RESEARCH INSTITUTE OF MOLECULAR PATHOLOGY
VIENNA BIOCENTER

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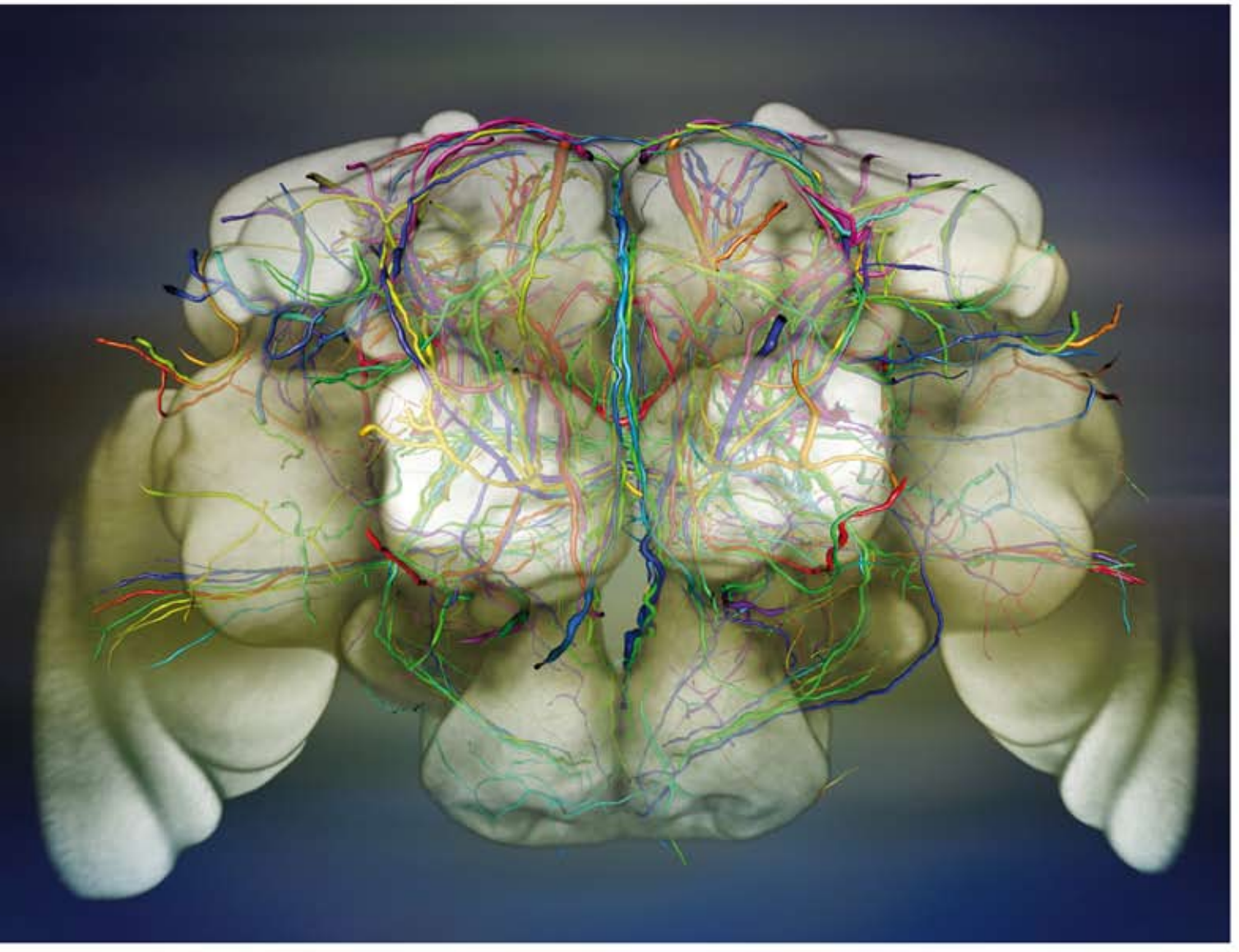

Boehringer Ingelheim

IMP
Research Institute of Molecular Pathology

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BARRY DICKSON
Managing Director/ Science

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The past year has been another exciting one for the IMP. As every year, we have seen a significant turnover of personnel at the IMP, which this year also extended to the Scientific Advisory Board. It was also another excellent year scientifically, particularly in the success of IMP scientists in bringing in prestigious large research grants. We've also seen a number of new developments that make the daily scientific life at the IMP easier and more enjoyable.

