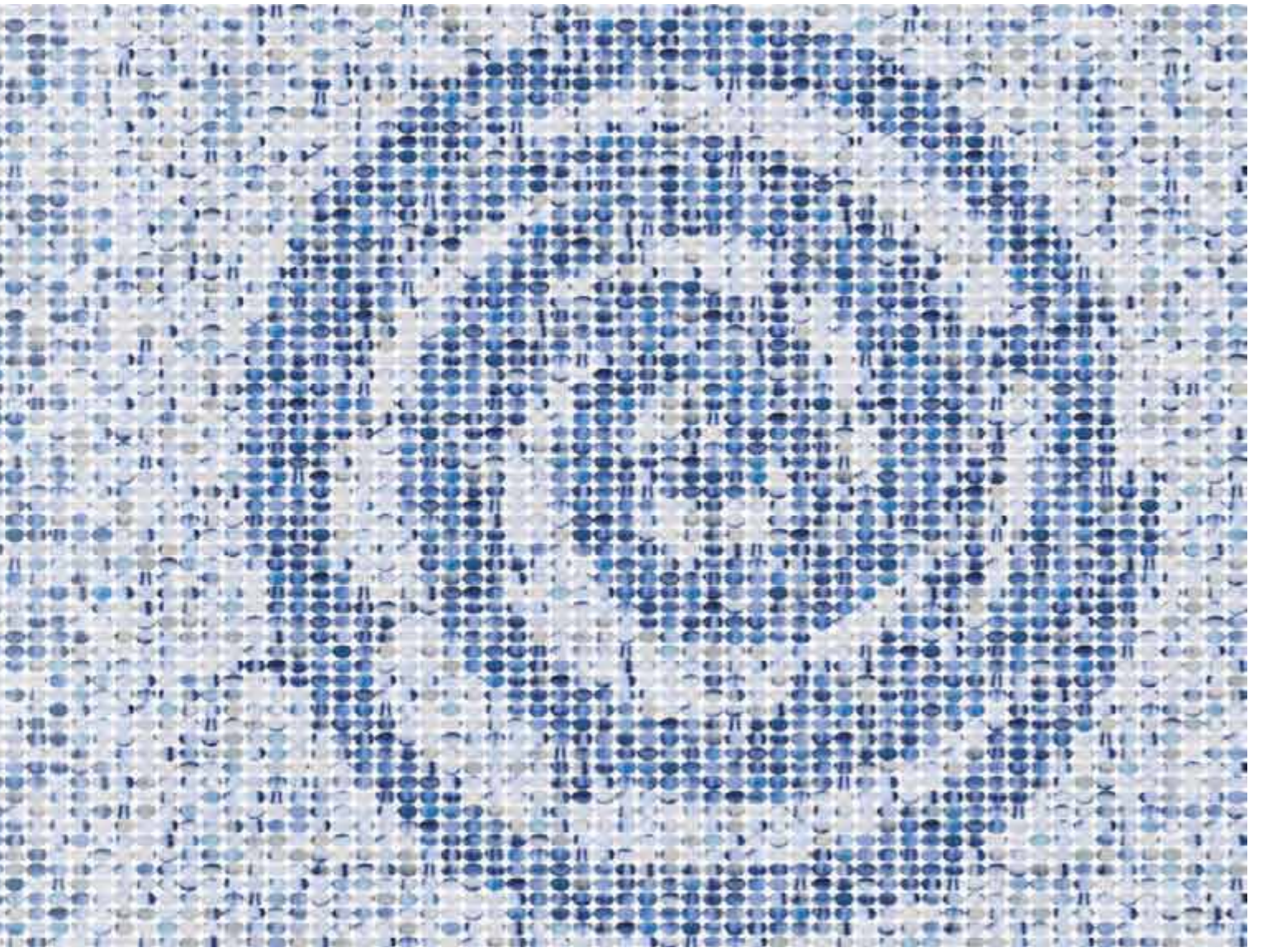
RESEARCH INSTITUTE OF MOLECULAR PATHOLOGY

VIENNA BIOCENTER

2011



Boehringer Ingelheim

IMP
Research Institute of Molecular Pathology

CONTENTS

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2011 has been a year of highs and lows for the IMP. New recruits, a key promotion, and several prominent awards marked a particularly successful year for the IMP scientifically. But it was also a year of profound sadness, with the untimely deaths of two of our dearest friends and colleagues.

We begin with the positives. As we already noted in last years' report, we were delighted to recruit 5 outstanding young scientists to the IMP in 2010: Wulf Haubensak, Andrew Straw, Alipasha Vaziri, Manuel Zimmer, and Johannes Zuber. Manuel and Andrew already started late last year; the other three joined us early in 2011. All five now have their groups up and running and you can find details of their research interests in this report. Alipasha is a joint appointment with the MFPL. This year we also added Tom Rapoport (Harvard University) and Norbert Kraut (Boehringer Ingelheim) to the Scientific Advisory Board.

As we have welcomed these new group leaders to the IMP, two others have moved on to new positions as their tenure here came to an end. Christine Hartmann, a group leader since 2001 has just accepted a full professorship at the University of Münster which she will take up in early 2012. Peggy Stolt-Bergner, the founding IMP fellow, becomes the head of the Structural Biology unit of the Campus Science Support Facility. We congratulate both Christine and Peggy and wish them well in these new positions. 2011 also saw the retirement of Gotthold Schaffner, who has been at the IMP since its foundation. Gotthold established the first scientific service at the IMP, initially providing expertise in molecular biology and later becoming responsible primarily for DNA sequencing. His extraordinary commitment to the IMP and our science is well known to anyone who has ever done an experiment here.

Jan-Michael Peters was promoted to Deputy Director in January. Jan joined the IMP as a group leader in 1996 and became a Senior Scientist in 2002. Jan's ground-breaking work on the mechanisms of mitosis and sister chromosome segregation is well known throughout the international scientific community. Less widely known is the critically important role Jan plays within the management of the IMP. He has also been a key figure in promoting the excellent scientific interactions we enjoy with Boehringer Ingelheim and also our colleagues on the Vienna Biocenter Campus. Jan's promotion recognizes both his outstanding scientific achievements and these leadership roles.

This promotion was not the only important recognition for Jan this year. In June, he was awarded the Wittgenstein Prize from the Austrian Science Fund. The Wittgenstein Prize is the most prestigious science prize in Austria, bringing with it not only national and international recognition, but also significant funds for further research. We congratulate Jan on this wonderful achievement, and proudly note that it is the 6th Wittgenstein award to recognize work done primarily at the IMP.

In 2007, the European Union established the European Research Council, giving it the mission of funding the highest quality scientific research in Europe through generous and highly competitive research grants. The IMP's Stefan Westermann was amongst the very first recipients of an ERC grant, obtaining a Starting Investigator award in its first year of operation. Alex Stark and I (B.J.D.) followed in 2009 with a Starting and Advanced Investigator grants, respectively. These awards had already made the IMP one of the most successful Austrian institutes in obtaining ERC awards. Now, in just one year, we managed to double this success, with an Advanced Investigator award to Meinrad Busslinger and Starting Investigator awards to two of our newest recruits, Andrew Straw and Manuel Zimmer.

The IMP continues its efforts to improve the scientific training we offer at all levels – from undergraduate student to senior investigator. The second installment of the Vienna Biocenter Summer School took place in 2011 and again was a great success. Much of this success is due to the creative energy of Dave Keays, the driving force behind the summer school since its conception. And plans are now underway to restructure and revitalise the PhD programme, in an effort spearheaded by Carrie Cowan and Meinrad Busslinger. The new programme will include a more structured series of lectures and workshops, and importantly also a written proposal and oral defense of the research project within the first year of PhD studies. The biooptics facility has led the way with the workshops, holding a two-week course in advanced light microscopy this autumn. With all these new initiatives, we have now also begun the search for a full-time coordinator of all scientific training activities. The new appointee, expected to be announced early in 2012, should bring new ideas and oversight to scientific training at the IMP, while also freeing faculty members from activities that, however important, distract from their core interest in scientific research.

Changes also continue within the scientific services. At the campus level, the Campus Scientific Support Facility has incorporated next-generation DNA sequencing, the Vienna Drosophila RNAi Center (VDRC) and the electron microscopy facility. Structural biology is currently being established by Peggy Stolt-Bergner, and ultraresolution light microscopy, preclinical phenotyping and scientific computing should all be added in 2012. Internally, with the retirement of Gotthold Schaffner, his General Scientific Service has been merged with the remnants of the Genomics service to form a new Molecular Biology Service headed by Martin Radolf.

Now, sadly, we must also come to the tragic events of 2011. In February, we were all deeply shocked by the sudden passing of Beate Peters, IMP alumna and wife of Jan-Michael Peters. Beate's vitality and friendship was an inspiration to all who knew her, and our thoughts and sympathy have been with Jan and his family as they have dealt so admirably with this tragic loss.

And in July, barely a year after his retirement, Hartmut Beug, too, sadly passed away. Hartmut had devoted his scientific career to the battle against cancer, a battle he also fought personally in the last few years of his life. Hartmut, one of the founding scientists at the IMP, was a respected and valued colleague and friend for all of us. He inspired us not only through his discoveries on the mechanisms of epithelial-to-mesenchymal transitions that are a hallmark of cancer metastasis, but also through his genuine passion for all things scientific, not least the scientist him- or herself. Shortly after his retirement, Hartmut, together with his wife Margrit, established "The Beug Foundation for Metastasis Research" to support original research in cancer metastasis and translational medicine. The first call for projects will be announced in the autumn of 2012, with further information available soon at: *www.beugstiftung-metastase.de.*

In closing, we thank all members of the IMP for their continued motivation and commitment throughout this turbulent year, particularly the staff in our joint scientific and administrative services. Scientists and support staff alike contribute to the scientific success of the IMP, and we can all look back proudly on another year of great progress in unravelling the mysteries of life at their most fundamental molecular and cellular levels. We all share the passion for scientific discovery, and above all thank our sponsor, Boehringer Ingelheim, for providing us with the privilege and responsibility of pursuing this passion.

THE SONG OF THE FLY

In 1967, Jim Morrison famously sang that he wanted to hear "the scream of the butterfly". By coincidence, and possibly unknown to Morrison, the same year saw the publication of *Nerve Cells* and *Insect Behavior*, in which Kenneth Roeder described how moths produce ultrasound "screams" in response to predation by bats. Despite Roeder's work, however, insect songs remain comparatively little studied. Forty years later, Anne von Phillipsborn came to Barry Dickson's lab at the IMP with a much less modest goal than Morrison's: to hear the song of the fly. Not only was her wish granted – and fairly quickly – but as a result of her work in Vienna we now have a far better picture of how flies generate their songs. Her results were published earlier this year in *Neuron* (Vol. 69, pp. 509-522).

Anne was interested in neurobiology and developmental biology long before she came to Vienna. She had previously worked on axon guidance in chicken cells but after completing her PhD she decided to switch to investigating an entire organism rather than continuing with isolated cells. The fruit fly thus represented an obvious model system for study, although Anne had never really paid much attention to it before. As she says, "on my first day in Vienna I saw a live fly under the microscope for the first time and it was love at first sight!"

When she joined Barry's group Anne had the choice between working on the fly's olfactory system and studying acoustic aspects of its courtship behaviour. During courtship, male flies use a wing to generate a sophisticated "song" and as a keen musician – she played the violin as a child and still enjoys going to concerts – Anne was naturally attracted by the idea of investigating this. Her initial plan was to examine how female flies hear and assess their mates' music but when she came to Vienna she elected instead to look at the other side of the problem, how male flies produce their songs.

A PhD student in the group, Jai Yu, was mapping the neurons that control the various aspects of sexual behaviour, including singing (see *Current Biology* 2010, Vol. 20, pp. 1602-1614). It was already known that a particular group of neurons, intriguingly known as the *fruitless* (*fru*) neurons, is primarily responsible for song production but understanding the precise function of each of the 100 or so classes of *fru* neurons was proving far more difficult. Anne planned to investigate what happened when individual neurons or groups of neurons were activated. She visited Oxford to learn Gero Miesenböck's method for using UV lasers to stimulate small numbers of neurons "but this was fairly tedious to perform and in retrospect it was not really suitable for a wide-scale screen." Nevertheless, Anne was preparing constructs to create flies for these experiments when a paper from another group (see *Nature* 2008, Vol. 454, pp. 217-220) appeared, describing the function of the TrpA1 ion channel in *Drosophila*. Barry's lab was one of several to realize that temperature-sensitive ion channels could be inserted into individual cells (such as neurons) or groups of cells, enabling them to be activated by slight increases in temperature. This method seemed ideal for use in a screen and in combination with the library of fly lines that was being generated at the IMP, Anne had the tools required for a far-ranging study of the functions of the various classes of *fru* neuron.

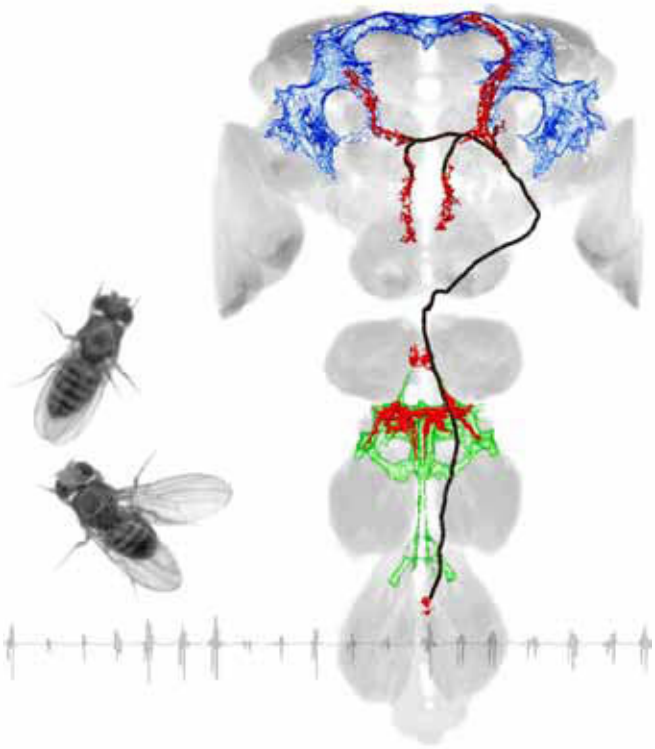
She used the new method to look at what happened when individual classes of *fru* neuron were activated. In the later stages of the project she was joined by Tianxiao Liu, who helped with the anatomical characterization. As Anne recollects, "It was a real pleasure to work with Tianxiao. He was very good at what he did and was incredibly hard working, always encouraging me to do a little bit more before going home." Anne and Tianxiao found that activation of some groups of neurons caused flies to sing, whereas when other neurons were stimulated the flies performed what Anne describes as "pseudo-copulations: they bend their abdomens and look as though they are trying to mate with imaginary females". Song production is relatively easy to quantify – songs can be recorded and the resulting oscillograms analysed – and this enabled the group to circumvent a frequent problem in behavioural science. "It is often difficult to come up with a reliable and unbiased way to compare the behaviour of different individuals. We could use audio traces to compare the songs of individual flies, then examine their brains anatomically to see exactly what was going on." This turned out to be extremely important: to characterize the role of single neuronal classes, it was frequently necessary to take a stochastic approach, i.e. to look at individual flies.

Anne's results established that five distinct classes of *fru* neuron are involved in producing the song. The so-called "P1" and "pIP10" neurons are located in the brain and were found to initiate the song. Anne believes that the P1 neuron is primarily responsible for deciding when to sing (i.e. for reacting to the presence of a female fly). The pIP10 neuron descends from the brain into the thorax, so could directly communicate with the final three groups of neurons, known as "dPR1", "vPR6" and "vMS11", which are located in the ventral nervous system. Activation of any one of them causes flies to sing, although the songs are distorted in particular ways, suggesting that a complex interplay of the three groups is required for the correct song to be produced. A further interesting point about flies' song is that it is only produced by males: under natural conditions females cannot sing. Anne's findings neatly explained why this is so. It had previously been shown that female flies lack the P1 neuron and Anne was able to show that the pIP10 and dPR1 neurons are also restricted to males. Without the neurons involved in song production, female flies are necessarily silent.

Impressive though the results are, two main questions remain to be addressed. The first is how the initial decision to sing is taken. How is the first neuron in the network (P1) activated? The second question relates to the precise pattern of the flies' songs. The courtship song of the fruit fly can be divided into two components: a "sine song" and a "pulse song". The sine song, a type of humming sound, is thought to attract the female's attention while the pulse song is known to be highly important in mating success. In other words, it is not sufficient for a fly to decide to sing for him to attract a mate: females critically judge the song pattern before responding. It is clear that neurons downstream of P1 must interact to generate the correct song pattern but how they do so remains a matter for conjecture. With the tools available at the IMP, few would bet against Anne and her colleagues in the Dickson lab making substantial progress on these issues in the near future.

In contrast to many people who work with *Drosophila*, Anne is genuinely interested in entomology. "When I came to Vienna, I thought of the fly as just a tool to learn more about behaviour but I'm now very interested in flies – and insects in general – and have started to read textbooks about them. Insects actually use sound in many ways to communicate and in the majority of cases they don't learn to do so. A lot must be hard-wired in their tiny brains – the machine for generating sounds is self-assembled during development based on a genetic master plan. In other words, male fruit flies instinctively know what they have to do to catch the girls. It's fascinating to speculate how this ability has arisen through evolution."

The song of male flies may seem a somewhat esoteric issue to investigate but it represents a good example of a type of behaviour that can be studied in the laboratory. Anne's work has shown that the underlying neural circuit can be mapped at cellular resolution and has revealed how circuits in the brain control the animals' behaviour by activating specific motor circuits. It is becoming increasingly clear that studies of relatively simple systems such as flies can yield important insights into key biological processes that are common to many species. Anne's results might thus one day lead to an understanding of the basic principles behind the evolution of neural circuits responsible for behaviour. Furthermore, the detailed description of neural circuits in flies may give insights into how neurons communicate with one another and how they interconnect and build networks and might lead to the discovery of fundamental properties shared by all nervous systems, including our own.



A Neuronal circuit for Drosophila courtship song
A Drosophila male singing to a female by vibrating it's extended wing (indicated by the pulse song trace) and a schematic of the Drosophila central nervous system with male specific neurons implicated in song production: P1 (blue) in the brain, the descending interneuron pIP10 (red) connecting brain and ventral nerve cord and vPR6 (green) in the wing neuropil.
picture by Anne C. von Philipsborn and Tibor Kulcsar.

Anne C. von Philipsborn together with her colleague and second author Tianxiao Liu.

IN THE LOOP

The mammalian immune system is truly remarkable. From a relatively limited amount of DNA, cells generate an almost unlimited variety of antigen receptors, ensuring that animals can recognize and react to just about any conceivable pathogenic threat. In 1987, Susumo Tonegawa was awarded the Nobel Prize for describing how this enormous diversity can be generated. Since Tonegawa's discoveries, one of the major goals of immunologists has been to understand the underlying molecular processes. This is one of the key interests of the group of Meinrad Busslinger at the IMP and the lab's progress was recognized by the receipt of a prestigious ERC Advanced Investigator Award by Meinrad this year.

The latest chapter in the story was published early in 2011 by Anja Ebert, a PostDoc in the group (*Immunity* Vol. 34, pp. 175-187). Anja performed her diploma and doctoral work in Halle in the group of Gunter Reuter, which had a close collaboration with Thomas Jenuwein at the IMP. During the course of her work she visited the IMP several times and was impressed by the quality of the institute's research and by its international flavour. She thus decided to move to the IMP for her PostDoc, although she elected not to join TJ's group but to work instead on something completely different. Having previously studied chromatin organization in Drosophila, Anja was looking to broaden her area of expertise. Meinrad's group was an obvious choice, especially as the lab was at that time moving into the field of epigenetics, thereby providing a link with her previous work.

Before Anja arrived, it was known that Pax5 controls the process by which the so-called "variable" (V) region of the immunoglobulin heavy chain locus is joined to the remaining portions of the gene, the D and J segments. Martin Fuxa in the group was studying the process of locus contraction, the "looping out" of large portions of DNA at a particular position (see *Genes & Dev.* Vol. 18, pp. 411-422). The gene locus for the immunoglobulin heavy chain (IgH) is vast and the problem of how very distant (2.5 MB or more away) portions of DNA could be joined to other gene fragments had proven a major obstacle to the understanding of the immunoglobulin gene recombination that Tonegawa had discovered. Locus contraction had been proposed to facilitate the recombination of very distant gene segments and Martin had shown that contraction is induced by Pax5. Work by Sasha Tarakhovsky's lab at the Rockefeller university indicated that Polycomb proteins are likewise required for proper recombination of the IgH locus. To investigate their role in locus contraction, Anja started to generate conditional knock-outs of Polycomb proteins in mice. "I spent my first two years generating three transgenic mice. The first construct turned out to be lethal, the second one had no phenotype and I'm currently characterizing the third mouse."

The main goal of Anja's work was to unravel the molecular mechanisms responsible for contraction of the IgH locus. To identify novel regulatory elements involved in the process, Anja used an unbiased chromatin immunoprecipitation (ChIP-chip) approach to map binding sites for Pax5 and other transcription factors at the locus. At the same time, Shane McManus in the lab mapped the sites of active histone modifications in pro-B cells, where the IgH locus is contracted, as well as in Pax-deficient pro-B cells, where the locus is in an extended conformation. Contrary to what was widely believed, he found that most V_H genes had no active histone marks. However, in pro-B cells (when the IgH locus is contracted) he found regions carrying active chromatin marks including the H3K4 trimethyl lysine mark immediately upstream of one of the 16 families of V_H genes, the so-called 3609 genes. This mark is frequently indicative of active gene transcription. Importantly, the presence of active histone modifications was found to be dependent on Pax5 expression. Anja showed that Pax5 and the general transcription factor CTCF (and other transcription factors) are bound at these sequences. In pre-B cells, the next developmental stage, when the locus is no longer contracted, Pax5 binding is lost although CTCF still binds the sequences.