We were very happy this year to be able to promote Tim Clausen from a group leader position to a senior scientist. Tim joined us back in 2002, bringing structural biology research to the IMP. His group quickly established a strong international reputation, particularly through their crystallographic studies of a family of bacterial proteins with the remarkable ability to act as both proteases and chaperones. These factors provide a mechanism for protein quality control. Proteins that are only slightly misfolded can be repaired by the chaperone activity, while the "hopeless cases", as Tim calls them, are destroyed by the protease activity. Tim's crystallographic studies have provided a detailed mechanistic insight into the inner workings of these "Dr Jekyll and Mr Hyde" proteins. More recently, while pursuing the regulatory mechanisms that activate these quality control processes, Tim made his most important discovery to date: the identification of a family of protein arginine kinases. New findings from Tim's group suggest that arginine phosphorylation may be an important regulatory mechanism in eukaryotic cells too, opening up exciting new possibilities that Tim and his group are now exploring. As a senior scientist, Tim will be able to pursue these studies with full vigour and a longer-term perspective, while also branching out into other new areas. His promotion also reflects the key role Tim has played in building up structural biology at the IMP and the Campus Vienna Biocenter more generally.

Anton Wutz also moved on to a senior position this year, taking up a position as member of the Wellcome Trust Centre for Stem Cell Research in Cambridge, UK. Anton had been a group leader at the IMP since 2001, using a clever series of genetic manipulations in mice to explore the mechanisms of X chromosome inactivation. Anton made many interesting discovery during his time at the IMP, the latest being the identification of the transcription factor SATB1 as a new key player in Xist-mediated gene silencing. We wish Anton and his group continued success as they pursue this exciting line of research further in Cambridge.

Other key personnel changes this year were the arrivals of Erwin Kunz, who took up the position as head of the Purchasing Department, and Karlo Pavlovic, as the new chief librarian. On a sadder note, we were all shocked this year by the sudden passing of Kashinath Mitra, who had managed the storeroom at the IMP for ten years. Mitra was not only a reliable and committed colleague, but also a cheerful and friendly character. We will miss him.

The annual Recess continues as the scientific highlight of the year, when IMP and IMBA scientists present their work to the members of both institutes and their respective Scientific Advisory Boards. Many aspects of the Recess continued this year in their usual format, including for example the Kirsten P. Rabitsch award for the most significant discovery by a PhD student – won this year by Evi Kiermaier in Stefan Westermann's group. But we also introduced a number of changes to the Recess format to provide even more opportunities for interactions between IMP scientists and SAB members. The most important change, however, was in the composition of the SAB itself. Fred Alt, who had been on the SAB for 8 years and chair for 4, came this year for the final time. Cori Bargmann has now taken over from Fred as chair. Additionally, we welcomed several new SAB members to the Recess for the first time: Angelika Amon, Anton Berns, Ewan Birney, Tobias Bonhoeffer, Dan Littman, and Venki Ramakrishnan. These new members cover research areas that have either been left open by recent departures from the SAB, such as cell biology, immunology, and oncology, or newer and expanding areas of research at the IMP, including computational biology, structural biology, and mammalian neurobiology. We were all particularly delighted when, in the week following the Recess, the Nobel Foundation announced that Venki Ramakrishnan was one of the winners of this year's Nobel Prize in Chemistry for his work on elucidating the structure of the ribosome and the mechanism of protein synthesis.