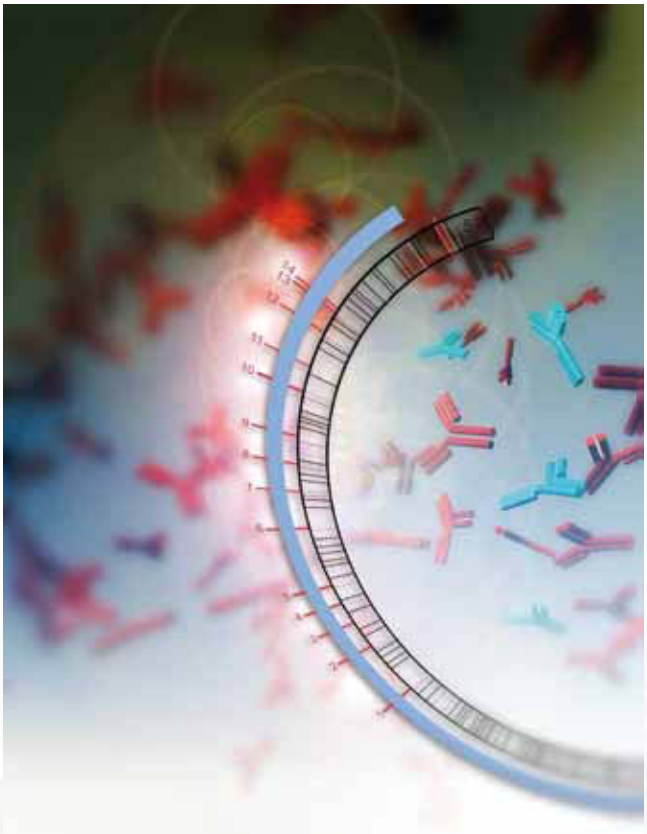
Anja Ebert and her colleagues about to compete in the dragon boat race.

The ChIP-chip experiments were complicated by the extremely repetitive nature of the locus, so it was important to confirm the results by means of another method. Anja turned to Hiromi Tagoh, who was developing DMS footprinting to study the binding of proteins to DNA in living cells. At the time, Hiromi was working at the University of Leeds but her collaboration with Meinrad's group proved so fruitful and enjoyable that she decided to move to Meinrad's lab. The DMS footprint experiments indeed confirmed that Pax5 bound to these particular sequences in the distal portion of the IgH gene, as Anja says, "exactly where you would want the protein to bind if you planned to loop the locus." Sequence analysis revealed the existence of 14 repeats in this area but nowhere else in the mouse genome. The group termed the sequences PAIRs (for Pax5-activated intergenic repeats).

A clue that transcription at the locus might be important came from Maria Novatchkova, who discovered a cDNA clone in an online database. This cDNA appeared to initiate within one of the PAIR elements and to run in an antisense direction. Anja looked in pro-B cells and was able to detect the corresponding antisense RNA. Furthermore, the RNA was not found in pro-B cells that have been engineered to lack Pax5 expression, nor is it found at subsequent developmental stages, when the IgH locus is no longer contracted and Pax5 no longer binds. In other words, the RNA is restricted to cells where the IgH locus is contracted. This represents very strong circumstantial evidence that transcription of the antisense RNA from the PAIR element is involved in contracting the locus or that the RNA itself somehow operates on the contracted IgH locus to regulate gene recombination.

All this sounds convincing and highly exciting but there is a major caveat: there is still no direct evidence that the antisense RNA or indeed the PAIR elements are functionally important for locus contraction. Although the work was invariably well received at conferences, it proved frustratingly hard to publish it in the absence of a proof of the function for the elements. Jasna Medvedovic in the group is using chromosome capture technology to investigate the importance of the PAIR elements in large-scale looping of the locus, although the size and repetitive nature of the locus is making her experiment extremely difficult to perform. The group is also pursuing an alternative transgenic approach, knocking out all 14 PAIR elements in the mouse and examining the effects. This work is also complicated by the nature of the locus and it is clear that the project will take some time to complete.

Many people might become frustrated by the problems of working with such an intractable system but Anja manages to maintain her enthusiasm. "Recombination of the IgH gene is an early step in the production of an almost infinite range of antigen receptors. Just thinking about how this unbelievable diversity is generated is enough to bring me back to the bench, no matter how many setbacks I suffer. And I still need to find out how Polycomb proteins are involved in the process!"



Circular diagram of the mouse Igh locus. The red curves denote the repeat elements containing the 14 PAIR sequences, which are located in the distal region of the locus.

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 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 in the early development of B cells. E2A and EBF1 function as B cell specification factors by activating B-lymphoid genes. Pax5, in turn, controls B cell commitment by restricting the developmental potential of hematopoietic progenitor cells to the B cell pathway. The function of these transcription factors is required throughout B lymphopoiesis, as E2A, EBF1 and Pax5 also control the generation of mature B cell types, including germinal center B cells (Figure 1). Notably, 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. These experiments identified Pax5 as the critical B cell identity factor that maintain B-lineage commitment throughout B cell development.

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 therefore want to elucidate the molecular functions of these transcription factors in pro-T cells by conditional mutagenesis, gene expression profiling, and ChIP sequencing.

Transcriptional networks

Global genomic approaches are ideal for elucidating the transcriptional network controlling early development of B cells and T cells. To achieve this aim, we define the regulatory landscape of pro-B and pro-T 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 B cell development.

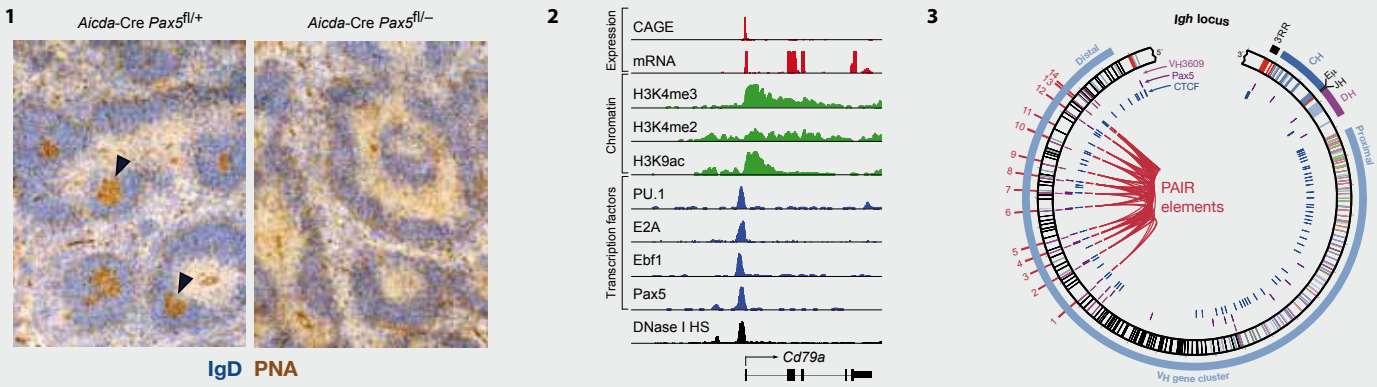

Figure 1: ***Essential role of Pax5 in germinal center (GC) B cell development****. The Aicda-Cre line deletes the floxed (fl) Pax5 allele in GC B cells of control Aicda-Cre Pax5$^{fl/+}$ mice eight days after immunization with sheep red blood cells. PNA^{+} GC B cells (arrows), which are detected by immunostaining in spleen sections, are formed in the presence of Pax5 in these control mice, but not in its absence in Aicda-Cre Pax5$^{fl/–}$ mice.*

Figure 2: ***Genome-wide identification of transcription factor target genes.*** *The Cd79a 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: ***Identification of regulatory PAIR elements in the distal V_H gene cluster of the Igh locus.*** *A circular diagram indicates the positions of the V_H, D_H, J_H and C_H gene segments, iE_μ enhancer and 3' regulatory region (3'RR) of the mouse Igh locus. The different colors denote members of the distinct V_H gene families. As shown by the inner concentric tracks, Pax5-activated intergenic repeat (PAIR) elements (red) are located upstream of V_H3609 genes (pink) and contain binding sites for Pax5 (purple) and CTCF (blue).*

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), organized in a complex manner (Figure 3), 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 that contraction of the *Igh* locus primarily depends on Pax5. Recently, we were able to show that Pax5 activates conserved intergenic repeats (PAIRs) in the distal V_H gene cluster, which most likely functions as a novel regulatory element in controlling *Igh* locus contraction (Figure 3). We are currently elucidating the molecular mechanism of *Igh* locus contraction by investigating the function of such *cis*-regulatory elements and by identifying novel *trans*-acting factors involved in this process.

FURTHER READING

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TIM CLAUSEN

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.

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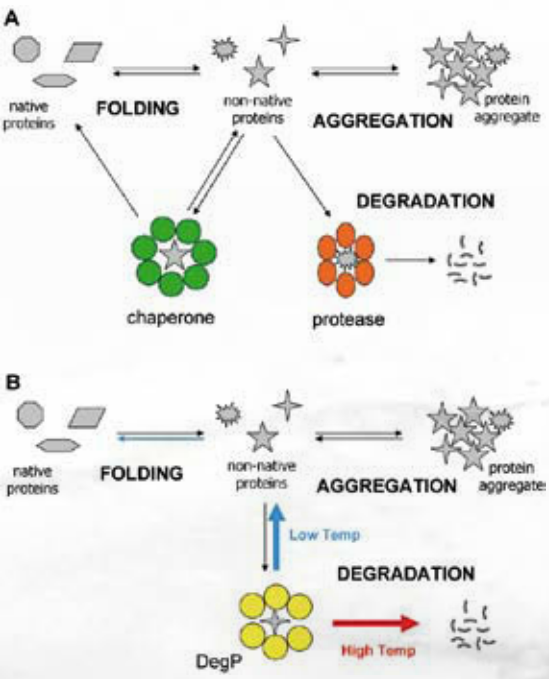

The CtsR/McsB stress response

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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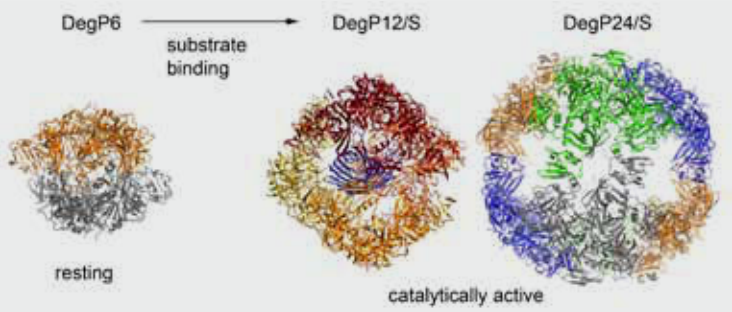

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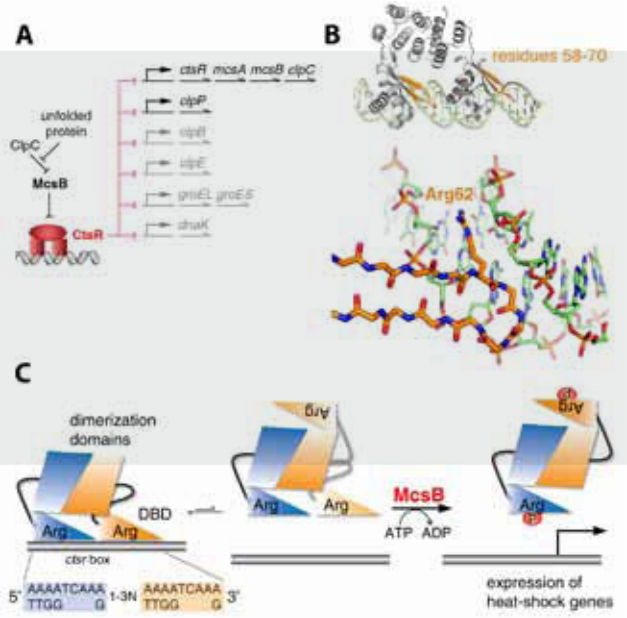

Figure 1: *Protein Quality Control (A) The scheme illustrates the different fates of misfolded non-native proteins. According to Gottesman and co-workers' "kinetic partitioning model", aggregate formation is counteracted by the action of proteases and chaperones. (B) DegP combines the antagonistic activities 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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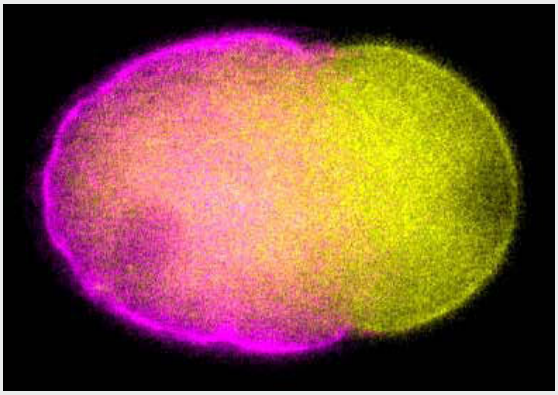

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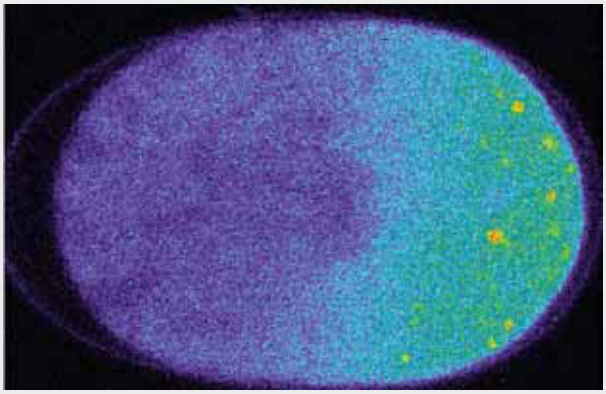

Figure 1: *Centrosome position (line) relative to the cortex (dots) during polarity establishment. The color scale indicates elapsed time (blue: -500 s, red: 300 s).*

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 [1] (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 [2], and are currently tracing the pheromone processing pathways that feed into these song circuits.

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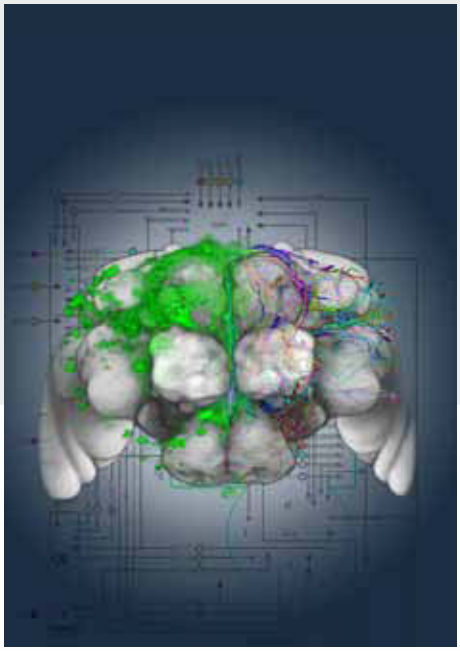

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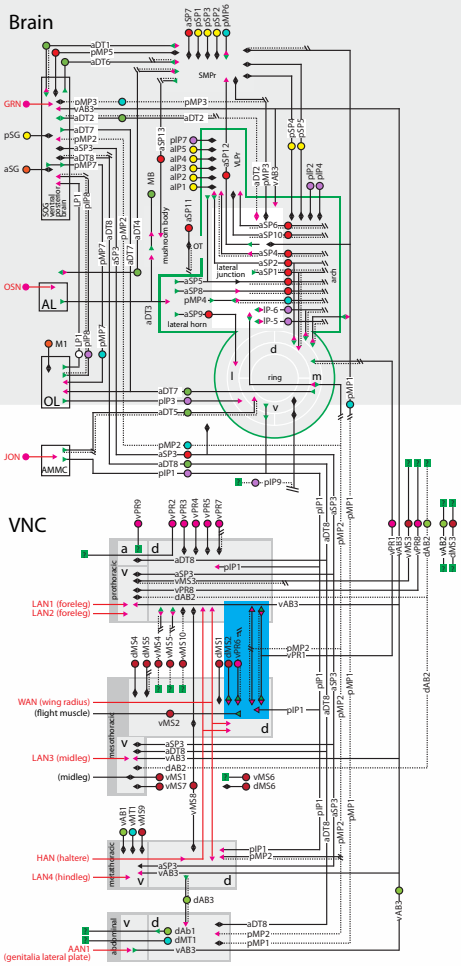

Figure 1: ***fru⁺ neurons in the CNS, shown in green.*** *Synapses are stained in magenta. This is a confocal image of the brain and ventral nerve cord of a fru^{GAL4} UAS-GFP male.*

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 [3]. 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

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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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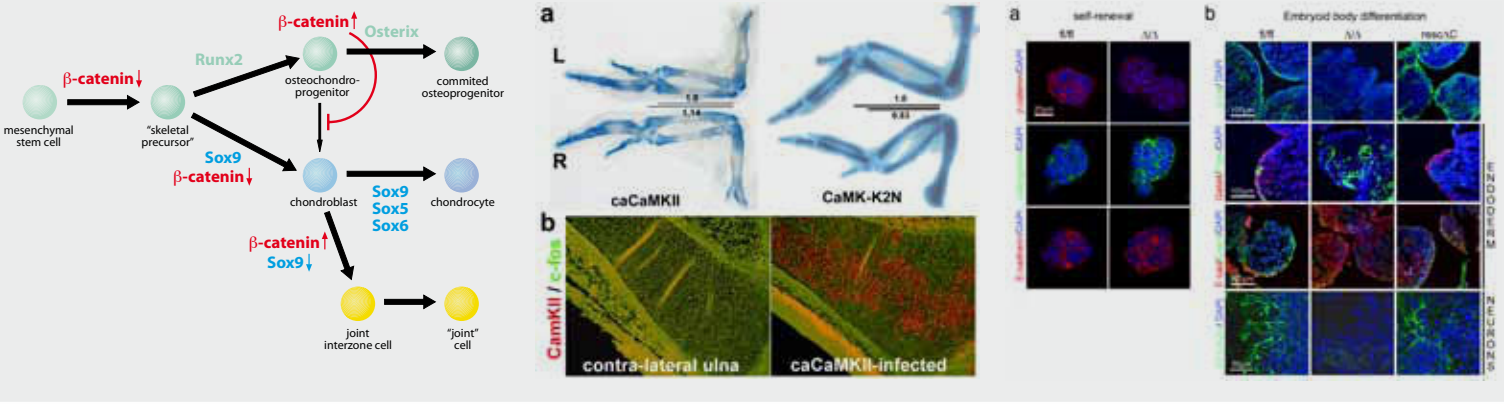

Figure 1: *β-catenin levels need to be modulated during skeletal lineage differentiation: only mesenchymal cells expressing low levels of β-catenin can differentiate into skeletal precursor which will give rise to osteoblasts, chondrocytes, and cells of the joint. Within the osteoblast and joint lineage, 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 threat and evokes defensive behaviors; emotions associated with reward induce the opposite. Emotions are a focal aspect of our mental selves, and linked to a variety of psychological conditions. But how are emotions wired in the brain? To investigate their underlying neural basis, we use molecular, 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 how genes and psychoactive drugs, in turn, 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 problem. 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 have established the limbic system as the central hub in emotion processing (LeDoux, 2000). It integrates sensory information, encodes emotional states, and instructs other brain centers to regulate physiology and behavior. However, it 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 address this problem, we combine genetic manipulation of brain circuitry to map circuit anatomy and function (Luo et al., 2008), using electrophysiological recordings (Du et al., 2009) for probing circuit interactions.

Circuit mechanics of emotions

In a first foray, we are screening for local limbic microcircuits that could serve as key emotion hubs. Pharmacogenetics, optogenetics, and viral tracing disclosed a local inhibitory circuit of two antagonistic neuronal populations in the lateral central amygdala (CEl) that 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 which alternates between two states: in the absence of a conditioned stimulus (CS), so called CEl-off neurons, identified by the expression of PKCδ (Figure 1A), are active, inhibiting their counterpart CEl-on neurons and amygdala output; in the presence of the CS, CEl-on neurons are active, inhibiting CEl-off neurons, which disinhibits amygdala output and fear signals to the brain stem (Figure 1B). In support of this model, initial pharmacogenetic experiments have shown that a reduction of CEl-off neuronal activity results in significantly higher amygdala output and conditioned freezing than usual. We are currently validating the proposed circuit mechanics with neural modeling (Figure 1C), optogenetic perturbations (Fig. 2A), electrophysiology and behavior. Using a similar strategy, we will screen for, and analyze, other local microcircuits in a similar manner to reveal general motifs in the neural circuit organization of emotion and brain function.

However, these microcircuits do not operate in isolation, but rather in cooperation with other brain structures. Cortical inputs, for instance, would make an excellent substrate for the top-down control of emotions by higher cognitive processes. Virus-based anatomical circuit tracing will identify this macrocircuit in- and output and establish core networks for emotions. Subsequent pharmaco- and optogenetic circuit manipulation in combination with in vivo electrophysiological recordings in Pavlovian fear conditioning will resolve circuit interactions in these networks.

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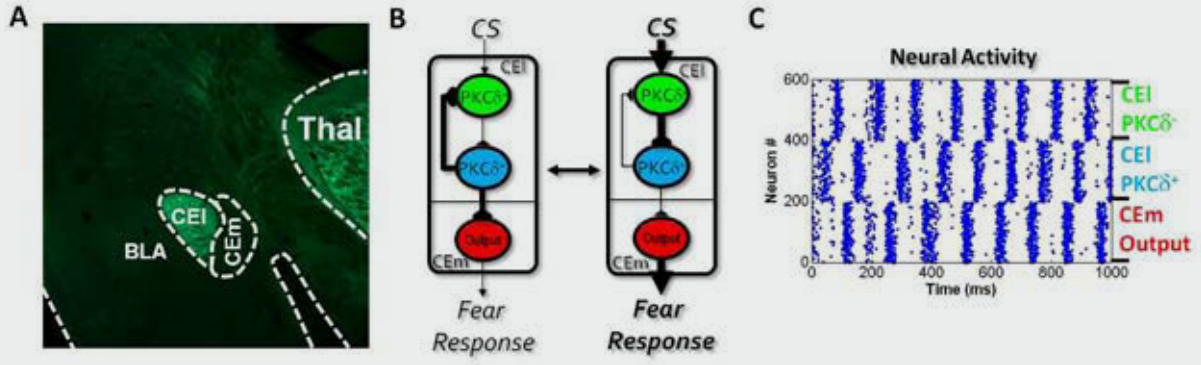

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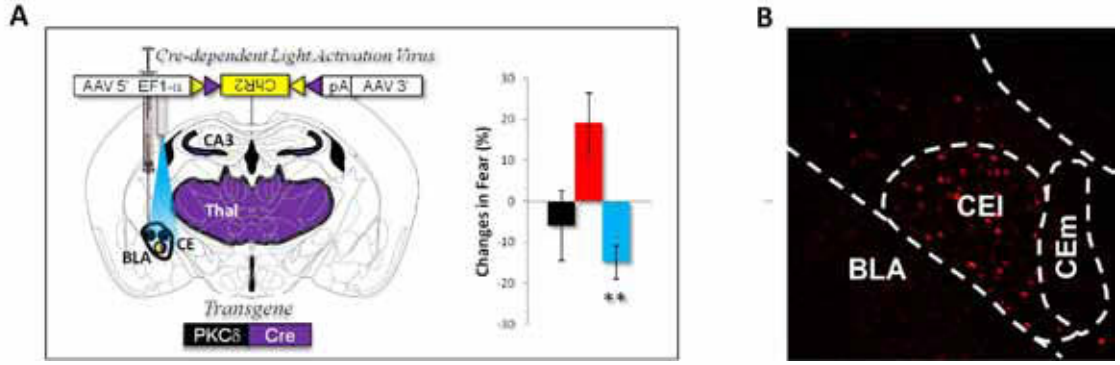

Figure 1: *CE circuitry gates fear. A, PKCδ identifies a subpopulation of neurons in CE. B, Neural circuit model of information flow through the CE. C, Simulation of neural activity in CE during inhibitory gating. BLA, basolateral amygala; CEl/m, lateral/medial central amygdala; Thal, thalamus.*

Figure 2: *CE circuit manipulation modulates fear. A, Left, Combinatorial viral/transgenic optogenetic targeting of CEl PKCδ+ neurons. Right, Light-induced changes in fear upon activating CEm output (red; Fig. 1B) or CEl PKCδ+ neurons (blue; Fig. 1B). B, Drug-induced neural activity and c-fos expression in CEl. ChR2, channelrhodopsin; BLA, basolateral amygala; CEl/m, lateral/medial central amygdala.*

In general, emotions like fear and reward states are represented in a two-dimensional space by their affective valence and arousal (Calder, Lawrence et al. 2001). We therefore hypothesize that emotions are encoded by a set of circuits for positive or negative valence and valence non-specific circuits for arousal, and that their relative activity determines emotional states and drives fear avoidance and reward-seeking behavioral responses. We will address this by comparing where pathways of fear and reward diverge and converge. The results will reveal how valence and intensity, the two principle dimensions of emotions, are represented in emotion circuits.

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. We surmise that the circuits identified above serve as an ideal field to study this problem. To this end 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 a target of psychoactive drugs, which alter the balance of neural activity in this network (Figure 2B).

Taken together, we hope 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 and disease conditions like anxiety disorders or addiction.

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KATRIN HEINZE

Nanoscale Functional Imaging of Living Cells

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Discoveries in bioscience are stimulated by the development of new scientific tools. We have focused on pushing low-invasive fluorescence techniques beyond their usual spatial and temporal resolution limits. Approaches such as these offer the prospect of studying the unperturbed dynamics and interactions of biomolecules under physiological conditions.

Ultra-fast Superresolution

Fluorescence techniques typically suffer from a resolution limit of ~300 nm due to the nature of light. To overcome the diffraction limit over a given spatial region, one requires an additional degree of freedom. Extensive efforts have been made to improve the resolution limit by using the temporal degree of freedom. These include approaches based on selective illumination or localization of emitters. The drawback is that imaging rates are limited by the scan speed or the intrinsic nature of the underlying photophysics (e.g. blinking, bleaching, and activation of emitters). We are thus still missing an imaging technique that provides high spatial as well as dynamic resolution. The goal of our research is to fill this gap.

An alternative approach towards superresolution is to sacrifice larger-scale features by scattering the near field of an object in a predictable fashion [Liu et al 2007]. We have shown how the loss of large-scale details can be compensated by use of off-resonance near-field lenses combined with real-time spectral analysis of the scattered fields [Elsayad et al. 2010]. We are currently working on the design and production of such devices.

Plasmonic rulers: When material science meets biology

An additional drawback of many "superresolution" techniques is the difficulty to obtain high resolution in axial (z) direction. This is compounded by the fact that the axial diffraction limit is already larger than that in the lateral (xy) direction. We have countered this problem by developing a technique that utilizes distance-dependent interactions of emitters with electronic excitations in certain metal-dielectric (MD)-coated substrates. The modified fluorescence rates can be accurately modeled, and the position of the emitters from the structure can be calculated on-the-fly by measuring the spectrum over several discrete wavelength ranges. 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 advantages of the technique named COCOS (Color-Coded Optical Nano-Sectioning) are its affordability and compatibility with conventional microscopy techniques. A disadvantage at the present time is that it is only effective in the 10- to 100-nm vicinity of surfaces. Results of a typical COCOS image and reconstruction of the average axial position of the protein Paxillin at fibroblast adhesions is shown in Figure1. The obtained average axial distance is in good agreement with previous EM sectioning studies, indicating that Paxillin is located at a distance of 20 to 40 nm from the substrate.

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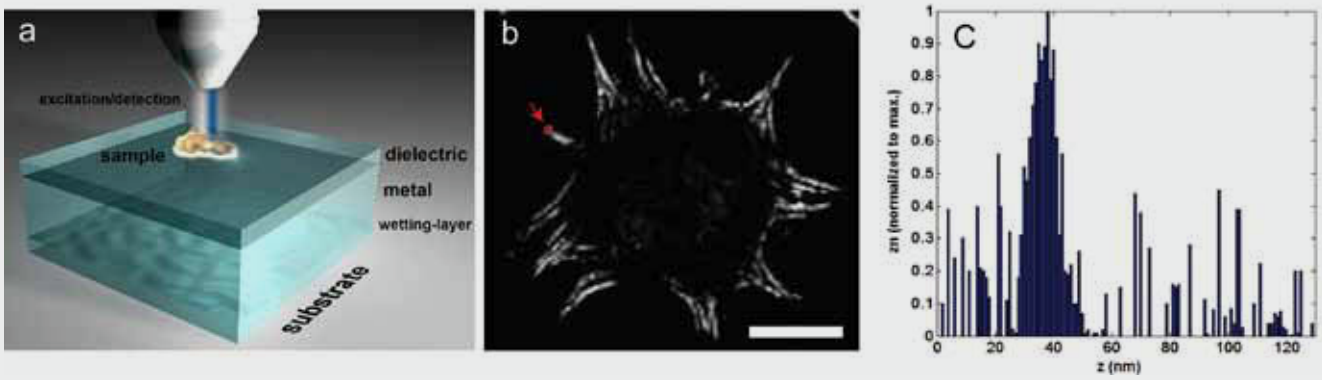

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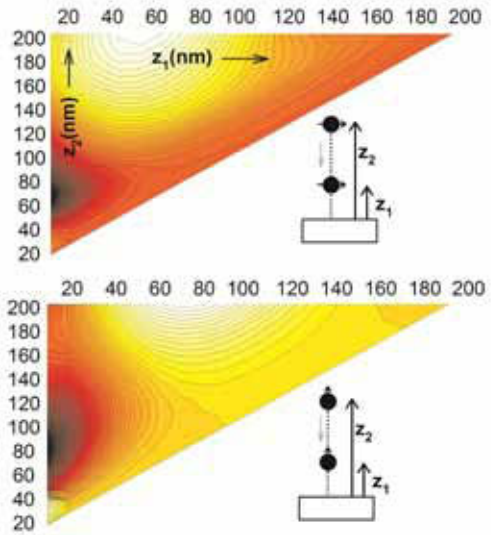

Figure 1: *Color-Coded Optical Nano-Sectioning (COCOS). Sketch of the COCOS setup used for imaging near the surfaces of coated substrates (a); Laser Scanning Microscopy image of an NIH 3T3 fibroblast cell on metal-dielectric substrate showing fluorescence of Alexa488 stained paxillin (b, λem=519, λexc=465nm, scale-bar=10μm); area indicated in red shows pixels over which the spectrum was analyzed to obtain the distance distribution in (c) inferred from Δλ =9.7nm resolution spectrum (λ=480–800nm).*

Figure 2: *Energy transfer rate in the presence of metal/dielectric surface. Plots show the enhancement in transfer rates (relative to the condition when the substrate is absent) from an excited state emitter at $z=z_2$ to a ground state acceptor at $z=z_1$. The two cases shown are for parallel (top) and perpendicular (bottom) emission/excitation dipole orientations relative to the substrate interface. Here, donor and acceptor were modeled as CFP and YFP. The structure was designed to have a cut-off frequency that coincided with the respective spectra. The false color map indicates an enhancement range from -0.3 (dark red) to >500 (white).*

It would be interesting to dynamically measure the separation between two molecules during a biological process. A popular technique for separations of ~10nm is Forster Resonant Energy Transfer (FRET), whereas separations on the order of the wavelength (larger than 300-600nm) can be inferred using conventional optical techniques. However, FRET cannot be used to measure distances larger than10 nm, leaving a "gap" in the ability of optical techniques to measure distances on the 10- to 100-nm scale. We have recently shown that the FRET distance between molecules in the vicinity of certain MD-coated substrates can be increased by more than one order of magnitude, thus completely filling this "gap". The effect is a result of an additional energy transfer mechanism, wherein emitters couple to collective electronic excitations in the MD which can subsequently excite acceptors. For our design of structures, this effect dominates inter-molecular energy transfer at distances of 10-200nm. Such coated substrates are biocompatible, and – compared to conventional FRET – even provide a more robust ruler with higher transfer efficiencies for smaller spectral overlaps of donor and acceptor. This paves the way for applications in biology as well as for designing artificial light-harvesting devices. Efforts are under way to develop lab-on-chip type devices that utilize this effect.

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DAVID KEAYS

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. 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). 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

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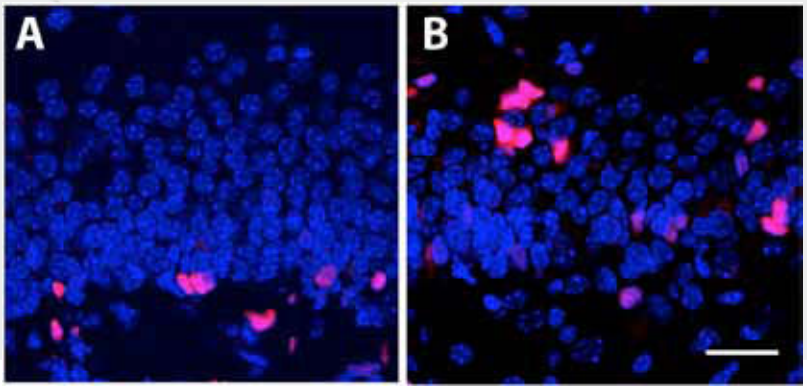

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Figure 1: *The Jenna mutant mouse. This mouse harbours a S140G mutation in the Tuba1a gene which results in defect neuronal migration during development. As a consequence the Jenna mouse is characterised by abnormal lamination of the hippocampus that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

discovery of magnetite in a range of other organisms that detect and respond to magnetic fields; most notably birds, fish and bees.

The Keays lab is investigating the magnetite based theory of magnetoreception employing the pigeon *Columbia livia* as a model system. Our current efforts are focused on the upper beak of the pigeon as the ophthalmic branch of the trigeminal nerve is required for magnetoreception in pigeons (Mora et al, 2004). To identify magnetic cells we have built a "magnetoscope" which enables us to identify cells based on their intrinsic magnetic properties. With the recent release of the pigeon genome we are complementing these cellular studies with transcriptomics and molecular profiling. 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 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 regime. 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 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 transgeneic RNAi to systematically test the function of every gene in *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 conditiong, 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 [1]. The molecular basis for this function remains however obscure. CPEB proteins are thought to regulate RNA trafficking and/or translation, and thus might contribute to local protein synthesis in activated synapses. To test this hypothesis for the *Drosophila* CPEB protein Orb2, we have generated *orb2*^{attp} allele (Fig.2), which allowed us to rapidly introduce any modification of the endogenous Orb2 protein in the tissue selective manner and test its role in courtship conditioning. With this system we can investigate the precise structural requirements for Orb2 function in long-term memory, examine its subcellular localization and trafficking, and identify specific RNAs or proteins that interact with Orb2.

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 have 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

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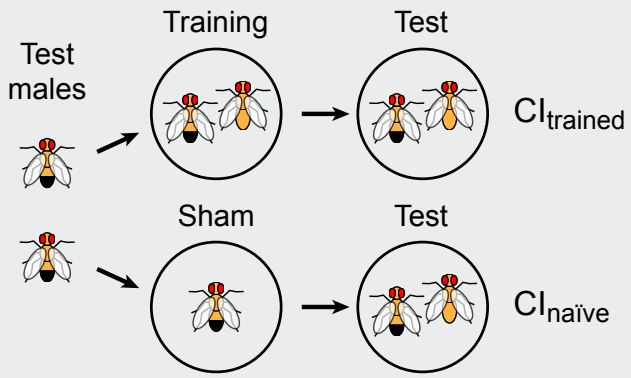

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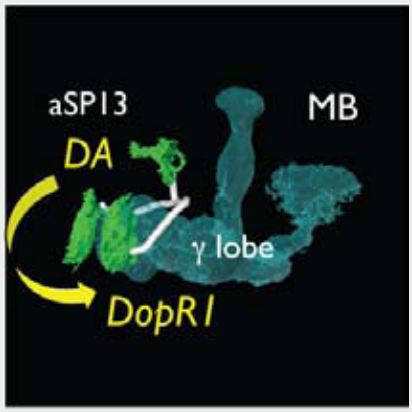

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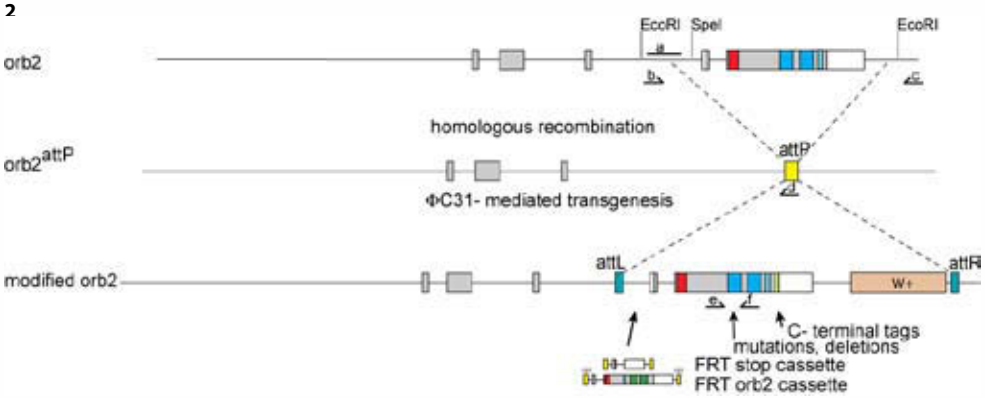

Figure 1: *Courtship conditioning. When tested with unreceptive mated females, males previously exposed during the training to mated females court less than naïve (sham-trained) males (CI = courtship index; $CI_{trained} < CI_{naïve}$)*

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

Figure 3: *Dopaminergic aSP13 neuron innervate MB gamma lobe. After experience with unreceptive mated female, dopaminergic neuron aSP13 release dopamine on the MB gamma lobe which enhances the behavioural response of the male to the pheromone cVA and the ability to discriminate between the virgin and mated female.*

on females after mating and distinguishes them from virgins. We have identified a specific class of dopaminergic neuron, aSP13 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 [2]. 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 *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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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

Many animal and plant pathogens share the same principles of infecting host cell organisms: they translocate specific bacterial toxins (collectively referred to as "effector proteins"), which originate from the bacterial cytoplasm, directly into the cytoplasm of a eukaryotic host cell. As a result, translocated effector proteins have the remarkable capacity to modulate various host-cell pathways, including endocytic trafficking, gene expression, programmed cell death, or cytoskeleton dynamics that induce membrane ruffling and subsequently render the host accessible to bacterial infection. At the heart of this process is the type-3 secretion system (T3SS), a protein-delivery machine that establishes intimate contact between the microorganism and the host cell, and permits safe and unidirectional passage of specific effectors. These systems are widespread among Gram-negative animal pathogens, including *Yersinia, Pseudonomas, Shigella*, enteropathogenic and enterohemorrhagic *E. coli* (EPEC and EHEC, respectively), or *Salmonella*, and the plant pathogens *Erwinia, Ralstonia* or *Xanthomonas*. They are essential for the onset of a variety of diseases ranging from diarrhea, bubonic plaque, even with fatal outcomes, to fire blight and bacterial wilt. While the task of translocating proteins from one compartment to the other has been basically solved in nature (for example the targeting and/or secretion of proteins through the Sec-system or the Tat-system), the contextual situation is complicated by the fact that the translocation must occur through a number of environments, which includes two bacterial membranes and one eukaryotic membrane, the periplasmic and the extracellular space. Consequently, the nature of a T3SS system is complex in terms of specific mechanistic details as well as the organization of all involved components. Using *Salmonella typhiumurium*, we are investigating the molecular mechanisms and structural framework required to translocate effector proteins specifically and safely into eukaryotic cells.

Architecture of the needle complex of the T3SS:

The core, and probably the most prominent structure of the T3SS (SPI-1), is the needle complex. It is a 'syringe'-like multi-component system. Overall, the needle complex is a large (approximately 30x80nm) cylindrical complex. In its native environment it is embedded in the inner as well as outer membranes, spans the periplasmic space, and protrudes into the extracellular environment with a needle filament. Its overall architecture provides a structural framework for a direct connection of bacterial and host cell cytoplasm, and delineates the secretion pathway through the needle complex. Although the needle complex is about 3.5 MDa in size, its overall shape is dictated by only five proteins. Nevertheless, mutually exclusive models of the individual protein organization have been described in the past. These models were rendered complex by a paucity of positional information, incorrect assumptions about the symmetry and stoichiometry of ring-forming base proteins, and consequent difficulties of modeling. Our laboratory 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

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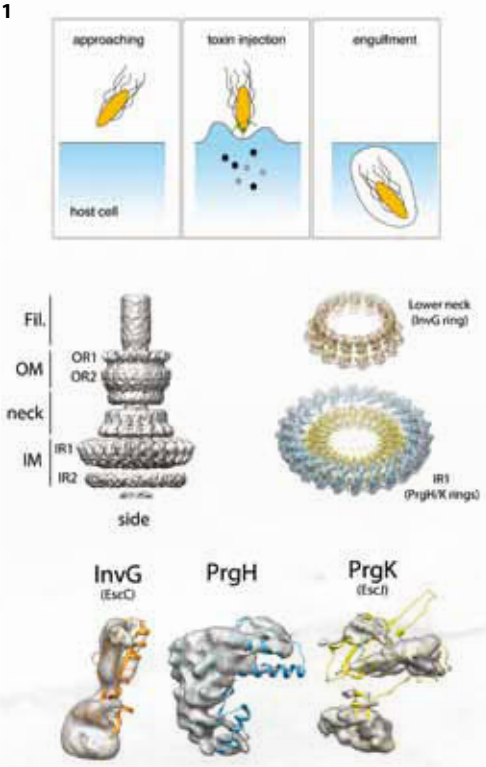

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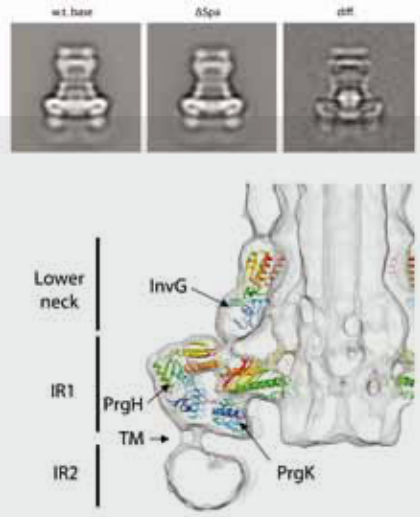

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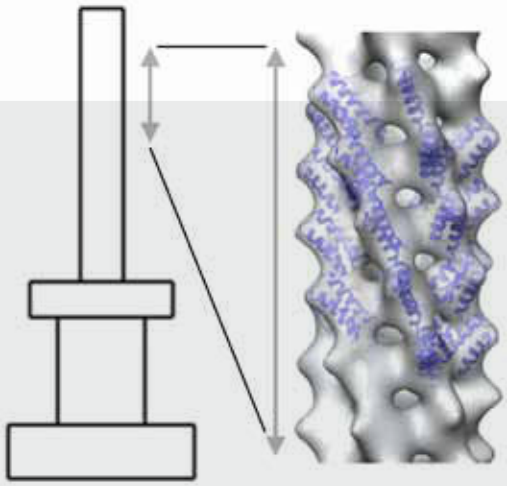

Figure 1: *Three-dimensional reconstruction of the needle complex to sub-nanometer resolution and docking of atomic structures of all available protein domains.*

Figure 2: *Formation of the socket/cup is dependent on the presence of export apparatus proteins (SpaPQRS, InvA). Single-particle analysis of w.t. and ΔSpa bases reveal marked differences in the cup and socket region.*

Figure 3: *Structure of the extracellular needle filament.*

organelle to sub-nanometer resolution by cryo EM and single particle analysis (Schraidt & Marlovits, 2011). The structure will serve as a basis to further understand the structural determinants required to form ring-like structures in membrane-embedded systems, and may also be used to design small molecules that interfere with the assembly pathway.