Scientific research at the IMP continues to progress well, reflected as usual in the institute's excellent publication record. This year also saw a bumper crop of major research grants being awarded to IMP scientists. Alex Stark, within his first year at the IMP, was awarded one of the prestigious European Research Council Starting Grants. This award is an important recognition of Alex's achievements and research plans, and will allow him to continue to pursue his goal of understanding genome regulation through both computational and experimental approaches. Jan-Michael Peters also brought in a major European research grant with the MitoSys project that he will coordinate under the EU's 7th framework programme. MitoSys is a successor to the highly successful MitoCheck project, also coordinated by Jan. More locally, Meinrad Busslinger, Krystyna Keleman, and Simon Rumpel were awarded grants from the Austrian Science Fund (FWF). Meinrad, Jan, Alex and Karl Mechtler also obtained grants from the Austrian Genome Research programme (GenAU), and two IMP teams (Krystyna, Alex, Jan and Meinrad) were awarded grants from the Life Sciences call of the Vienna Science and Technology Fund (WWTF). We congratulate all of these groups on their success, and thank the various funding agencies and our grant managers Tanja Winkler and Uta Pohn for their continued support of IMP science.

Another important development this year has been the introduction of a summer school for undergraduate students, scheduled to begin in 2010. This summer school will offer research opportunities, scientific lectures and a rich social programme to undergraduate students for a 10-week period in the summer. Our goal in establishing this programme is to spread the excitement of basic research – and in particular research at the IMP – to undergraduate students around the world. We hope that many of them, and their colleagues, will some day return to the IMP for their further scientific training. We are particularly grateful to David Keays, Simon Rumpel, Stefan Westermann, and Julius Brennecke for putting this programme together.

Finally, we are happy to note a few small changes that make daily scientific life at the IMP just that little bit easier and more enjoyable. A new Genomics core facility started operation at the beginning of the year, offering Solexa deep sequencing and microarray services. The IT department has introduced a number of changes this year that have greatly improved the quality of the IT service and infrastructure. This includes providing each student and postdoc with their own laptop, as well as improving the capacity and performance of the network. We were also able to open the campus Kindergarten this year, offering childcare from the age of three months and opening hours according to the needs of scientists.

We thank everyone at the IMP for their efforts in another busy and successful year at the IMP, and Boehringer Ingelheim for their generous and continued support of our research.

Barry J. Dickson
Harald Isemann

A TWIST IN THE (N-) TAIL – A NOVEL CLASS OF PROTEIN KINASE

Protein phosphorylation is a well known mechanism for rapidly and reversibly activating particular cellular functions in response to external stimuli. A large number of proteins are known to be the targets of so-called protein kinases, which transfer phosphate groups to the side-chains of histidine, aspartate, serine, threonine or tyrosine residues. Jakob Fuhrmann, a PhD student in Tim Clausen's group, has now identified a novel type of protein kinase that specifically phosphorylates arginine residues. In collaboration with the IMP/IMBA mass spectrometry unit he was able to show that phosphorylation of a regulatory peptide at an arginine residue had a biological effect and in doing so he uncovered what may turn out to be the founding member of a completely new class of protein kinases. His work was reported earlier this year (Fuhrmann *et al*. 2009, *Science* **324**, 1323-1327).

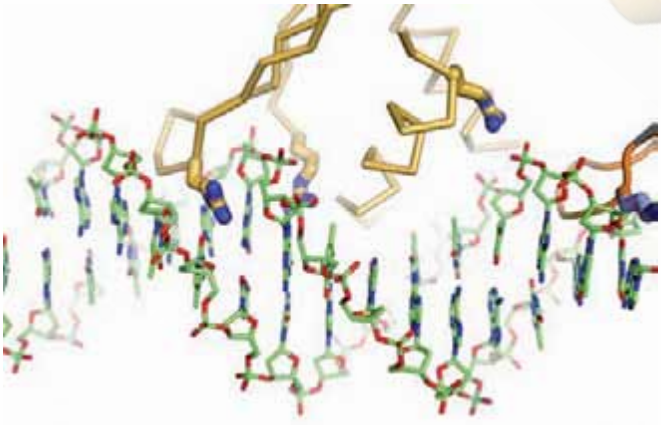
Unusually, Jakob performed his diploma work in a pharmaceutical company, Baxter Bioscience. For his PhD, however, he decided to move back to basic research and applied to the VBC PhD programme although he had no clear idea of what project to select. Tim Clausen was one of the group leaders to interview him and the two hit it off immediately. As Jakob says, "Tim was incredibly enthusiastic and was offering some fascinating projects. And he managed to make me understand that I didn't need a strong background in maths to do structural biology."