Assembly of the T3SS:

Our topological analysis revealed that additional proteins must be present. These constitute the cup/socket structure which is located in the center of the needle complex (export apparatus). Using mass spectrometry, we were able to identify five additional candidate proteins that co-fractionate in marginal quantities with purified needle complexes. Subsequent structural analysis revealed the absence of the cup/socket, suggesting that one or more of these proteins are required to build up the cup/socket (Figure 3). We were also able to show that these proteins nucleate the coordinated assembly of the needle complex (Wagner et al., 2010)

Structural Plasticity of the needle filament

Efficient effector protein translocation is known to occur only after host cell contact. Therefore, it is conceivable that the extracellular filament is a key player in the transmission of this information, probably due to small conformational changes throughout the filament. This hypothesis is supported by mutations found in the homologous Shigella needle filament, which convert the system into a constitutively "on" state. If this is true it would be justified to presume that the filament is provided with a certain degree of structural heterogeneity in order to accommodate the required conformational plasticity for signal transmission. We therefore analyzed the structure of the needle filament by cryo electron microscopy (Figure 3) and discovered that the structure is, indeed, highly variable (Galkin et al., 2010).

Although the design of the TTSS appears to be conceptually simple, many questions remain unanswered: How dynamic is the entire assembly process? How are substrates recognized by the needle complex? What is the molecular mechanism of protein translocation? We have started to address some of these questions. 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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Mitosis and chromosome biology

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Yan Sun / MitoCheck / MitoSys Project Manager

[1] until March 2010

To pass the genome from one generation to the next, eukaryotic cells first replicate their DNA, then bi-orient chromosomes on the mitotic spindle, and finally separate their sister chromatids, thus permitting division of one cell into two genetically identical daughter cells. We wish to understand 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 repair of DNA damage, 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, known as Wapl.

How does cohesin control chromatin structure and gene regulation?

Although cohesin is best known for its role in mediating cohesion, we and others have discovered that cohesin plays an important role in gene regulation. We suspect that these functions are the reason why cohesin binds to chromatin before cohesion is established, and why cohesin associates with DNA even in post-mitotic 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 the 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. We intend to test whether cohesin plays a general role in forming chromatin loops and understand the mechanistic basis of this function.

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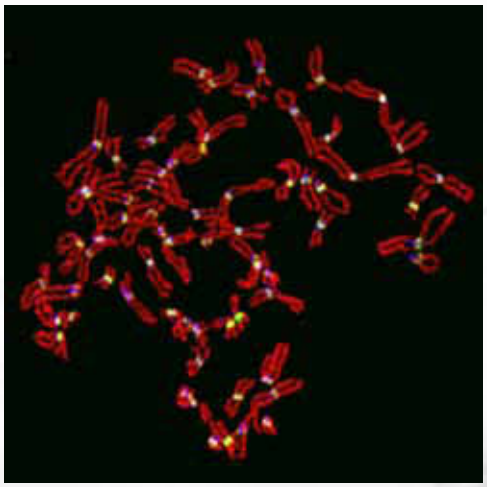

How is sister chromatid cohesion dissolved during mitosis?

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 was identified several years ago, its function and importance for chromosome segregation are still unknown. We therefore generated a conditional Wapl "knockout" mouse to study the role of the prophase pathway *in vivo*.

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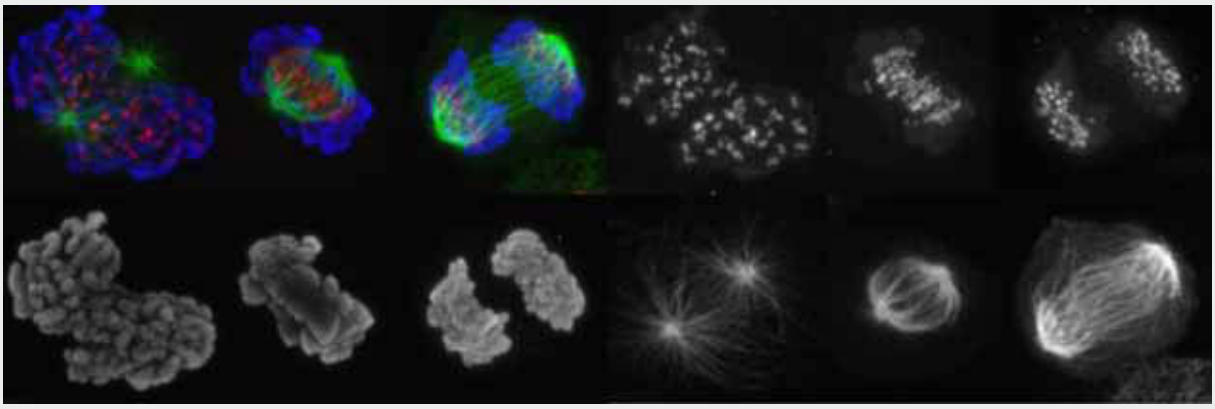

Figure 1: *Human mitotic chromosomes stained for condensin (red), cohesin (blue) and the centromere-specific histone Cenp-A (green). Courtsey of Peter Lenart.*

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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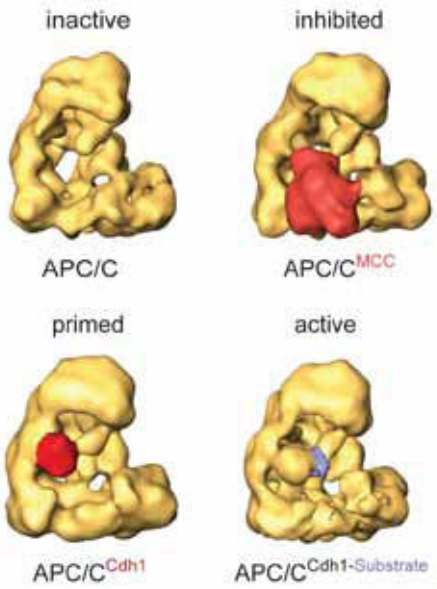

How does the APC/C initiate anaphase?

In metaphase, when all chromosomes have been bi-oriented, 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. Subsequent destruction of these proteins by the 26S proteasome allows activation of separase, cleavage of centromeric cohesin, and sister chromatid separation. Until chromosome bi-orientation is complete, 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, many of its aspects are poorly understood: how this complex is inhibited by the SAC, how the inhibition is relieved in metaphase, and how active APC/C recruits and ubiquitylates its substrates. We are using biochemical assays and electron microscopic analyses of the APC/C in different functional states to address these questions.

MitoCheck /MitoSys

Although mitosis has been studied for more than a century, our molecular understanding of this complicated process is far from complete. During the last five years the MitoCheck consortium funded by the European Union has developed and used genomic and proteomic approaches to study mitosis. The consortium employed RNA interference screens to identify proteins required for mitosis in human cells, tagging of genes in bacterial artificial chromosomes (BACs) for 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 can generally be used for high-throughput analysis of other processes in mammalian cells. In the future we will develop quantitative assays for mitosis in a new project funded by the European Union, known by the name of MitoSys.

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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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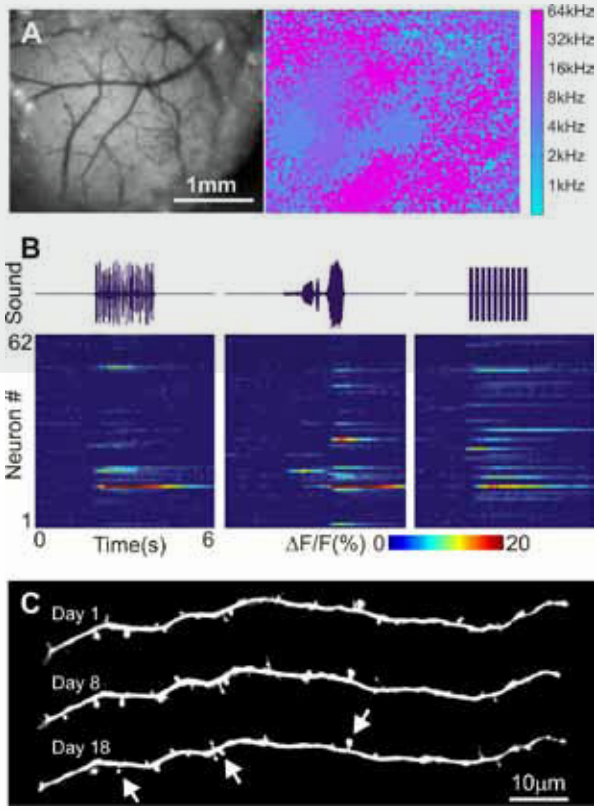

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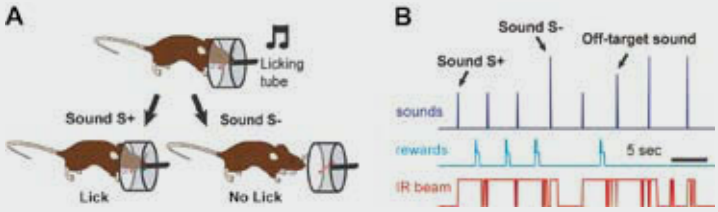

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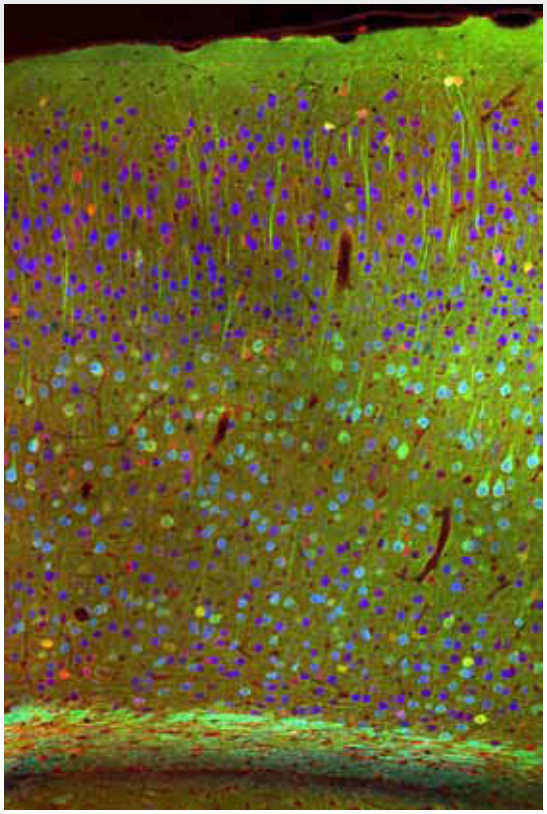

Figure 1: ***Imaging the auditory cortex.*** *(A) Mapping tonotopic organization of the auditory cortex using intrinsic imaging. (B) Using calcium imaging to monitor sound-evoked responses in multiple neurons simultaneously. Average population activity of 62 neurons in response to three different sounds. (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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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. We focus on transcription factor binding during embryonic mesoderm and muscle development (Zinzen et al., 2009). 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. Our results further suggest that the transcription factors vielfaltig/zelda and tramtrack are important determinants of transcription factor binding in the early embryo (Yáñez Cuna et al., submitted). We are also establishing tissue-specific ChIP-Seq in *Drosophila* to determine the tissue-specific targets of the circadian clock factors 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. As of today, we have assayed more than 2000 enhancer candidates and have seen an activity-rate of ~40% with diverse activity patterns throughout embryogenesis (Figure 1). Interestingly, the activity rate increases from about 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 have also established a high-throughput screen based on next-generation sequencing (NGS) to measure enhancer activity in specific cell types, and will analyze the sequences using bioinformatics and machine learning tools.

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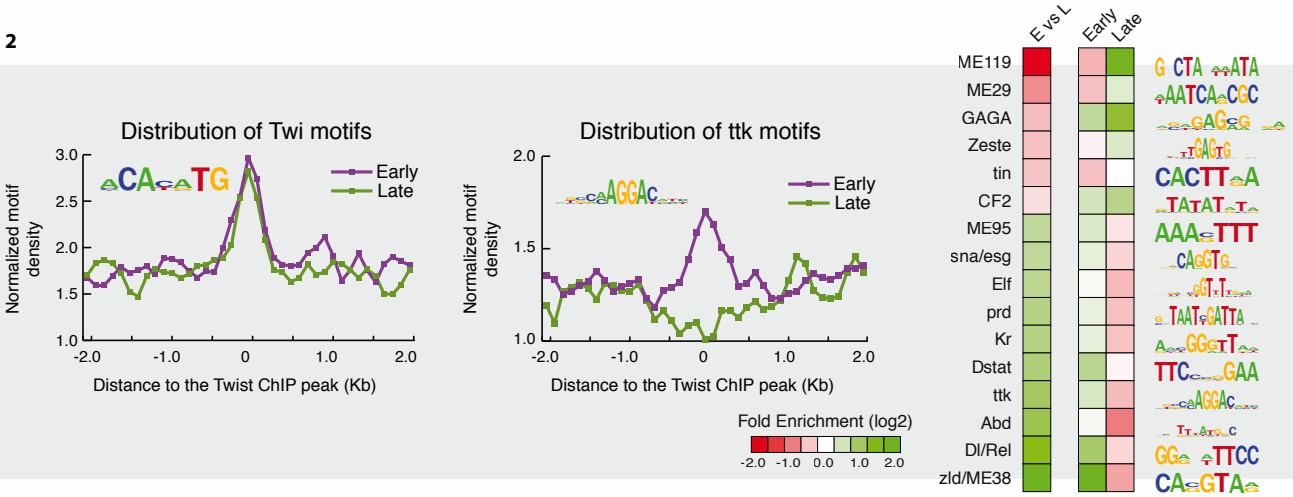

Figure 1: *Enhancer screen in transgenic Drosophila embryos. Shown is a sample of embryos that stain positively in our enhancer assay and display diverse activity patterns. Embryos are at different stages and shown in lateral orientation, anterior to the left. The inset shows a magnification of a blastoderm 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., Genome Research, in revision; ChIP-chip data from Zinzen et al., 2009).*

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).*

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 *Drosophila 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. It also includes more than 10,000 low-occupancy sites near the detection limit, which tend to mark enhancers of later developmental stages. We have developed computational methods to score motif conservation across different *Drosophila* genomes. These enabled us to discover novel motif types, as well as identify functional targets of many transcription factors and microRNAs with a high degree of certainty. 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. They will also allow us to integrate microRNA-mediated regulation into regulatory networks and understand their role in tissue-specific expression programs.

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 groups on campus and abroad.

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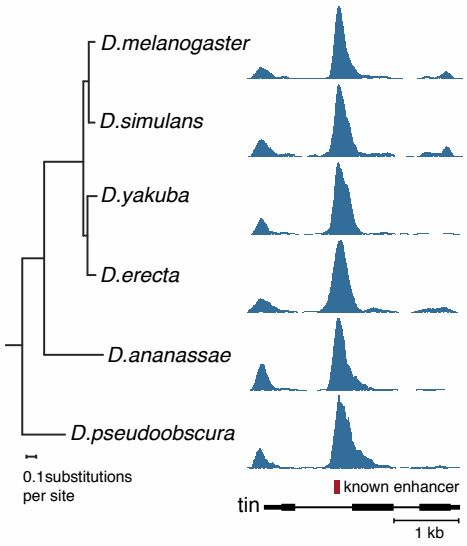

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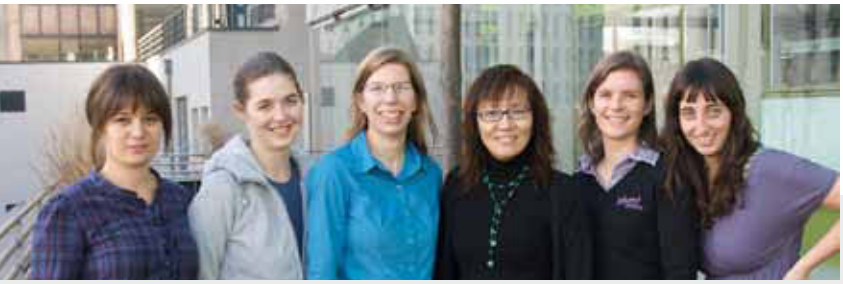

PEGGY STOLT-BERGNER

Transfer of Substrates Across the Cell Membrane

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All cells are surrounded by at least one impermeable lipid bilayer, and have evolved numerous types of proteins to allow them to interact with their environment by transducing signals and transporting substrates across this membrane. We are interested in understanding the molecular mechanisms these proteins use to accomplish their tasks.

Characterization of peptide transporters

Integral membrane proteins perform many vital cellular functions including energy metabolism, nutrient uptake, and signal transduction, yet comparatively little is known about their structure and the molecular mechanisms these proteins use to carry out their functions due to the challenges inherent in working with such hydrophobic proteins.

We are focusing on the structural and functional characterization of secondary active transporters, which drive the transport of substrates across the membrane by coupling this process to the energetically favorable transport of a proton or ion. While recent work has begun to uncover some of the details underlying secondary transport mechanisms, there are still few structures available, especially when considering proton-coupled transporters. Therefore, in many cases important questions remain unresolved, such as how substrate and ion translocation are coupled, how substrate specificity is determined, and what conformational changes occur during transport.

The peptide transporter (PTR) family represents a group of proton-coupled secondary transporters responsible for bulk uptake of amino acids in the form of di- and tri-peptides, an essential process employed across species ranging from bacteria to humans. In order to identify amino acids critical for peptide transport in a prokaryotic PTR member, we have screened a library of mutants of the *E. coli* peptide transporter YdgR using a high-throughput substrate uptake assay. We have identified 31 single point mutations that result in a full or partial loss of transport activity. Additional analysis, including homology modeling based on the crystal structure of the *S. oneidensis* peptide transporter $PepT_{So}$, identified E56 and R305 as potential periplasmic gating residues. In addition to providing new insights into transport by members of the PTR family, these mutants provide valuable tools for further study of the mechanism of peptide transport.

Facilitation of membrane protein crystallization

Structural studies on membrane proteins are challenging primarily due to the instability of these proteins when outside of the native membrane environment. Moreover, the conformational heterogeneity of membrane transporters can contribute to their instability. In parallel with attempts to crystallize secondary transporters, we are interested in developing methods to stabilize membrane proteins in order to facilitate their crystallization and biophysical characterization.

Mutagenesis to increase protein stability and conformational heterogeneity has been used successfully to solve several membrane protein structures. Despite the obvious success of this approach, the available methods depend upon having a known ligand that binds with high affinity to the protein of interest, and which can be radioactively or fluorescently labeled, making it less applicable to membrane proteins that associate with their substrates only weakly, or where a labeled substrate is not available. In order to overcome this limitation, we have attempted to develop a method to identify conformationally locked mutants in a manner independent of the protein's activity, by using determination of

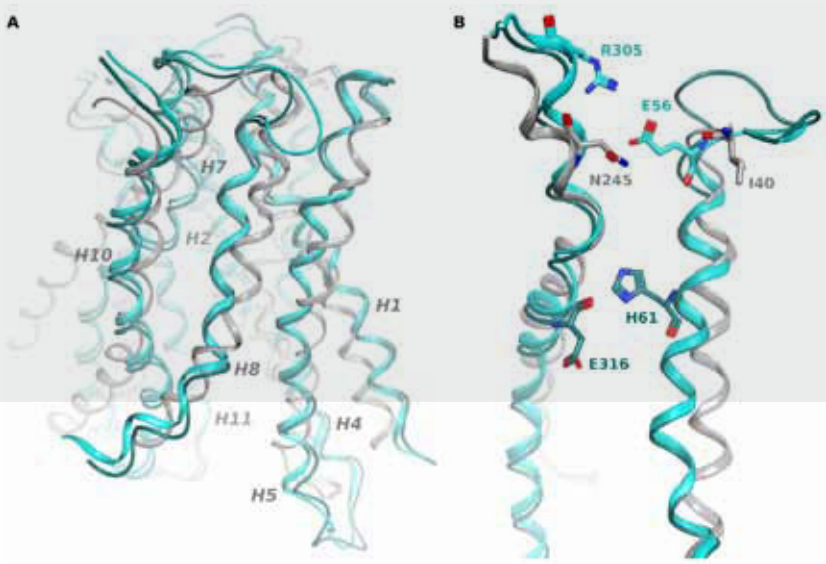

Figure : *Putative periplasmic gating residues of YdgRA YdgR homology model based on the structure of homologue $PepT_{so}$ was aligned with the crystal structures of LacY (pdb code 2CFQ) and $PepT_{so}$ (pdb code 2XUT) to compare the positions of the periplasmic gating residues from LacY (I40, N245) and $PepT_{so}$ (H61, E316) with E56 and R305. A. View perpendicular to the membrane. The backbone of the YdgR homology model (cyan) is aligned with that of the LacY structure (light gray) and that of $PepT_{so}$ (dark cyan). For clarity only helices lining the substrate cavity are shown, and are labeled in gray. B. The isolated helices 2 and 7 of the aligned structures, colored as in A. Gating residues from each protein are shown in stick conformation. (from Malle et al (2011) J Biol Chem 286:23121)*

protein aggregation temperatures. As a model system we have chosen the *E. coli* peptide transporter YdgR, which has very low affinity for its substrates, making this protein an unlikely candidate for approaches based on protein activity measurements. We have identified four mutants with increased thermostability using this method, however, only one was confirmed to be thermostable using alternative assays. We then compared this mutant to a targeted mutant designed based on homology with the LacY transporter, and early results indicate that both mutants preferentially adopt a single conformation. These mutants provide new tools for structural and functional studies on this transporter.

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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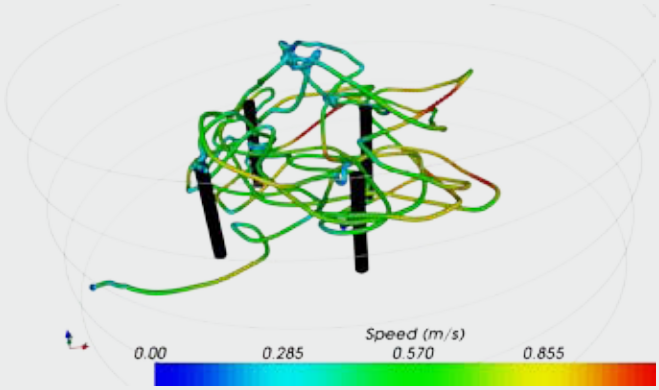

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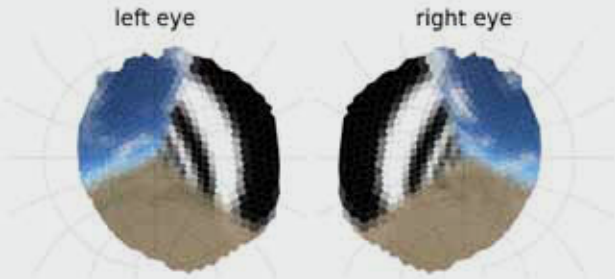

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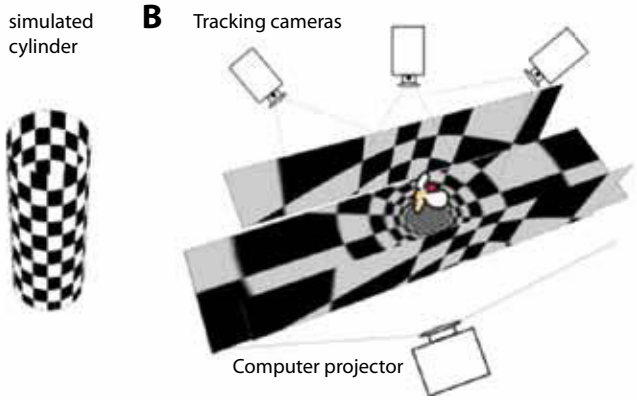

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Figure 1: *Top view of a 3D fly trajectory captured as a fly explored its environment, a 2 meter diameter cylinder and four vertical posts.*

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: the fundamental principles of protein and bio-molecular dynamics on the nano-scale, and the 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 is one of the main aspects of our research.

Light sculpting for high-resolution and ultrafast functional 3D imaging:

Using the frequency degree of freedom of light allows decoupling of lateral and axial resolution in lens-based microscopy, resulting in a large field of view while retaining high axial resolution. This light sculpting approach, also known as temporal focusing, was recently combined with super-resolution microscopy (PALM) to extend it to 3D imaging [1]. Temporal focusing may also serve as a new tool for high-speed scanning-less imaging of biological samples. Furthermore, it provides a tool for targeted single-cell-specific control of neuronal responses when combined with optogenetics, opening new ways for studying a wide range of fundamental neuroscience questions. We recently implemented a scheme for two-photon activation of channelrhodopsin (ChR2) by incorporating the temporal focusing approach [2], and used it to study the neuronal mechanisms of theta-phase-related oscillatory behaviors in hippocampal circuits [3]. This method has provided, for the first time, targeted single-neuron spatial resolution and millisecond temporal resolution. We plan to expand this method and create a flexible tool for high-throughput, fast, and specific two-photon optogenetic control and readout of neuronal activity.

Quantum-coherence-assisted ion transport and selectivity in ion channels:

Despite a large body of work, open fundamental questions concerning the mechanism of ion selectivity and transport of the selectivity filter of the potassium channel (KcsA) remain unanswered. We are interested to understand the details of the underlying molecular mechanism and the potential involvement of quantum coherence in this process. Based on our recent theoretical model, we hypothesize that an interplay between quantum coherence and environmental noise is responsible for the extreme ion selectivity of the potassium (K^+) channel and its high throughput rate. In this model, the nearly degenerate binding states of K^+ in the selectivity filter are in a coherent superposition. Environmental noise temporarily lowers the potential barrier between these states so as to allow tunneling only for specific ions. This would provide an explanation for the high throughput and selectivity of KcsA.

Our goal is to experimentally identify the signatures of transient interactions of K^+ with binding sites of the selectivity filter during ion conduction. Advanced spectroscopic techniques such as 2D femtosecond spectroscopy (2D IR) provide the necessary picosecond time resolution. We showed the feasibility of this approach by demonstrating the IR absorption and Raman spectroscopic signatures of potassium-binding model molecules (Fig. 2) that resemble ion coordination states in KcsA. The narrowing and frequency red shift, and diagonal narrowing of 2D IR line shapes on K^+-binding show the induced ordering and changes in the electrostatic environment.

To guide our experiments on the real system we performed molecular dynamic-based simulations of FTIR and 2D IR spectra of the entire KcsA complex. We found that, by

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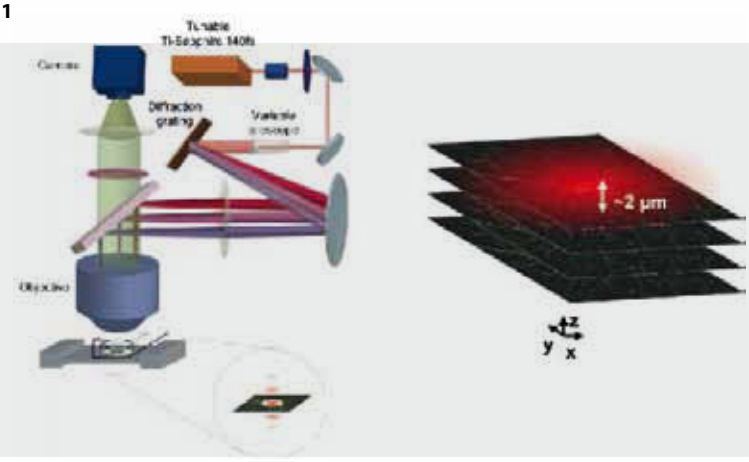

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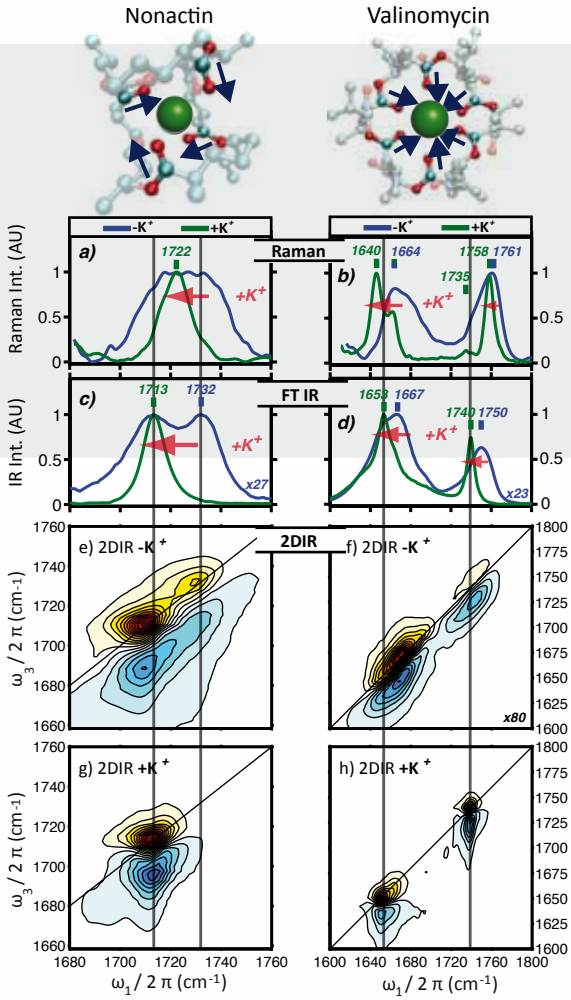

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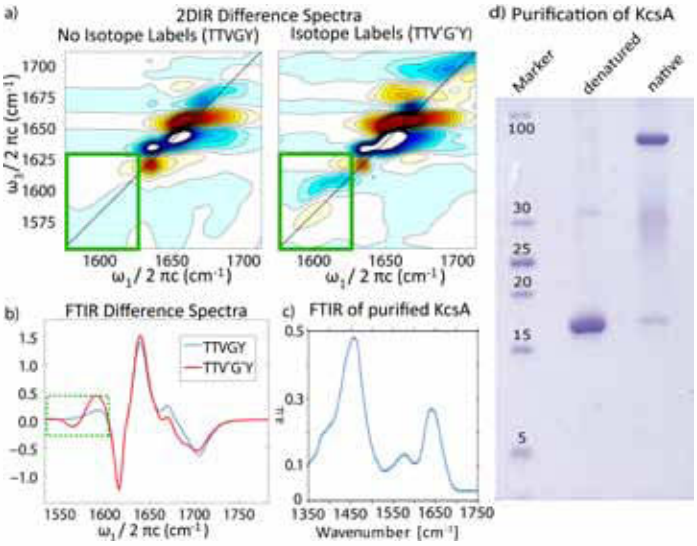

Figure 1: *Left: Typical setup for a temporal focusing microscope. Right: Light sculpting provides images with a large field of view while retaining high axial resolution.*

Figure 2: *Molecular structure (top) of K^+ binding model compounds nonactin and valinomycin, corresponding Raman (a, b), FTIR (c, d) and 2D IR spectra (e-f) in the carbonyl vibrational spectrum with and without K^+ indicate induced ordering and structural rearrangements.*

Figure 3: *Preparations for 2D IR spectroscopy of KcsA. Modeled a) 2D IR and b) FTIR spectra of KcsA show an observable K^+-binding signature on isotope labeling (green box). c) Experimental FTIR of unlabeled KcsA. d) Heterologously expressed KcsA can be purified as a folded tetramer (MW monomer 18kDa, Marker in kDa).*

combing isotope labeling with 2D IR spectroscopy, the signatures of K^+ interaction with individual binding sites could be observed experimentally in KcsA as well. Modeling of all possible isotope label combinations of the selectivity filter identified specific labeling combinations that would maximize our anticipated experimental signatures (Fig. 3a-c). As the next step we recently set up heterologous expression, purification (Fig. 3e) and deuteration for FTIR of purified KcsA (Fig. 3d), which has served as an intermediate step in performing 2D IR spectroscopy of an entire functional KcsA channel.

Ultimately detailed information about ion transport, selectivity, and underlying protein dynamics on the atomic scale and in high-time resolution will - in conjunction with mutational studies - lead to a new understanding of these processes and how they are facilitated by the interplay of quantum effects and environmental noise.

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STEFAN WESTERMANN

Molecular Mechanisms of Kinetochore Function

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Eurkaryotic 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.

Construction of a kinetochore in the test tube

Understanding kinetochore function requires study of its constituent parts, 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 is hierarchically assembled 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: the Dam1 complex, a specialized microtubule-binding component of the budding yeast kinetochore (Figure 1), oligomerizes to form a ring around microtubules *in vitro*. The ring slides along the microtubule lattice and remains attached to the plus-end even during microtubule disassembly. These properties make the Dam1 ring a very efficient force coupler at the kinetochore. A challenge for the future is to understand how the Dam1 ring is connected to the rest of the kinetochore, visualize the structure of the fully assembled interface, and analyze how it is regulated - for instance by mitotic kinases.

Building simplified chromosome segregation systems in the cell

To define functional modules within the complex kinetochore architecture, we have adopted a reductional approach in designing simple kinetochores *in vivo*. By artificially recruiting individual kinetochore components to engineered binding sites on circular plasmids and on native yeast chromosomes, we were able to 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.

A further challenge for the future is to understand how kinetochore structure and function are modulated throughout the cell cycle. The basic signals that couple cell cycle progression with the regulation of kinetochore function have remained elusive. Combining time-resolved analysis of post-translational modifications with yeast genetics is expected to disclose general principles of regulation.

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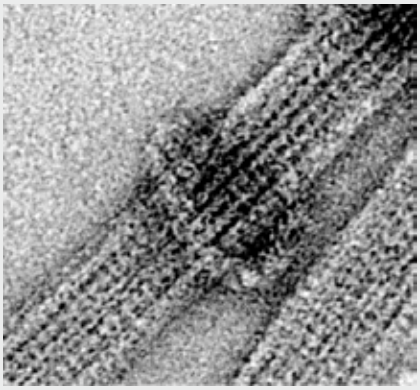

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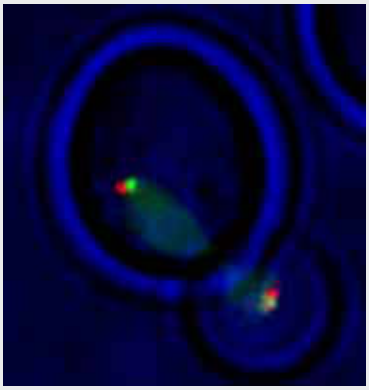

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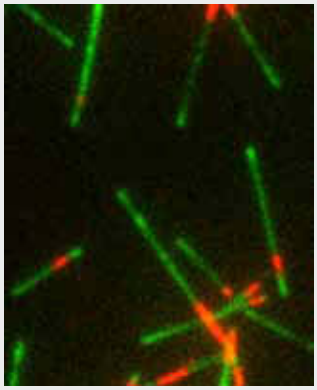

Figure 1: *The 10-protein Dam1 complex oligomerizes into a ring around the microtubule in vitro. Negative stain electron microscopy of a Dam1 ring decorating a taxol-stabilized microtubule.*

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 bright field image was pseudo-colored in blue to reveal the outline of the yeast cell.*

Figure 3: *Total internal reflection fluorescence (TIRF) microscopy of dynamic microtubules growing from stable microtubule seeds (red). The growing segments are decorated with the yeast EB1 protein Bim1p (green), which accumulates at the growing ends of the microtubules.*

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? What features allow it to follow a polymerizing microtubule end as well as stay connected during disassembly? How does the kinetochore modulate microtubule dynamics? To analyze this process we reconstituted dynamic microtubules *in vitro* and visualized the interaction of individual kinetochore components using total internal reflection fluorescence (TIRF) microscopy. This technique allows the observation of individual kinetochore complexes and microtubule-binding proteins with single-molecule sensitivity to reveal their mode of interaction with dynamic plus-ends. Our initial analysis has focused on the yeast EB1 protein Bim1p (Figure 3). We showed that this protein uses a microtubule-binding interface composed of a calponin-homology domain and a flexible basic linker to autonomously track growing microtubule ends *in vitro*. Multi-site phosphorylation of the linker domain by the Aurora kinase Ipl1p steers the interaction of Bim1p with microtubules and critically regulates the quantity of Bim1p on the mitotic spindle *in vivo*. In the future we aim to reconstitute additional kinetochore plus-end tracking systems to define functional dependencies.

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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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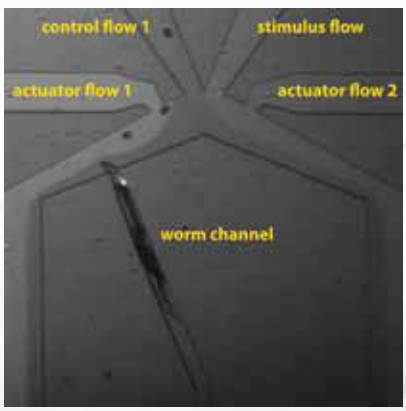

To study behavior, we are filming worm populations that experience downshifts and upshifts in environmental oxygen levels. Image processing and further computer analysis is used to quantify simple locomotion responses. To measure the neuronal activity of individual neurons, we use real-time fluorescence 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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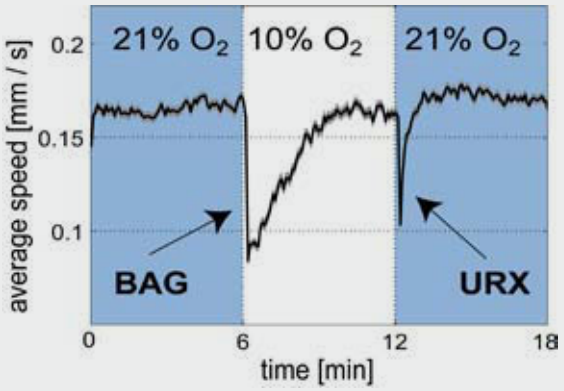

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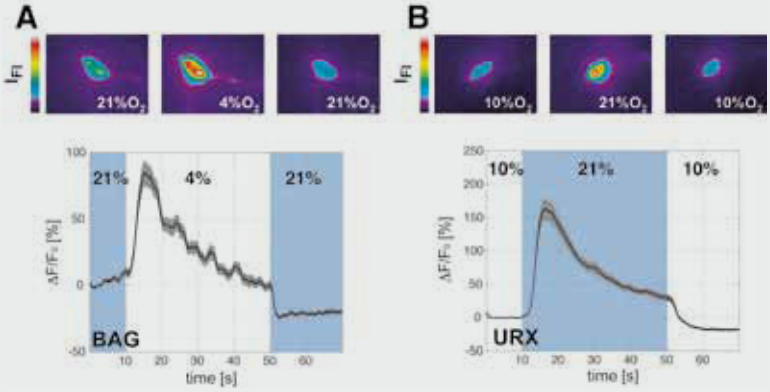

Figure 1: ***A microfluidic device for worm immobilization and stimulus delivery.*** *We designed a worm immobilization channel from an optically transparent silicon polymer. The channel is connected to a microfluidic delivery network. A worm is pushed into the immobilization channel so that its nose 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

Cancer drug target discovery using *in-vivo* RNAi

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The genetic complexity and heterogeneity of human cancers complicate the search for effective targeted therapies. The advent of RNA interference (RNAi) has provided a new tool to reveal cancer-specific genetic vulnerabilities in a systematic and unbiased way. Our lab uses an integrative approach combining new in-vivo RNAi technologies and genetically defined cancer models to identify and evaluate new therapeutic targets in leukemia and other cancers.

Tractable and traceable models of human AML

Acute myeloid leukemia (AML) accounts for ~80% of all adult acute leukemias and remains the most common cause of leukemia death. Extensive oncogenomic studies have revealed over 100 AML disease alleles, which occur in different combinations and impact the outcome of clinical therapy. To dissect this genetic complexity, our lab uses a rapid mosaic approach to generate experimentally tractable AML models that are based on defined compound mutations, implement bioluminescence monitoring and enable studying the disease in a physiologic environment (Fig. 1). In previous work we have established that these models can accurately mirror the pathology, disease course and treatment response of human AML. More recently, we have used these models to decipher a Myb-driven gene expression program, which leukemia cells hijack to evade normal cell-fate programs and maintain a state of aberrant self-renewal. Remarkably, partial and transient suppression of Myb - while being well tolerated in normal myelopoiesis - can cure advanced chemotherapy-resistant AML (Fig. 2), suggesting that strategies to inhibit Myb-dependent expression programs would provide highly effective therapeutics, even in otherwise refractory cases.

Tet-on RNAi technology for modeling targeted therapies *in vivo*

To systematically identify genetic dependencies in pool-based shRNA screens and to subsequently use RNAi for studying the consequences of acutely inhibiting a target in an intervention setting (i.e. in established tumors *in vivo*), our lab applies a Tet-on conditional shRNA expression system that implements several recent technological advances. For example, the recently established "Sensor assay" provides a new approach to rapidly identify potent shRNAs in a high-throughput format. Results from a Sensor-based evaluation of ~20,000 unbiased shRNAs have led to the establishment of improved shRNA-specific design algorithms. Further advances include retroviral Tet-on shRNA expression vectors optimized for studying deleterious phenotypes (TRMPV series), a targeted knock-in platform for generating Tet-shRNA transgenic mice, and an established workflow for multiplexed shRNA screening *in vitro* and *in vivo*. In addition, we recently have established a robotics- and deep-sequencing based pipeline for rapid and cost-effective production of single-clone validated shRNA libraries. While we together with several collaborating groups will continue to further improve RNAi technologies, our recent advances have now established a robust toolkit to identify and evaluate candidate drug targets *in vivo*.