Jakob initially started to work on the mechanism of protein degradation by the bacterial ClpCP protease complex. The current dogma was that this complex represented a nonspecific protein-shredder, with the required specificity provided by various "adaptor" molecules that bound to it and somehow controlled access to the enzyme's active site. One of the known substrates of ClpCP was a transcriptional repressor known as CtsR and it was believed that the so-called McsB protein was the adaptor molecule that enabled ClpP to degrade CtsR. It was obvious that a structural analysis would be useful to understand the mechanism so Jakob attempted to clone the genes from a number of sources and to use the clones to express the proteins. It turned out that the genes from the thermophilic organism *Bacillus stearothermophilus* yielded stable proteins when expressed *in vitro* so Jakob selected them for further investigation.

The first result of his studies was that the interaction between CtsR and McsB was surprisingly weak for McsB to be an adaptor, so Jakob and Tim turned to a consideration of other possibilities. It was already known that McsB showed kinase activity and targeted CtsR. Preliminary experiments showed that McsB did indeed phosphorylate CtsR *in vitro*; furthermore, when CtsR was phosphorylated by McsB it was no longer able to bind DNA. Phosphorylation of CtsR by McsB thus deactivated this latter protein (and so could activate the genes that CtsR normally repressed). Removing the phosphate group(s) restored CtsR's DNA-binding activity, so CtsR seemed to be operating as an McsB-dependent switch.

Jakob and Tim now had a working hypothesis for McsB function – it phosphorylated CtsR and thereby prevented it from binding to DNA – but testing this proved surprisingly difficult. According to published information (see Kirstein *et al*. 2007, *EMBO Journal* **26**, 2061-2070), McsB transferred phosphate moieties to tyrosine residues so Jakob approached Karl Mechtler's group to help him identify which tyrosines in the CtsR sequence were phosphorylated. Unfortunately, even the most sophisticated mass spectrometry experiments failed to find any evidence for phosphotyrosine in CtsR. As Jakob notes, "It took us more than a year before we recognized that McsB actually phosphorylates arginine residues. Because of what we had read in the literature, we were all completely stuck on the idea of tyrosine phosphorylation and simply couldn't look for another explanation. This was probably the most important lesson I learned: not to accept everything that is published as true but to keep an open mind and question everything – including my own results, of course."

Part of the difficulty in recognizing that CtsR is phosphorylated on arginine residues stemmed from the fact that phospho-arginine is very difficult to detect. Most standard mass spectrometry procedures involve sample preparation under conditions where



The image represents the CtsR/DNA complex highlighting the arginine phosphorylation sites. All these arginine residues are directly involved in DNA minor or major groove interactions (DNA: green; CtsR backbone: gold; arginine phosphorylation sites are shown in stick representations)

phospho-arginine is unstable. The breakthrough depended on two events. First, it was possible to exclude tyrosine as the site of CtsR phosphorylation so the group switched its attention instead to arginine residues. Secondly, Andreas Schmidt in Karl's group adapted the protocols for sample preparation. After several iterative cycles of optimization, CtsR samples were finally – and to the elation of the group – revealed to contain phosphorylated arginine residues. And Andreas was able to show that a single highly conserved arginine residue – Arg62 – was the primary site of modification. Two other arginine residues, Arg28 and Arg49, were also found to be phosphorylated, albeit to a lesser degree.

While Andreas was revealing the presence of phospho-arginine by mass spectrometry experiments, Jakob was continuing his attempts to co-crystallize the CtsR protein with DNA fragments to undertake a detailed structural analysis of the complex.