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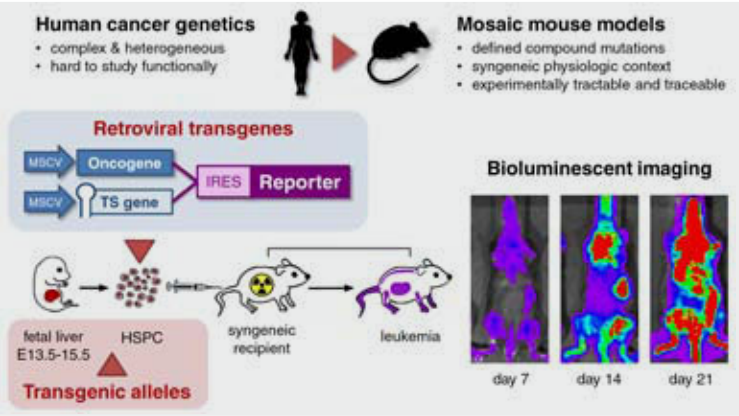

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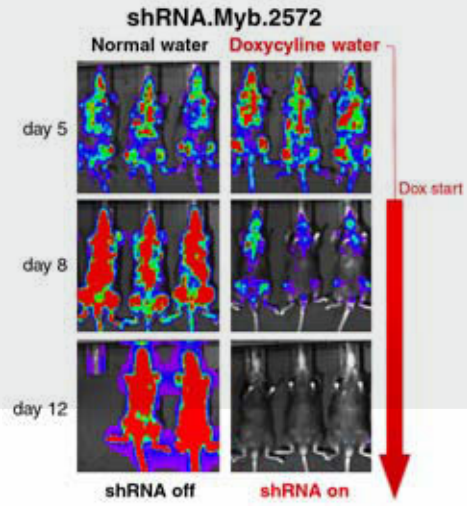

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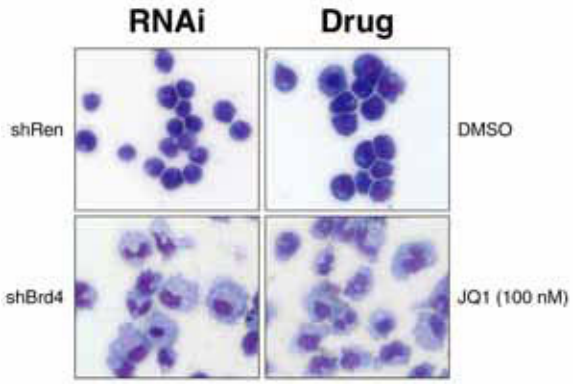

Figure 1: *Mosaic mouse models of human AML. Genetic aberrations found in human AML are introduced in defined combinations into murine hematopoietic stem and progenitor cells (HSPC), which are subsequently transplanted into lethally irradiated syngeneic recipient mice. Bioluminescent reporter genes are co-transduced using bicistronic expression vectors and are used to monitor resulting diseases in vivo.*

Figure 2: *RNAi-mediated Myb suppression cures aggressive AML. Bioluminescent imaging of recipient mice transplanted with clonal Tet-on competent MLL-AF9;Nras^{G12D} AML cells harboring a potent Myb shRNA in a Tet-inducible vector (TRMPV-Neo). Following disease onset (day 5), shRNA expression is induced by addition of doxycycline to the drinking water. While the disease rapidly progresses in untreated control mice, all dox treated animals show complete disease remission following Myb suppression.*

Figure 3: *RNAi- and small-molecule mediated suppression of Brd4 similarly induce terminal myeloid differentiation. Light microscopy of May-Grünwald/Giemsa-stained MLL-AF9;Nras^{G12D} leukemia cells after 2 days of doxycycline-induced shRNA expression or 2 days of treatment with the Brd4-inhibitor JQ1 (100 nM). In both cases Brd4 suppression triggers terminal differentiation of leukemic blasts into mature macrophage-like cells, demonstrating that RNAi can accurately predict the effects of small-molecule inhibitors.*

A chromatin-focused RNAi screen identifies Brd4 as a drug target in AML

In a first screen, we recently have applied our new platform to systematically probe the dependency on chromatin-associated genes in MLL-AF9;Nras^{G12D}-driven AML. Through generating and screening a custom shRNA library targeting 250 chromatin regulators we have identified the bromodomain containing protein Brd4 as a critical requirement for AML maintenance. Suppression of Brd4 using shRNAs or the small-molecule inhibitor JQ1 led to robust anti-leukemic effects, accompanied by terminal myeloid differentiation (Fig. 3) and elimination of leukemia stem cells. Moreover, we found that these effects are in large part triggered by an acute breakdown of Myc expression, whose transcription directly depends on Brd4. Together, these results identify small-molecule suppression of Brd4 as the first pharmacological strategy to inhibit Myc in cancer and unambiguously highlight the utility of RNAi screening for discovering cancer-specific genetic vulnerabilities and guiding the development of rational cancer therapeutics. Following these pioneering studies, our current major aim is to extend these screening efforts to different genetic subtypes of AML, new focused shRNA libraries and, eventually, other types of cancer.

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BIOOPTICS

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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 in planning experiments and implementing 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 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 scanners 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 intensive Advanced Practical Microscopy Course is organized on a yearly basis, including hands-on sessions and lectures by internal and external faculty members.

Image Processing and Analysis

Five state-of-the-art computer workstations are available at the BioOptics Facility, operating most types of common commercially available 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. Organized courses are also provided. 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/

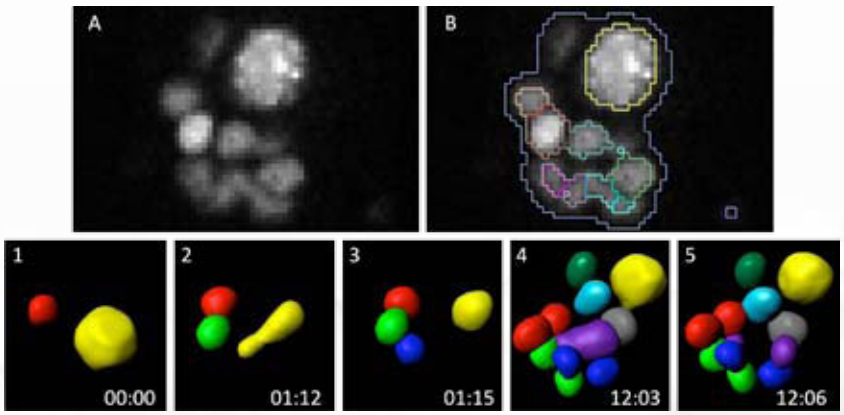
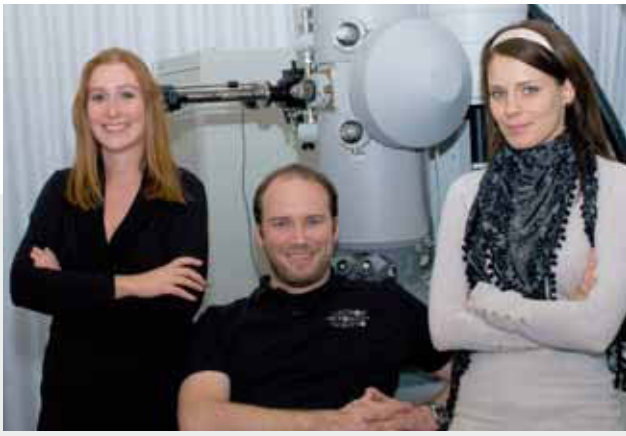


Figure: Using Fluorescence activated cell sorting (FACS) Drosophila neural stem cells, specifically labeled with a nuclear GFP, were isolated from larval brain tissue and subsequently, live-cell imaging has been performed on a spinning disk confocal to monitor cell-cycle time and cell growth of these neuroblasts. Cells were segmented in 3D and the neuroblast and its offspring were linked over time. Panel A shows a single slice of an image stack, panel B the respective segmentation and classification. The stills 1 to 5 show different surface rendered time points of the movie; the neuroblast is highlighted in yellow and its offspring is labeled by different colors. Daughter cells that itself underwent a round of division show the same color. The time is indicated in hours and minutes.



ELECTRON MICROSCOPY

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The Electron Microscopy Facility provides a wide range of preparation techniques for tissues, cells, and purified molecules for transmission electron microscopy, as well as facilities for microscopy, data management and image processing. Access to scanning electron microscopy is provided via external collaboration.

Preparation of Specimens

Know-how, training and instrumentation for a wide variety of preparation techniques for visualization of ultrastructure in tissues or cells and of biomolecules by transmission electron microscopy (TEM) are being offered by the Electron Microscopy Facility to researchers at IMP, IMBA and GMI. Techniques routinely used at the facility include the production of support films, negative staining, rotary shadowing of sprayed molecules, chemical fixation, immersion freezing, high pressure freezing, freeze substitution, embedding in epoxy- and acrylic resins, and ultrathin sectioning of resin-embedded or frozen samples.

Microscopy

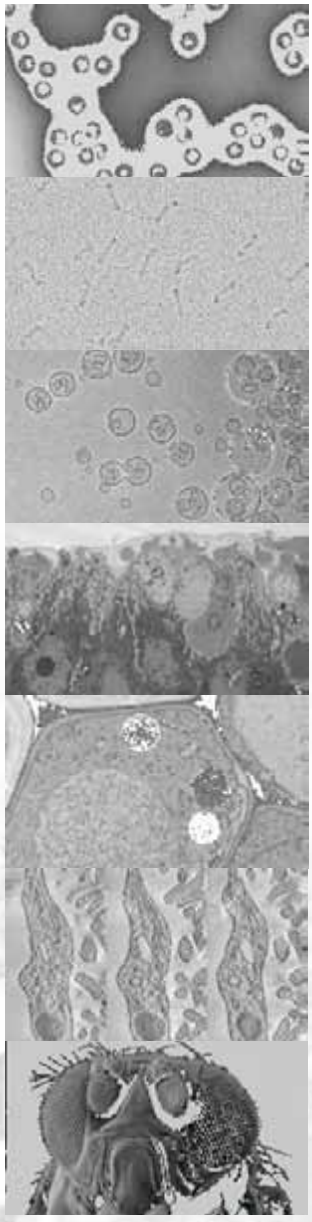
The Facility is equipped with two instruments: The FEI Morgagni is a robust and easy-to-use 100 kV TEM, equipped with an 11-megapixel CCD camera. It is tailored to meet routine requirements at the Facility's multiuser environment. Advanced applications run on the FEI TF30 Polara. This 300 kV TEM, unique in Austria and equipped with the most advanced imaging systems, was funded by a Vienna Spot of Excellence grant and became fully operational at the beginning of 2008. It is primarily used for cryo-TEM of molecules and cells, electron tomography, and electron energy loss spectroscopy.

Software Development and Image Processing

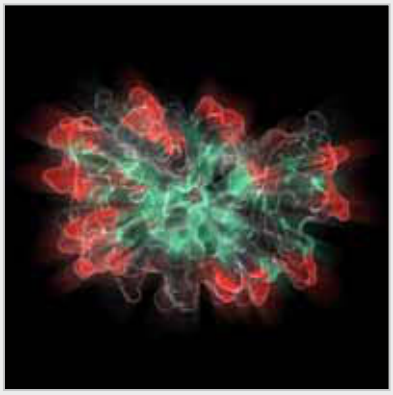
To support users with data management, a Web-based project-oriented database system named MIMAS was developed by, and is being run at, the Facility. Electron micrographs from both microscopes, including meta data, can be stored on and accessed from this database on a user-restricted basis. Furthermore, software solutions for automated image acquisition and for status monitoring of the microscope were developed on the Polara. Workstations and training are provided for image processing of EM data, especially those obtained by electron tomography.

Guenter Resch / Head of Electron Microscopy Facility

Marlene Brandstetter / Technical Assistant
Nicole Fellner / Technical Assistant
Karin Trimmel / Trainee



A: *Negatively stained rotavirus-like particles (Cornelia Gänger, Ringrose Group, IMBA)*
B: *Glycerol sprayed and rotary shadowed α-actinin molecules.*
C: *DPPC liposomes visualized by cryo electron microscopy.*
D: *Drosophila melanogaster intestine also showing stem cells from a chemically fixed specimen (sample: Ryan Conder, Knoblich Group, IMBA).*
E: *Cells from high pressure frozen Arabidopsis thaliana root (sample: Matzke Group, GMI).*
F: *z-Sections from a three-dimensional reconstruction by electron tomography showing the endothelial lining in a blood vessel.*
G: *Scanning electron micrograph of Drosophila melanogaster.*



BIOINFORMATICS

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Thomas Burkard / Computational Biologist
Wolfgang Lugmayr / Software Engineer
Maria Novatchkova / Computational Biologist
Alexander Schleiffer / Computational Biologist

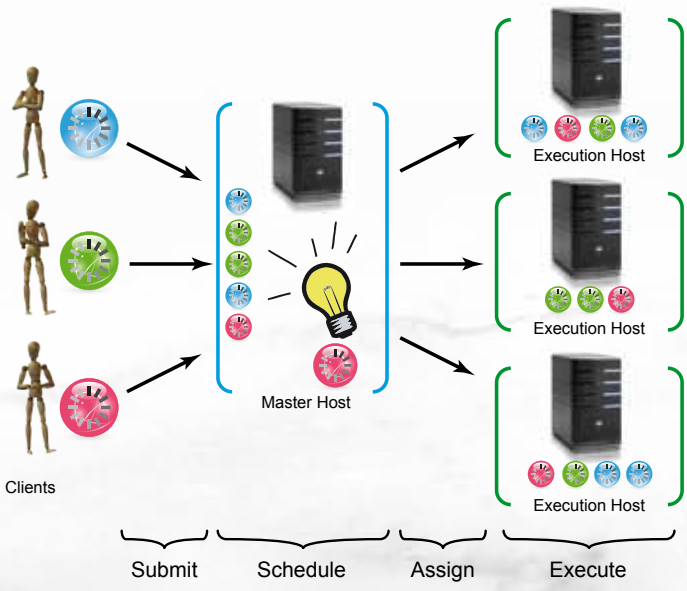
The Bioinformatics unit assists research groups in molecular-biology-related fields by providing sequence analysis services, scientific data mining, hardware and software infrastructure, and training in bioinformatics.

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. We engage in custom software and database development, and design computational and mathematical solutions that can cope with higher load and memory requirements. To perform complex sequence analysis tasks, we also 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, Flymine) 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, the main computing cluster has been updated to a state-of-the-art processing system using batch and parallel computing environments. The cluster is managed by the Sun Gridengine (SGE) software, which provides policy-based workload management for a large number of jobs and nodes. Software installed and maintained on the bioinformatics cluster 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 on dedicated clients to the Sun Gridengine (SGE) master, which is responsible for running the jobs on the cluster nodes.

GENOMICS

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Martin Radolf / Engineer
Harald Scheuch / Engineer

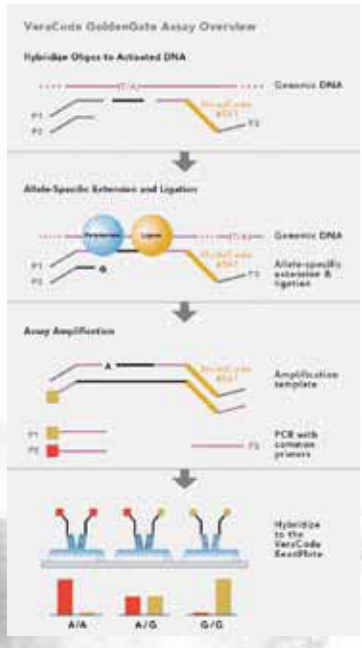
After successful establishment of the Next Generation Sequencing Service, Andreas Sommer and his team joined the newly formed CSF. We routinely offer hybridization and analysis of self-spotted cDNA and Agilent Arrays for gene expression analysis for various model organisms.

Robotics:

We established a high-throughput plasmid DNA isolation platform using the Xiril 100 robot, which is also used to pipette 384 RT PCR reactions and isolate genomic DNA in a 96-well format.
In cooperation with Zuber Lab we have established a pipeline for generating single-clone validated oligonucleotide-based libraries. Our approach combines on-chip oligonucleotide synthesis, liquid handling robotics, and a barcoding strategy for deep-sequencing-based single clone validation. In one application the pipeline will be used for rapid and cost-effective production of custom-designed shRNA libraries. The Agilent Bravo pipetting robot was mainly used to process in situ reactions together with the Stark group, PCR set-up, and purification. Like Precision XS from Biotek, the Bravo liquid handling station is used to pipette reactions for SNP genotyping.

SNP Genotyping

Together with the Nordborg Group from GMI we have designed a 384 SNP Illumina assay that can be used as a "universal" genotyping panel for Arabidopsis. We use Illumina 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.



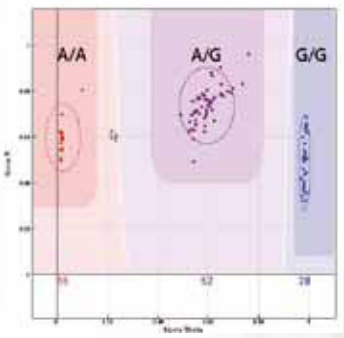


Figure: *Three oligo-nucleotides are synthesized for each SNP: two allele-specific oligos that distinguish the SNP, and a locus-specific sequence just downstream of the SNP.*

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
Johannes Stadlmann / Postdoc
Werner Straube / Postdoc
Debora Broch-Trentini [2] / Phd Student
Thomas Taus / Diploma 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

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

Mass Spectrometry for Systems Biology

Genome-wide transcriptome analysis has provided systems-level insights into gene regulation. However, comparable analysis of post-transcriptional processes has been hampered by the limited depth of quantitative proteomics. We use chromatographic separation and iTRAQ (isobaric tag for relative and absolute quantitation) technologies to overcome these limitations. In a collaboration with the Knoblich group, we have been able to determine the brain-specific expression of more than 6000 proteins in the fruit fly *Drosophila melanogaster*, corresponding to about 70% of all potentially transcribed protein-coding genes in the brain. Our data demonstrate that quantitative proteomics yields important insights into gene regulation beyond transcriptomics, and serves as a rich source for achieving a systems-level understanding of post-transcriptional gene regulation.

Localization of Phosphorylation Sites

In addition to protein identification the detection and correct localisation of phosphorylation sites is an important task in mass spectrometry-driven proteomics. We have developed a novel software tool, phosphoRS, which permits probability-based localisation of phosphorylation sites. The algorithm assigns probabilities to all potential phosphorylation sites on phospho-peptides and works for all types of peptide fragmentation modes.

Functional Analysis of Arginine Phosphorylation

Recently, the first arginine kinase was described. This kinase, McsB, is a major regulator of stress response in Gram-positive bacteria. So far, only one substrate of McsB, the class-three transcriptional regulator (CtsR), has been studied in detail. Other targets have not been identified yet. In a collaboration with the Clausen group we develop novel methods in order to isolate and systematically detect *in vivo* arginine-phosphorylated proteins by mass spectrometry. This has enabled us to study the pivotal role of this posttranslational modification in the bacterium *Bacillus subtilis* as a response to adverse growth conditions. Furthermore, we aim to investigate the occurrence of this phosphorylation in higher organisms and thus uncover novel regulatory mechanisms.

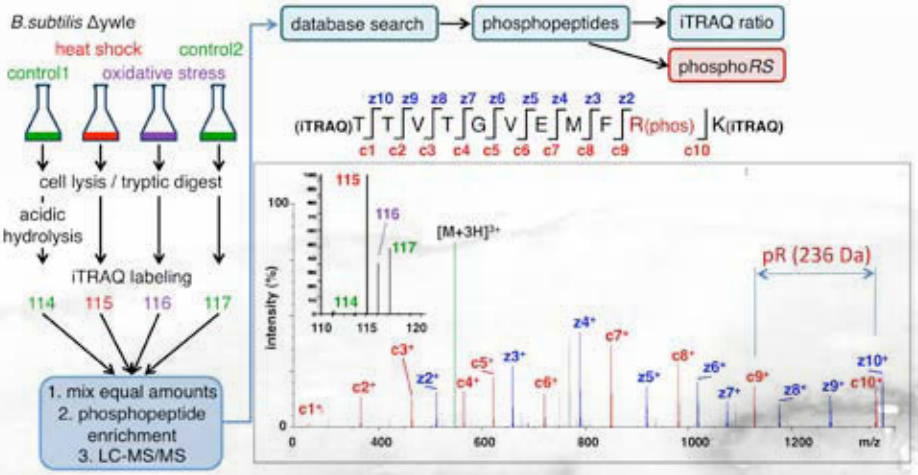

Figure: *Determination of in vivo McsB activity by quantitative proteomics*
B. subtilis arginine phosphatase-deficient cells (Δywle) were subjected to heat and oxidative stress. Phosphorylation on arginine R265 of elongation factor Tu was detected from the MS/MS spectrum (right panel). iTRAQ quantification revealed an upregulation of this phosphorylation under heat shock conditions.

SERVICE DEPARTMENT

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The Service Department offers a variety of high-quality rapid services to IMP, IMBA and GMI scientists. The large part of our work involves DNA sequencing, fly food production and preparation of various media and solutions.

Gotthold Schaffner will be retiring from active service at the end of this year. The Media Kitchen (which will be an independent service group in the future) and the Fly Food unit, (which will also be an independent group in the future) prepare substantial quantities of reagent-quality solutions and media for cell cultures, flies (more than 1,800,000 tubes and bottles per year) and other organisms.

The Service Department also prepares a number of selected reagents such as DNA molecular weight markers, enzymes, a variety of transformation-competent E. coli strains; and maintains a stock of cloning vectors, sequencing primers and other reagents.

Production of antibodies

In collaboration with IMP group members, Ivan Botto, a member of the Service Department, produces and isolates many different monoclonal antibodies from hybridoma cell lines. This activity takes up a substantial part of the department's recources.

Sequencing and DNA isolation

The 48-capillary ABI 3730 DNA Genetic Analyzer was supplemented by an ABI 3730XL with 96 capillaries early this year, as the 48-capillary sequencer had clearly reached its limits. We sequenced approximately 140,000 samples during the first nine months of this year. This substantially higher demand was mainly due to screening projects, the fly library, as well as new groups at IMBA, IMP, and GMI.

The quality and concentration of DNA samples is still a problem, even when prepared by sophisticated Qiagen kits like Midi-, Maxi- or Minipreps. The same is true for incorrect primer sets or poorly documented plasmid constructs from external sources. Sequencing is performed even more rapidly now because of the tripled capacity. It has thus become much easier and faster than analyzing samples by restriction digests and running them on an agarose gel. This concurs with the new style of working with large quantities of samples. Clean-up 96-well microtiter plates, filled automatically with a BioTek benchtop minirobot, Sephadex G50 superfine slurry, and centrifugation conditions have to be optimized further. The reason for the occasional, but sometimes quite obvious "dye blobs" one encounters when using DNA samples of low quality is still unclear. The larger quantity of contaminants as compared to PCR products, which yield a much stronger signal than double-stranded plasmid DNA, obviously plays a major role.