Although the DNA sequence to which CtsR binds was known, obtaining good crystals turned out to be an extremely laborious process and Jakob estimates that he had to try out about 40-50 fragments differing in length and sequence before he obtained crystals that diffracted to sufficiently high resolution. Finally, though, the crystals were obtained and the structure solved. CtsR binds to DNA as a dimer and the protein is comprised of two domains: a C-terminal domain that seems to be involved in dimerization and an N-terminal DNA-binding domain. And the three arginine residues that Andreas had shown to be phosphorylated are located at positions where they could potentially play an important part in binding to DNA. Arg62 lies deep within the minor groove of the DNA, while the other two arginine residues could interact with the major groove.

Jakob already knew that McsB phosphorylation of CtsR was sufficient to prevent DNA binding, so an obvious question was whether phosphorylation and dephosphorylation of Arg62 had the same effect. He thus replaced Arg62 with a glutamate residue (which looks similar to a permanently phosphorylated version of the protein) – the mutant protein was completely unable to bind DNA. Replacing Arg62 with lysine (which is of similar size and shape but cannot be phosphorylated) did not affect the protein's ability to bind DNA.

By this stage, Jakob had a large amount of circumstantial evidence to suggest that McsB phosphorylated certain arginine residues of CtsR but conclusive proof that this is the case required experiments performed *in vitro* using synthetic components. He thus designed a model peptide that mimicked the area around Arg62. This peptide was indeed phosphylated by McsB (and NMR experiments further confirmed that the arginine residue was phosphorylated), while peptides lacking arginine or carrying chemically modified arginine at position 62 were not affected. This key experiment finally established beyond doubt what Jakob and Tim had reluctantly come to accept: McsB is a protein kinase that phosphorylates CtsR not on tyrosine residues but exclusively on arginine residues.

It is difficult to understand how unexpected this finding was. Before Jakob's work no protein kinase had been shown to act specifically on arginine residues and the discovery that McsB does so has huge ramifications. The obvious next question is how many other such proteins there are in nature and in what organisms they are distributed. CtsR is involved in the heat-shock response of most gram-positive bacteria, many of which are pathogenic to humans. If it turns out that McsB is only found in such bacteria, it is conceivable that it would represent a completely new target for highly selective antibiotics, ones that would have no side-effects at all on the host organisms or on the gram-negative bacteria in the intestine. As Jakob says, "selective inhibitors of McsB would only prevent bacteria from activating their stress response. When bacteria invade higher organisms the host immune response is activated and this puts the bacteria under stress. If we could inactivate the bacterial stress-response mechanism we would perhaps impair the harmful bacteria without damaging anything else. When the bacteria are thus weakened it would be much easier for the immune system to kill them."

Of course, it is quite conceivable that phosphorylation of arginine residues will turn out to be more widespread. Jakob is quick to point out that previous mass spectrometry experiments to examine phosphorylation sites have used samples prepared under conditions where phospho-arginine would lose its phosphate group, so it is quite conceivable that higher organisms also have protein arginine kinases and that McsB may turn out to be the first member of a new class of protein kinase. There are hints that histone H3 might be phosphorylated on arginine residues – thereby raising the intriguing notion that arginine phosphorylation may somehow be involved in epigenetic regulation – but the evidence is still only indirect. With the refined methods, it should be possible to address this point in detail. Jakob is extremely excited at the possibility that his work might open up a new field. As he sees it, "however the story unfolds we'll have something interesting. If protein arginine kinases are widespread we can study their mode of action, while if they are restricted to gram-positive bacteria we'll have a handle on potential new antibiotics."

But there is interesting work to be done even before the distribution of protein arginine kinases in nature is resolved. Unlike the majority of DNA-binding proteins, which tend to bind as symmetrical dimers to DNA sequences comprising inverted repeats, CtsR has been shown to bind direct repeats. It binds as an asymmetric dimer to the DNA with the two CtsR molecules featuring very different interdomain interfaces, one hydrophobic and one hydrophilic. Jakob is currently studying the precise nature of the interaction between CtsR and DNA, which he feels is likely to provide us with clues on how proteins that bind to direct DNA repeats could recognize and bind to their target sequences.