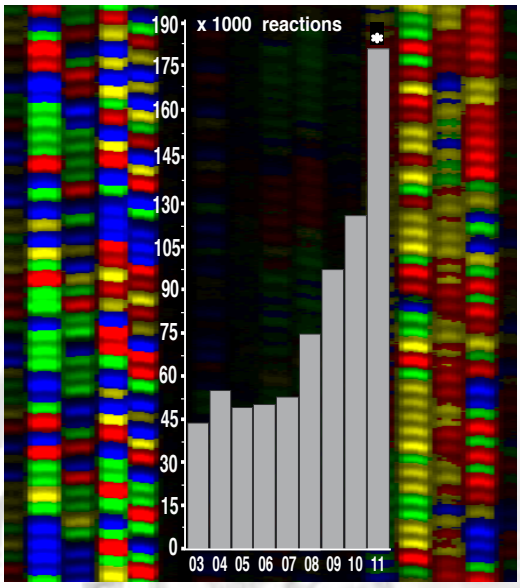

Figure: *A sequencing run on an ABI 377 PRISM and number of reactions analyzed on ABI 3100 (since 2001) and ABI 3730 (since June 2004), performed with dye deoxy terminators (v3.0 since 2001) from 2003 to 2011 (scale 0 to 190,000).*
**calculated from January 2011 to September 2011 data*

Gotthold Schaffner / Head of Facility

Ivan Botto / Technical Assistant
Zuzana Dzupinkova / Technical Assistant
Markus Hohl / Technical Assistant
Shahryar Taghybeeglu / Technical Assistant
Christa Detz-Jaderny / Technician Media Kitchen
Dagmar Faustenhammer / Technician Media Kitchen
Ulrike Windholz / Technician Media Kitchen
Christine Giesel / Technician Fly Food Preparation
Janusz Petri / Technician Fly Food Preparation
Franziska Stransky / Technician Fly Food Preparation
Oliver Botto / Freelancer Fly Food Preparation
Thomas Haydn / Freelancer Fly Food Preparation
Anna Windholz / Freelancer Fly Food Preparation

HISTOLOGY

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Vukoslav Komnenovic / Head of Facility

Mihaela Zeba[1] / Technical Assistant

[1]*on maternity leave*

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.

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

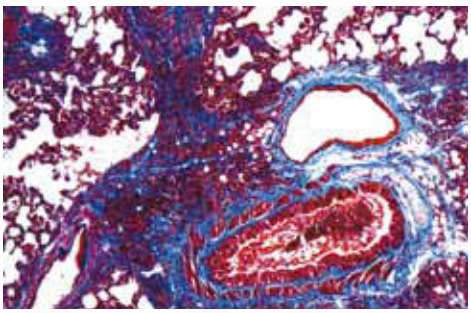
In our group we have developed a high throughput method to cut paraffin and frozen tissues. Using this method, we could increase the quality and also the quantity of services.

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), I-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).

1



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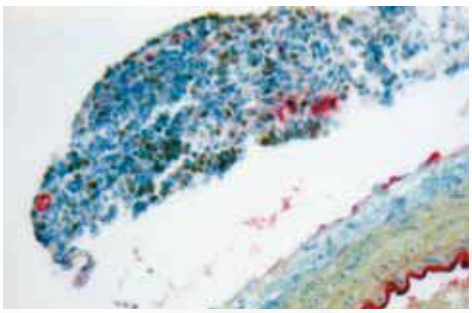

Figure 1: *Trichrom blue staining. The picture shows 4 different signals, light blue for Lung fibrosis, red for Mast cells and Neutrophils, pink for Myeloiud cells and dark blue for the Nuclei.*

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

transgenic@imp.ac.at

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 morulae, 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 Meinrad Busslinger.

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

Figure 2: *Mouse blastocysts.*

MAX PERUTZ LIBRARY

library@imp.ac.at

Karlo Pavlovic / Librarian

Jürgen Sallachner / Trainee

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

The main task of the library is to provide comprehensive scientific literature pertaining to the areas of research pursued at the institutes. The Max Perutz Library holds approximately 3500 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. All book holdings can be searched for systematically in the online catalog, where each item is described in respect of its availability and accessibility. The most heavily used kind of literature resource are the licensed online journals: Approximately one full text view per scientific affiliate per day strikingly illustrates its value.

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 licensed by the library.

Study environment

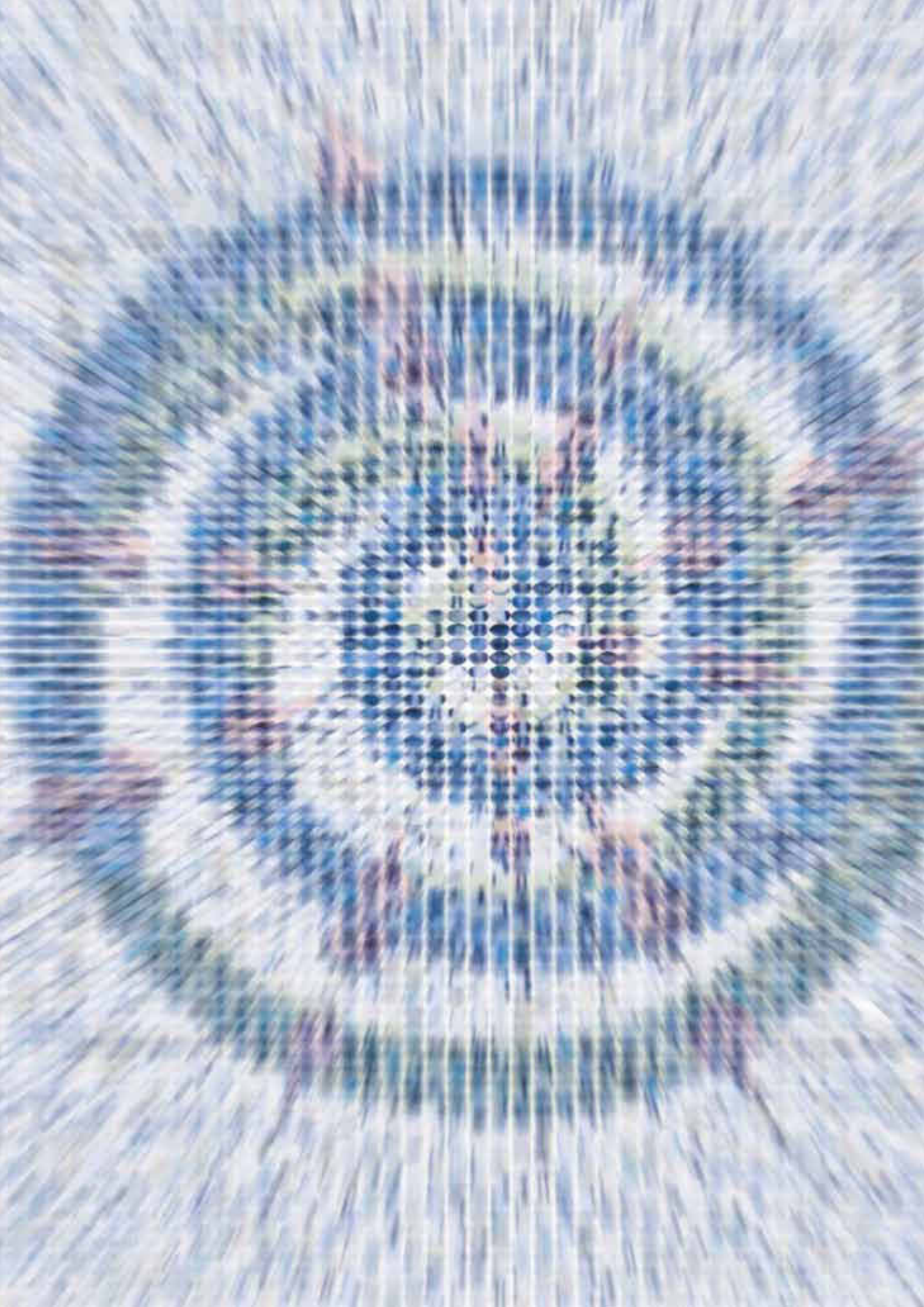
The reading room serves as a quiet and well-equipped place for reading, writing or just relaxing. Twenty-four study desks and a cozy lounge as well as two public computers, wireless LAN and a printing facility are provided.

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.



CSF – General Information

www.csf.ac.at

The Campus Sciences Support Facilities GmbH (CSF) is the most recent member to join the wide range of research institutions and companies that make up the Campus Vienna Biocenter. The CSF was founded in the beginning of 2011 as the first ever publicly funded provider of scientific infrastructure in Austria. The CSF funding was granted jointly by the City of Vienna and the Federal Ministry for Science and Research in response to the Vision 2020 application, and comprises 52 million euros over 10 years, divided between eight core facilities.

During the first year the CSF has established an administrative team and incorporated the first two Core Facilities, the **Vienna *Drosophila* RNAi Center (VDRC)** and the IMP-IMBA Deep Sequencing Unit (now known as **Next Generation Sequencing, NGS**), who started providing services through CSF in March 2011.

Additionally, the core facility concepts foreseen in Vision 2020 were revised, in close cooperation with the users, and the next three units are scheduled to start their Campus-wide services at the beginning of 2012:

The Structural Biology Facility (SBF) will offer three different services: Protein Expression in eukaryotic systems, Protein Crystallisation and Large Scale Screening. Peggy Stolt-Bergner has been recruited as the head of this service.

Andras Aszodi, head of the **Scientific Computing Core (SCC)**, will support core facilities and researchers with a streamlined collection of data analysis and visualisation tools.

Electron Microscopy (EM) will be integrated into the Ultra-Resolution Imaging unit. Günter Resch, head of the Electron Microscopy, is bringing his expertise to the start-up process.

Next Generation Sequencing (NGS)

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Andreas Sommer / Head of the NGS

Benjamin Almeida
Carmen Czepe
Renate Landwehr
Ido Tamir

Next Generation Sequencing, also known as Massive Parallel Sequencing, has become a key analysis method for a large number of biological research areas. The capacity to expand analysis from more or less defined genomic regions to genome-wide studies has boosted the pace of research discovery and has enabled researchers to obtain a global view on biological processes.

As of March 2011, sequencing resources were shifted from the joint Genomics facility to the newly founded CSF Next Generation Sequencing Unit. A team was built up consisting of a head of the unit, two bioinformaticians and two lab technicians. Also, billing was introduced to cover reagent costs and hands-on personnel time.
A HiSeq instrument, Illumina's sequencing platform with maximal throughput, was installed and is currently used for most long read applications, while the three GAIIx Systems cover short and single read sequencing.

Opening the gates to the whole VBC campus brought in new users and applications as well as an increased demand for sequencing. By the beginning of November, 950 samples from 39 scientific groups had been submitted and a total of 650 lanes sequenced.
The NGS Unit expanded its bioinformatic tool repertoire adding TopHat and Cufflinks for RNA-seq analysis as well as a local installation of Galaxy, a web-based analysis platform. Galaxy workshops and a Sequencing Symposium with invited speakers from all Viennese sequencing institutions were organized in addition to regular User Meetings.

Vienna *Drosophila* RNAi Center (VDRC)

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Reinhard Klug / Stock maintenance supervisor
Thomas Micheler / Software developer
L. Zemann/V. Salva[1] / Administration
Barbara Müllner / Technician
Andreas Gansch / Technician
Judith Utner[1] / Technician
Alisher Tashpulatov / Technician
René Zörnig[2] / Technician
Irina Kolarov / Technician
Michaela Eckmann / Technician
Kristina Belogradova / Technician
Irene Penzenauer / Technician
Sonja Lang / Technician
Florence Maxwell / Technician
Rainer Keck[1] / Technician
Yulia Barinova[1] / Technician
Angela Graf[1] / Technician
Svetlana Zorinyants / Technician
Sandor Urmosi – Incze / Technician
Elena Popowich / Technician

[1] 50%
[2] 80%

Genome-wide RNAi

RNAi can be effectively triggered in Drosophila by spatially and temporally controlled expression of a dsRNA from a transgene that contains a long inverted repeat under control of a genetic promoter, the GAL4-responsive element (Figure1). The initial creation of a genome-wide transgenic RNAi library [1] has revolutionized Drosophila genetics. We have generated two independent genome-wide transgenic RNAi libraries for Drosophila, allowing researchers to systematically study gene functions in specific tissues at specific developmental stages. The VDRC maintains and further develops these libraries, and distributes RNAi lines to Drosophila researchers world-wide.

In addition to the original library based on P-element transgenesis, we have generated a second genome-wide collection by phiC31-mediated transgenesis and made it available to the *Drosophila* community in April 2009. The VDRC currently has 1821 registered users world-wide and has delivered a total of 704,926 RNAi lines to the *Drosophila* community.

Currently, the VDRC maintains and makes available 31,879 *Drosophila* lines, consisting of:
• 21,152 lines in the GD RNAi collection, constructed by P element mediated transgenesis
• 10,727 lines in the KK RNAi collection, constructed by phiC31 mediated transgenesis into preselected single genomic locus, VIE260b, on chromosome II.
• 33 miscellaneous stocks used for the construction of both collections

Additionally, the VDRC provides:
• 13,841 DNA constructs used for the generation of the GD collection

Collectively, the GD and KK libraries cover a total 13,264 *Drosophila* genes (93.1%), with the GD collection covering 11,972 genes (84.6%) and the KK collection covering 9502 genes (71.49%). For most of the genes, more than one independent RNAi line is available through the VDRC.

Administratively, the VDRC was initially operated jointly by the IMP and IMBA. From March 1, 2011, the VDRC formally became independent from IMP and IMBA and joined the Campus Science Support Facilities (CSF). This administrative move does not affect any of the operating procedures of the VDRC, but will provide for more secure longterm funding.

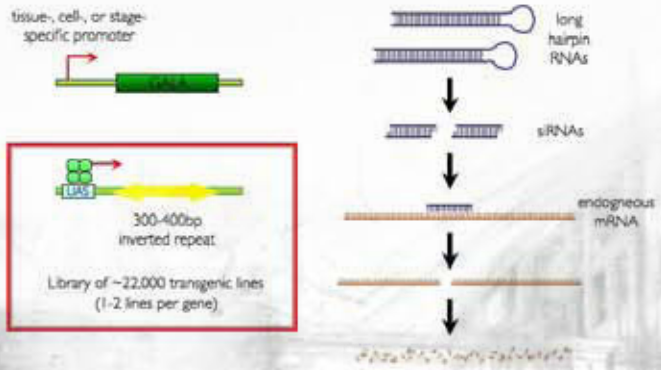

Figure: *RNAi in Drosophila.*

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Seminar Speakers

JANUARY

10.01.11 Peter Jonas
IST Austria
Dendritic integration and transmitter release in fast-spiking hippocampal interneurons

12.01.11 Tomaso Poggio
Massachusetts Institute of Technology
Learning and Vision in Brains and Machines

20.01.11 Mitsuhiro Yanagida
Kyoto University, Okinawa Institute of Science and Technology
Metabolic control of chromosome dynamics and cell division

27.01.11 Lumir Krejci
Masaryk University
Homologous recombination and its quality control

FEBRUARY

02.02.11 Hans Clevers
Hubrecht Institute
Wnt, Lgr5, stem cells and cancer

03.02.11 Daniel Gerlich
ETH Zurich
Bridging spatial and temporal resolution gaps in the study of cell division

24.02.11 Michael Rosbash
Brandeis University
Circadian Rhythms and Gene Expression: Molecules, Neurons and Circuits

25.02.11 Arnd Pralle
Dept. of Physiology & Biophysics, State University New York at Buffalo
Remote triggering of ion channels and neurons through magnetic-field heating of nanoparticles

MARCH

02.03.11 Gilles Laurent
Max Planck Institute for Brain Research
Dynamics and Circuits for Olfactory Coding

03.03.11 Walter Schaffner
University of Zurich
A career in gene regulation - what went right, what went wrong

10.03.11 Nipam Patel
University of California, Berkeley
Developmental and Evolutionary Insights from Newly Emerging Model Organisms

24.03.11 Jerzy Paszkowski
University of Geneva
Epigenetic control of retrotransposition

28.03.11 Rudolf Oehler
Anna Spiegel Center of Translational Research
Advances, Challenges, and Limitations in Clinical Proteomics

30.03.11 Ruth Birner-Grünberger
Center of Medical Research, Medical University of Graz
Lipo-proteomics: functional proteomics in lipid research

30.03.11 Eileen Furlong
EMBL
cis-regulatory networks during development: Is there a code?

APRIL

06.04.11 Natalia Soshnikova
University of Geneva
Epigenetic regulation of Hox genes during vertebrate development: repression, activation and insulation?

06.04.11 Steven Gygi
Harvard Medical School
A tissue-specific atlas of the mouse phosphoproteome

11.04.11 Mike Walker
University of Auckland
Animal magnetism: Structure, function and use of the magnetic sense in animals

14.04.11 Toru Hirota
Japanese Foundation for Cancer Research
How chromosome assembly is triggered in cells entering mitosis

14.04.11 Yang Shi
Harvard Medical School
Histone demethylases: mechanisms and link to mental retardation

15.04.11 David Sherrat
University of Oxford
in vitro and in vivo single-molecule studies of chromosome replication and segregation

21.04.11 William Martin
Heinrich-Heine-University, Duesseldorf
Eukaryote origin, endosymbiosis, and the energetics of genome complexity

26.04.11 Peter Reddien
Howard Hughes Medical Institute
Title to be announced

28.04.11 John Tainer
Scripps Research Institute
An allosteric paradigm for DNA damage response networks: A dawn for mechanistic systems biology in disease prediction and intervention

29.04.11 Werner L. Straube
Max Planck Institute of Biochemistry
A practical approach towards high-throughput absolute quantitation using high resolution mass spectrometry

MAY

04.05.11 Steven Henikoff
Howard Hughes Medical Institute
Histone variant dynamics and epigenetics

05.05.11 Tomoyuki Tanaka
College of Life Sciences, University of Dundee
Kinetochore-microtubule interactions: the means to the end

06.05.11 Gary Brouhard
McGill University
How to Make a Microtubule: Control of Microtubule Architecture by Doublecortin

11.05.11 Rui M. Costa
Instituto Gulbenkian de Ciencia
Learning and organizing new action repertoires

12.05.11 Yuh Nung Jan
University of California, Howard Hughes Medical Institute
Dendrite morphogenesis and functional implications

13.05.11 Edgar Serfling
University of Würzburg
NFAT Transcription Factors Control the Fate of Lymphocytes upon Immunoreceptor Stimulation

19.05.11 John Doebley
University of Wisconsin
Evolution under Domestication: An example from Maize

26.05.11 David Dougan
LaTrobe University, Melbourne
Regulated protein degradation in bacteria: AAA+ proteases and the adaptor proteins that control them

27.05.11 Iris Salecker
MRC, National Institute for Medical Research, London
Regulation of layer-specific targeting in the visual system of Drosophila

JUNE

01.06.11 Geert Kops
University Medical Center Utrecht
Maintaining Genomic Stability: How Mitotic Kinases Regulate Chromosome Segregation

08.06.11 Christian Machens
Ecole Superior Normale, Paris
Computational and optogenetic dissection of the oculomotor integrator circuit

09.06.11 Gero Miesenboeck
University of Oxford
Lighting Up the Brain

14.06.11 Oliver Bimber
Johannes Kepler University Linz
The Potential of Light Fields for Microscopy

16.06.11 Nikolai Mischeriko
University Medical Centre Utrecht
Characterization of post-translational modifications of the transcription-related protein complexes TFIID and SAGA from Saccharomyces cerevisiae

16.06.11 Alexander Tomasz
The Rockefeller University
Accelerated Evolution: Tracking Genes and Phenotypes in Antibiotic Resistant S. aureus

17.06.11 Christian Huber
University of Salzburg
High-resolution liquid chromatography-mass spectrometry of intact proteins: challenges and perspectives for proteome analysis

21.06.11 Francois Nedelec
EMBL Heidelberg
Modeling the dynamic architecture of a meiotic spindle

22.06.11 Julie Cooper
Cancer Research UK, London Research Institute
Telomeres and the challenges to chromosome integrity

29.06.11 Bjoern Kampa
Center for Brain Research, University of Zurich
Representation of visual scenes in local cortical circuits

30.06.11 Neil Hunter
University of California
Making A Connection Between Chromosomes During Meiosis

JULY

01.07.11 Maxime Bouchard
McGill University
Morphogenesis and cancer of the urogenital system: Gata3, LAR phosphatases and friends

07.07.11 Lewis Cantley
Beth Israel Deaconess Medical Center
PI 3-Kinase and Disease

14.07.11 Eric Selker
University of Oregon, Institute of Molecular Biology
Control of DNA methylation and heterochromatin formation in Neurospora

15.07.11 Joseph Schlessinger
Yale University School of Medicine
Cell Signaling by Receptor Tyrosine Kinases: from basic principles to cancer therapy

18.07.11 Saurabh Sinha
University of Illinois at Urbana-Champaign
Exploring the evolutionary origins of homotypic binding site clustering with a quantitative model of cis-regulatory function

28.07.11 James Crowe
Vanderbilt Vaccine Center
Genetic and Structural Basis for Neutralization of Viruses by Human Antibodies

AUGUST

29.08.11 Julian Heng
Monash University
Transcription factor control of neuronal migration during mouse brain development

SEPTEMBER

01.09.11 Alberto Kornblihtt
University of Buenos Aires
Chromatin and transcription regulate alternative splicing

07.09.11 Michael Eisen
Howard Hughes Medical Institute
Starting the Engines: Activation and Regulation of Transcription in the Early Drosophila Embryo

08.09.11 Joanna Wysocka
Stanford School of Medicine
Stem cells, enhancers and emergence of epigenomes in development

15.09.11 Maarten Fornerod
Erasmus University Medical Center
Chromatin interactions with the nuclear periphery

20.09.11 Alexander Fleischmann
Center for Interdisciplinary Research in Biology, Paris
Genetic Analysis of Olfactory Processing and Function in Mice

21.09.11 Kees Murre
University of California, San Diego
Long-Range Genomic Interactions and the Generation of Antigen Receptor Diversity

22.09.11 Peter Donnelly
Oxford University
The Evolution of Recombination Hotspots

29.09.11 Frank Schnorrer
Max Planck Institute of Biochemistry, Martinsried
The Making of Flight Muscles

OCTOBER

11.10.11 Julia Zeitlinger
Stowers Institute for Medical Research
Predicting future gene expression during Drosophila development based on chromatin status

12.10.11 Stanislas Leibler
Rockefeller University
Survival in the face of unknown: Lessons from microbes

14.10.11 Timothy Mitchison
Harvard Medical School
How does a large cell find its center?

19.10.11 Björn Brembs
Freie Universität Berlin
Action – outcome evaluation: the genetics of Drosophila self-learning

20.10.11 Enrico Coen
John Innes Centre
From Genes to Shape

24.10.11 Martin Wild
University of Auckland
Magnetoreception and long distance navigation in birds: is the trigeminal system involved?