Jakob hopes to complete his thesis soon – he has started to write up his results – but is still keen "to finish the work we've started". We have so many promising leads at the moment, and I personally hope to be able to contribute to solving at least some of the questions that our discovery has raised."

Text by Graham Tebb

Jakob Fuhrmann and his daughter Lisa

DRIVING THE DIVIDE – THE YEAST KINETOCHORE

While modern cars look superficially similar to the glorious "motor cars" made famous in early black-and-white films, they have attained an amazingly high level of complexity. Numerous back-up systems guard against the event of problems and sophisticated electronics increase efficiency by governing all operations (and thereby ensure that even minor problems can only be fixed by a visit to a garage). Their basic function – moving people from one place to another – has stayed the same but understanding how it is accomplished would be much facilitated if we could go back in time and examine the earlier, simpler models.

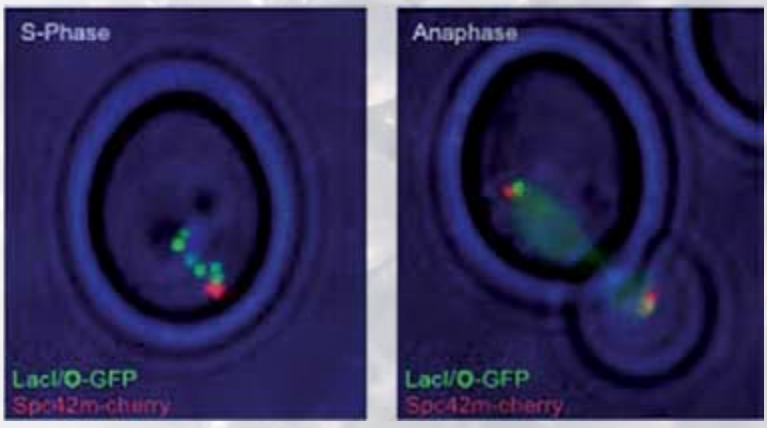
In a way, the kinetochores of eukaryotic cells are analogous. When cells divide, they need to ensure that their DNA is transferred equally to both daughters. In normal cell division, or mitosis, chromosomes must first be replicated and then moved to the appropriate parts of the cell so that following division each of the progeny inherits a single copy of each chromosome. How precisely this takes place has represented one of the "hot topics" of cell biology almost since the discovery of DNA.

A considerable body of work – much of it performed by the groups of Kim Nasmyth and Jan-Michael Peters at the IMP – has shown how sister chromatids are held together by cohesins until the appropriate time in the cell cycle for them to be pulled apart by the microtubules that ultimately bring them into the daughter cells. However, the machinery that connects chromosomes to the microtubules had until recently proved largely intractable to study. The main difficulty is similar to that with modern cars: the level of sophistication. The complex, or kinetochore, is simply too large and too complicated for normal biochemical or genetic methods to yield real insights into its function. All this may have changed, though, with the publication of a paper by Eva Kiermaier, a graduate student in Stefan Westermann's group at the IMP (Kiermaier *et al.*, 2009, *Nature Cell Biol.* , **11**, 1109-1115).

Eva came to the IMP from Munich, where she studied Biochemisty. She had performed her Master's work on the cell cycle "although this was fairly clinically oriented. For my PhD I wanted to stay in the field but move more into basic research. And I really wanted to go and live in an attractive town." Vienna seemed to fit the bill and the IMP's strong tradition in cell-cycle research made the institute an obvious first choice for her study. She thus applied to the VBC PhD programme to work in Stefan's group, attracted also by the possibility of working with yeast.

At that time, Stefan had recently joined the IMP and was still in the process of building up his laboratory. Eva rapidly settled in and was happy to "play with yeast – it was a pleasant change from having to pay so much attention to sterile conditions for cell-culture work." She concentrated initially on two related projects, a "safe" purification and biochemical characterization of Spc105, one of the proteins in the yeast kinetochore, and a far more risky project to identify a "minimal kinetochore". As she puts it, "I thought this was highly unlikely to work but it was clear that it would be very interesting if it did." The advantage of working on two projects in parallel was that she was able to switch directions for a while when she came up against problems with one project, which helped prevent her from becoming frustrated with the work. And ideas to solve problems often came to her while she was working on the other project.

Stefan had worked on the Dam1 complex during his post-doctoral studies and a considerable amount of circumstantial evidence suggested that it played an important part in attaching chromosomes to microtubules and thus might be central to kinetochore function. Stefan's suggested way to try to identify a minimal kinetochore was to fuse candidate proteins (such as the members of the Dam1 complex) to a DNA-binding domain (the tetracyclin repressor protein, TetR) and see whether the resulting proteins could promote the correct segregation of a yeast mini-chromosome that lacked the so-called centromere sequences that normally direct kinetochore binding. To Eva's (and Stefan's!) surprise, two such fusion proteins – with Ask1 and Dam1 – did indeed help mini-chromosomes to segregate at rates that were far higher than those shown by negative controls, although they could not approach the accuracy of segregation shown by wild-type mini-chromosomes. Control experiments confirmed that the result was specific for Dam1 subunits because just recruiting other microtubule binding proteins had no effect on segregation. With fusion proteins derived from the Dam1 complex, the mini-chromosomes aligned along the spindle axis and travelled to opposing spindle pole bodies, similarly to normal chromosomes during cell division. And further experiments proved that the fusion proteins were actually integrated into the Dam1 complex and that they did not work by permitting the mini-chromosomes to "piggyback" onto native chromosomes (in certain mutant strains, the Ask1-TetR fusion protein allows mini-chromosomes to segregate correctly even though native chromosomes do not).



Visualization of mini-chromosome segregation by lacI-GFP binding to the lacO-array inserted to the mini-chromosomes.

By this stage, Eva was not the only person in Stefan's group working on the project. Sophie Woehrer had joined the lab as a diploma student and "was extremely helpful. Her gels were much prettier than mine and having her doing experiments in parallel with me gave us both the confidence that what we were seeing was reproducible by different pairs of hands. It was also good to have someone share the microscope duty so neither of us suffered too much from having to spend too long staring down lenses." The work was proceeding surprisingly smoothly and Eva's fall-back project was not receiving a great deal of her attention. But then came the shock: thanks to a chance meeting at a conference, Eva, Sophie and Stefan learned that Andrew Murray's group at Harvard had come up with a similar system to study yeast kinetochores (based on intact chromosomes and LacI fusions instead of mini-chromosomes and TetR fusions) and was about to prepare a manuscript for publication. As Eva puts it, "his work was really frighteningly similar to ours. Our mini-chromosome system had some advantages but it seemed as though his group was more advanced than we were and that we might be scooped for publication."

Although Eva was concerned by the news, Stefan was actually quite relieved. In his words, "the result was so surprising and the chance that this could work so slim that I was happy to see that someone else, using a different artificial system, had come to the same conclusion". Fortunately, Murray proved amenable to the idea of both groups' submitting papers together for possible back-to-back publication, which they did in August 2008. Perhaps the manuscripts were somewhat rushed as a result of the circumstances under which they were written but in the end the journal rejected them both. Eva freely concedes that there were "perfectly plausible reasons: the ideas were interesting but the story was still incomplete." By now, though, the two groups were communicating regularly and at the end of the year they were ready to submit revised versions. This time the reviews were much more positive, although the referees still wanted additional experiments from both groups. There followed what Eva describes as "the only really stressful time for Sophie and me. We wanted to be sure that we did everything the referees had asked of us – their suggestions were all good, although some of them were fairly wishy-washy and it was hard to be absolutely sure what they wanted." By Easter, however, the necessary experiments had been performed – thankfully with the correct results! – and the manuscripts from both groups were accepted for publication (see also Lacefield *et al*., 2009, *Nature Cell Biol.* , **11**, 1116-1120).