24.10.11 François Spitz
EMBL
Exploring the regulatory architecture of the mammalian genome

27.10.11 Sir Philip Cohen
MRC Protein Phosphorylation Unit, University of Dundee
Novel and unexpected roles for the IKK-related kinases in the regulation of innate immunity

NOVEMBER

09.11.11 Joszef Csicsvary
IST Austria
Spatial Learning Associated Reorganisation of Hippocampal Circuits

21.11.11 Ilona Grunwald-Kadow
Max Planck Institute of Neurobiology
Smelling C02 - Drosophila as a model system for nervous system development and function

24.11.11 Nikolaus Pfanner
University of Freiburg
Mitochondrial protein import: from proteomics to functional mechanisms

28.11.11 Silvia Arber
Biozentrum - University of Basel
Organizational Principles of Antagonistic Motor Circuits

DECEMBER

02.12.11 Melina Schuh
MRC Laboratory of Molecular Biology
Oocyte + Actin: Old Love - New Affairs

07.12.11 Sebastian Carotta
Walter and Eliza Hall Institute of Medical Research
Transcriptional regulation of lymphoid development

12.12.11 Scott Williams
Rockefeller University
Asymmetric cell divisions in mammalian epidermal development

12.12.11 Dirk Hockemeyer
The Whitehead Institute
Elucidating telomerase function using genetically defined human stem cell models

14.12.11 Rushad Pavri
Rockefeller University
The transcriptional basis of antibody gene diversification by AID

14.12.11 Yadin Dudai
The Weizmann Institute of Science
Consolidation of Long-Term Memory: Does it ever end?

14.12.11 Cecilia Cotta-Ramusino
Harvard Medical School
Genetic Interrogation of the DNA damage response

15.12.11 Frank Chan
Max Planck Institute for Evolutionary Biology
Selecting for Giants --- Genetic Dissection of an Adaptive Complex Trait in Laboratory and Wild Mice

19.12.11 Luisa Cochella
Columbia University
Spatial and temporal specificity of miRNA function during development in C. elegans

19.12.11 Dimitrios Anastasiou
Beth Israel Deaconess Medical Center & Harvard Medical School
Pyruvate kinase M2 and cancer metabolism

20.12.11 Xiaoke Chen
Columbia University
How does the brain know what the tongue knows?

21.12.11 Florian Schmitzberger
Harvard Medical School
Structure of the Ctf19-Mcm21 complex reveals a recurring module in kinetochore architecture

Awards & Honors

Meinrad Busslinger

• *ERC Advanced Investigator Grant by the European Research Council "Transcriptional networks controlling lymphocyte development"*

Barry Dickson

• *Marie Curie International Training Network: "Systems neuroscience of Drosophila: from genes to circuits to behaviour"*

Tomoko Fujii (Peters Group)

• *PRESTO grant by the Japan Science and Technology Agency*

Karl Mechtler

• *Elected president of the Austrian Proteomics Society*

Jan-Michael Peters

• *Wittgenstein Award by the Austrian federal government*

Oliver Schraidt (Marlovits Group)

• *Vienna Biocenter PhD Award*

Andrew Straw

• *ERC Starting Grant by the European Research Council. "Linking neural circuits to visual guidance in flying flies"*
• *WWTF Cognitive Sciences Call: "Algorithms, neural circuitry, and genetics of high-level visual behaviour in the fly"*

Alipasha Vaziri

• *WWTF Young Research Group Vienna: "Multi scale understanding of biological function"*

Manuel Zimmer

• *ERC Starting Grant by the European Research Council. "Neuromodulation of Oxygen Chemosensory Circuits in Caenorhabditis elegans"*

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In order to maintain the highest standard of research, the IMP has installed a process of review and feedback: the Scientific Advisory Board (SAB), consisting of internationally recognized scientists. The Board meets yearly at the IMP, and, together with IMP researchers, discusses the quality, significance, and main focus of research conducted at the IMP.

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The IMP and Its Surroundings

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

The Campus Vienna Biocenter

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

Close ties have been knit between the IMP and IMBA: the institutes are not only linked physically 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.

Recess 2011

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 72-75).
"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

"Learning and Vision in Brains and Machines" Tomaso Poggio, Massachusetts Institute of Technology

Tomaso Poggio, currently a Professor at MIT, USA, is one of the pioneers in the field of computational neuroscience and has been working in this particular field for the past 40 years! His work has touched numerous aspects, ranging from the most basic neuroscience problems like dendritic integration to computer vision applications like face detection software. In the Birnstiel lecture, Tomaso discussed the recent research interest of his lab which deals with computational modeling of learning and vision. His lab has come up with a computationally simplistic model which is based entirely on anatomical and physiological data from experiments on humans and monkeys. In spite of its simplicity the model works surprisingly well at complex visual recognition tasks like detecting whether an animal is present or absent in an image. The model worked as good as human volunteers carrying out the same task! This now provides a validation of the known data and also a framework for future experiments in this field. Tomaso even gave glimpses of future directions of this work in which they are trying to model even more complex tasks like visual attention.

NEW IMP GROUP LEADERS

Johannes Zuber started his new group at IMP in January. He is a trained medical doctor and spent the last six years in the laboratory of Scott Lowe at the Cold Spring Harbor Laboratory, NY. Johannes uses genetically defined mouse models of acute myeloid leukemia and other cancers to dissect the impact of individual mutations on tumorigenesis, disease progression and treatment response.

Wulf Haubensak started his group at IMP in January. Since 2003 he was a member of the team of David Anderson at the California Institute of Technology. At the IMP Wulf investigates the underlying neural basis of emotions, applying molecular-, pharmaco-, and optogenetic methods to map neural circuits for emotional behaviors in mice.

Alipasha Vaziri, who is a joint group leader of IMP and MFPL, started in April. Before, he worked at the Howard Hughes Medical Institute, USA. At the IMP and MFPL Alipasha aims to understand biological function on a multi-level scale: the fundamental principles of protein and bio-molecular dynamics on the nano-scale, and the dynamic interactions in cellular networks giving rise to macroscopic function and behavior on the organism level.

MAX BIRNSTIEL LECTURE

"Dynamics and Circuits for Olfactory Coding" Gilles Laurent, Max Planck Institute for Brain Research

Gilles Laurent's lab in Caltech has been studying the structure and function of the insect olfactory system for more than twenty years and made major contributions in the understanding of this system. In his talk, he showed how mathematical modeling of the olfactory circuit could help put together past experimental observations to build a working odor information processing system. He also showed how the model could be useful in making predictions about missing functional elements of the circuit, which were then identified experimentally in his lab. Furthermore, he showed some experimental evidence of a plasticity process in a higher olfactory center which in normal conditions constantly stabilizes the system by self-tuning but can also support learning of behaviorally relevant associations upon the action of reinforcement signals. Gilles Laurent is now director of the Max Planck Institute for Brain Research in Frankfurt.

01/11

IMP DEPUTY DIRECTOR

Jan-Michael Peters, Senior Scientist at IMP, has been promoted to the position of Deputy Scientific Director this January. Jan joined IMP in 1996 and has been a Senior Scientist since 2002.

02/11

MAX BIRNSTIEL LECTURE

"Wnt, Lgr5, stem cells and cancer"
Hans Clevers, Hubrecht Institute, NL

Hans Clevers is director of the Hubrecht Institute (Utrecht, Netherlands) and made many important discoveries in the field of Wnt signaling, cancer and stem cell biology. Recently he characterized the stem cell compartment of the gut, stomach and hair follicle. In his lecture, he discussed the characterization of intestinal stem cells and their niche in the crypt of the villi of the gut. He reported that a single stem cell could give rise, in an in vitro culture system, to an organoid structure resembling the intestinal villus and, upon transplantation, to reconstitution of the gut, which may lead to important medical applications of stem cell transplantation in the future.

03/11

VBC SPRING CONCERT

This years' VBC Spring Concert took place on March 23. The first half of the concert featured three piano duets, several solo performances on cello and piano, and was rounded off with a Neapolitan love song, two Turkish folk songs and two jazz pieces. The second half featured the "MolBio Orchestra" which performed classical pieces, including Mozart's Clarinet Concerto in A major and the Waltz No. 2 by Shostakovich, followed by modern pieces including the Overture from "Phantom of the Opera" and a medley from "Indiana Jones".

© VBC Amateur Dramatic Club

AN EVENING OF SHORT PLAYS

The Vienna Biocenter Amateur Dramatic Club invited all VBC employees to attend the show, "Smorgasbord", on May 4 and 5. The theatrical banquet of four short plays included "The Proposal" and "The Boor" by the Russian playwright Anton Chekhov, "Press Cuttings" by the Irish playwright George Bernard Shaw and "Die Panne" by the Swiss playwright Friedrich Dürrenmatt.

VBC PHD RETREAT

In the last week of June, 75 PhD students and three invited speakers met for a common/joint PhD Retreat in the southeast of Austria, near the Hungarian and Slovenian borders. The students presented their projects in a poster session and the speakers gave three entertaining, versatile and very interesting talks. Julie Cooper talked about her life as a group leader in the US and the UK and about her postdoctoral experiences in the labs of Tom Cech and Paul Nurse, two Nobel laureates. Thomas Höger explained how he ended up as the CEO and CFO of Apogenix, a small company that took a novel drug from the research lab through to clinical trials. Barbara Pauly who works as an editor for EMBO-reports gave an insight into the journals' side of publishing and into a typical day at the office of an editor.

04/11

MAX BIRNSTIEL LECTURE

"A tissue-specific atlas of the mouse phosphoproteome"
Steven Gygi, Harvard Medical School

Steven Gygi's lab at Harvard Medical School develops and applies new technologies in mass spectrometry and proteomics. Among these techniques are ICAT (Isotope-Coded Affinity Tags), the paradigm for chemical labeling using isotope-coded tags for relative quantification, AQUA (isotope-coded synthetic peptides for Absolute QUAntification), and methods for detecting protein phosphorylation and for the assignment of phosphorylation sites. In his talk, Steven discussed the application of state-of-the-art proteomics methods to study the proteome and phosphoproteome of nine mouse tissues. The numbers of proteins identified varied between 5574 (pancreas) and 7644 (spleen) depending on the type of tissue. When data from all nine tissue samples were taken together, 12039 proteins could be identified, among these 6296 phosphoproteins harboring nearly 36,000 phosphorylation sites. In this study, spectral counting was performed to provide a semi-quantitative measure of protein abundance. The data is available as a web-based resource at http://gygi.med.harvard.edu/phosphomouse/index.php. Although Steven has extensive experience with SILAC (stable isotope labeling with amino acids in cell culture), he concluded his talk with a statement that he expects an advent of isobaric labeling techniques such as iTRAQ (isobaric tags for relative and absolute quantification) and TMT (tandem mass tags) in quantitative proteomics because these techniques offer the benefit of multiplexing, i.e. the possibility to analyze samples parallel.

05/11

MAX BIRNSTIEL LECTURE

"Histone variant dynamics and epigenetics"
Steven Henikoff, Howard Hughes Medical Institute

On May 4th we had the pleasure to host Steven Henikoff, currently an Investigator of the Howard Hughes Medical Institute, a Member of the Basic Sciences Division of the Fred Hutchinson Cancer Research Center and Affiliate Professor of Genome Sciences at the University of Washington. Steven performs in his laboratory an extraordinary wide range of experimental research – not only has he made important contributions to understand epigenetic inheritance, nucleosome dynamics, chromosome structure and centromere evolution but also DNA methylation and imprinting in plants. He has also made a name for himself as an inventor, designing innovative techniques such as tools for computational analysis, in vivo mapping of nucleosome turnover and functional genomics which has proven to be invaluable not only in his laboratory, but to researchers around the world. Some of his more recent studies have aimed to understand the elusive relationship between chromatin and epigenetic inheritance. In large part, the focus has been on the universal set of histone variants that replace canonical histones independent of replication, the topic of his Max Birnstiel Lecture.

06/11

WITTGENSTEIN AWARD FOR JAN-MICHAEL PETERS

On June 21 IMP senior scientist and deputy director Jan-Michael Peters was awarded the Wittgenstein Award 2011, which is Austria's top research prize. Science Minister Karlheinz Töchterle and FWF-President Christoph Kratky presented the award. The Wittgenstein Prize is worth 1.5 Million Euros and is strictly confined to research. The endowment will enable Jan-Michael Peters - whose interest has focused on the molecular mechanisms of cell division - to investigate further how DNA is packaged within the chromosomes.

IMP SUMMER PARTY

This year's summer party was blessed with beautiful weather. After an initial get-together with food and drinks the games were opened. Dodgeball was the first one and was taken up enthusiastically. The purpose of the game is that players of one team eliminate players of the opposing team by hitting them with a ball while avoiding being hit themselves. In the speedy Dragonboat race and the following Softball Variation all participants could prove their team spirit and fitness. Everybody had fun and the well-deserved dinner including the trophy ceremony rounded off a wonderful day

MAX BIRNSTIEL LECTURE

"Starting the Engines: Activation and Regulation of Transcription in the Early Drosophila Embryo."
Mike Eisen, UC Berkeley and HHMI

The Max Birnstiel Lecture in September was given by Mike Eisen (UC Berkeley and HHMI), who is also one of the co-founders of the open-access journal PLoS Biology. Mike presented his latest work on transcriptional regulation in the early Drosophila embryo. He showed that deep sequencing of embryonic RNA from a cross of two different wildtype Drosphila strains can be used to discriminate transcripts that originate from the maternal and the paternal allele, thereby also classifying zygotic and maternally deposited RNAs. In the 2nd part of his talk, he identified the maternally deposited transcription factor Zelda as an important factor of early zygotic chromatin organization and transcription: early binding sites of unrelated transcription factors were enriched in Zelda motifs and Zelda was indeed found to bind to most of these sites, potentially opening them or keeping them open.

07/11

VBC SUMMER SCHOOL

For the second time this year, the VBC Summer School provided the opportunity for undergraduate students to spend ten weeks at one of Europe's leading research centers. From June 23 to August 29, twenty-six students representing nineteen different countries worked on their assigned research projects in one of the labs at IMP, IMBA, GMI and MFPL. The program was accompanied by a series of lectures and many social activities. Again, the Summer School was a tremendous success and concluded with a scientific mini-symposium in which each student presented his or her results and competed for an award.

08/11

"TWELFTH NIGHT"

On 24th and 25th August 2011, the Vienna Biocenter Amateur Dramatic Club performed its annual outdoor Shakespeare production, which was this year "Twelfth Night". Regarded by many as the finest of the Bard's comedies, this piece involves slapstick humor, wordplay, music and a large dose of anarchy to create a matchless atmosphere of mirth. The play took place on the lawn of the Campus Vienna Biocenter and thanks to the beautiful weather both the show and the barbecue afterwards were very well-attended. More than 100 people turned out for each performance.

09/11

MAX BIRNSTIEL LECTURE

"Long-Range Genomic Interactions and the Generation of Antigen Receptor Diversity"
Kees Murre, University of California, San Diego


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MAX BIRNSTIEL LECTURE

"Survival in the face of unknown: Lessons from microbes"
Stanislas Leibler, Rockefeller University

The Max Birnstiel lecture "Survival in the face of the unknown: lessons from microbes" was given by Stanislas Leibler from the Rockefeller University and Princeton University. Stan is a physicist who has applied quantitative methods and theory to a variety of biological problems including self-organization of microtubule structures, communication in complex neuronal networks, and most recently, how bacterial populations respond in fluctuating environments. He thinks about biological systems in novel ways - for instance, applying economic theory to predict protein folding - and his talk tomorrow will show the sorts of discoveries that can be made when one approaches long-standing questions from a different perspective. Stan's work on how microbes persist through environmental stresses has assessed phenotypic heterogeneity in individual bacteria to find switches encoded in the network that enable rare survivors. Now he is extending these studies to long-term population analyses using some exciting technical developments.

THINK ALTERNATIVE!

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 9th VBC International PhD Symposium was held on November 3-4, 2011. It was entitled "Think Alternative! Insights from Unconventional Model Organisms". The idea was to discover the opportunities and benefits that are offered by systems other than the classical genetic models, such as fruit flies, Arabidopsis or mice. The symposium covered a broad range of research topics, including regeneration, epigenetic inheritance, germline specification, development, evo-devo, neurobiology, behavior, and even paleogenomics.

10/11

11/11

12/11

RECESS

From October 5-7, 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. In 2011 two new members have joined the IMP's Scientific Advisory Board: Tom Rapoport, Professor of Cell Biology at the Harvard Medical School, and Norbert Kraut, Vice President Lead Discovery at the Boehringer Ingelheim Regional Center Vienna. The IMP would like to thank all its SAB members for their commitment and advice.
IMP SAB members: page 67 in this booklet

MAX BIRNSTIEL LECTURE

"Consolidation of Long-Term Memory: Does it ever end?"
Yadin Dudai, The Weizmann Institute of Science

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Cover Illustration

The cover shows ~2 hour old embryos from transgenic Drosophila lines (Vienna-Tiles [VT]-library) in which a Gal4 transcriptional reporter is visualized by in situ hybridization (lateral orientation, anterior left, dorsal top). 5000 embryo images (76 distinct ones) are arranged to a mosaic o the IMP logo using the the software 'MozoDojo' (Pierre Chachatelier). Courtesy of Gerald Stampfel, Evgeny Kvon, and Alex Stark.

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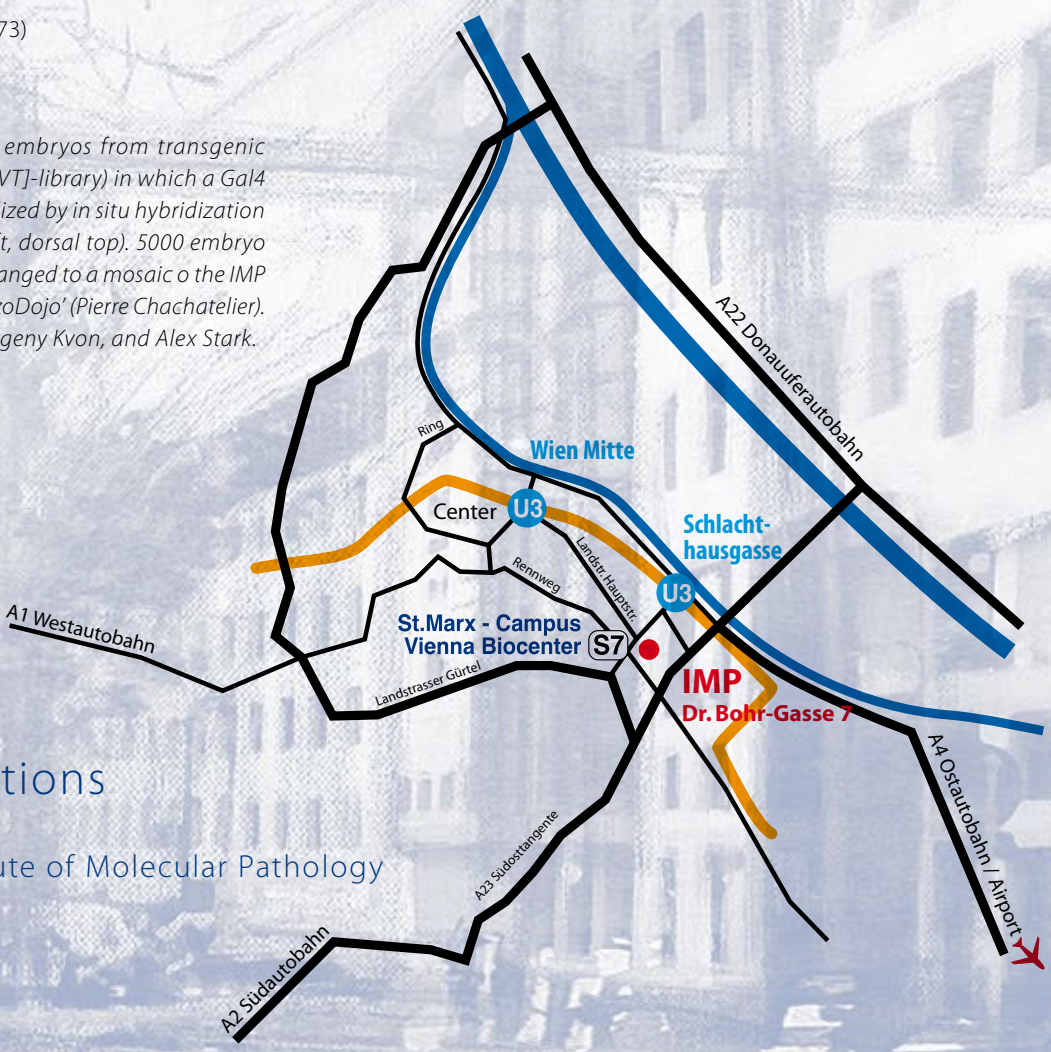

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