What both groups had shown was that the ten-protein Dam1 complex represented a basic functional kinetochore. This is an extremely significant finding that opens the way for many further studies. An obvious question is why normal kinetochores are so much more complicated: even the "simple" yeast kinetochore contains over 80 proteins and mammalian systems are more complex still as they contain additional layers of regulation. It seems likely that the additional proteins are required for fine tuning and to make the system sufficiently robust.

As Eva notes, "our simplified kinetochores are able to connect microtubules to chromosomes and thus to promote chromosome segregation but only for a few cell cycles, after which the whole thing breaks down." There is some evidence to indicate that the mini-chromosome-TetR fusion protein system operates independently of evolutionarily conserved inner kinetochore proteins and of the conserved "KMN" network , although the experiments are difficult to perform because the mutants used are intrinsically very sick. Furthermore, the mini-chromosome system does not seem to be under the control of the normal mitotic checkpoint (which delays the onset of cell separation until all chromosomes are correctly attached to microtubules). It seems likely that the remaining proteins in yeast kinetochores are involved in the regulation of kinetochore function, both spatially and throughout the cell cycle. The availability of the "basic kinetochore" that Eva, Sophie and Stefan have described means that it is now possible for the first time to create and analyse different artificial kinetochores, so providing a handle on the function of individual kinetochore proteins.

The future direction of the field is clear but what does the future hold for Sophie and Eva? Sophie is about to start a PhD with Kazufumi Mochizuki at the IMBA, next door to the IMP, while Eva plans to submit her thesis within the next few months and then plans to move on to a post-doc in the USA or in London. "I love science and really like the idea of trying to understand how the cell works. Although I recognize that I'll be very lucky to find somewhere as good as the IMP to work at in future, a post-doc would be the best way for me to carry on contributing to the field."

Text by Graham Tebb

Eva Kiermaier at her colleague's hens night

HARTMUT BEUG

Tumor progression: abnormal developmental plasticity/ reprogramming?

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In leukemia and in the development of carcinoma, several oncogenes / tumor suppressor genes cooperate to induce abnormal survival, proliferation, and developmental / behavioral plasticity in tumor cells, which may adopt properties of stem cells. Using both genetically modified mice and in vivo-like cell culture models, we focus on molecular mechanisms that transform hematopoietic stem cells into leukemia-initiating cells, and reprogram epithelial cells into primitive mesenchymal, stem cell-like cells during the progression of carcinoma and metastasis.

Mechanisms in hematopoietic progenitor renewal and leukemogenesis.

Human leukemia involves the cooperation of mutated transcription factors/ chromatin regulators with mutated/overexpressed receptor tyrosine kinases/signal transducers In erythroid cells. Progenitor renewal requires cooperation of the EpoR, c-Kit, and the glucocorticoid receptor (GR) in primary mouse erythroblasts and in immortal but diploid, and *in vivo*-like murine ES cell-derived erythroblasts (ESEPs). The latter are almost indistinguishable from erythroid progenitors *in vivo* by antigenic and functional characteristics, and were used to characterize erythropoiesis defects caused by lack of Stat5, p38 MAPK and Flk-1. We also purified hematopoietic stem cells (HSC) from multilineage mouse leukemias caused by the human chronic myelogenous leukemia (CML) oncogene BCR-ABL p210 and constitutively active Stat5, showing that they function as leukemic stem cells (LSC's). These cells could be expanded in culture on a long-term basis without losing their ability to cause multilineage leukemia upon transplantation. In contrast, HSCs infected with BCR-ABL p185 (causing human B-ALL) initiate leukemia, but are lost during disease progression through differentiation into committed immature B-cell progenitors which function as LSCs in secondary transplants, although constituting the bulk of leukemic cells (Fig 1). We now focus on potential molecular and cellular differences between these two LSC types and normal HSCs by expression profiling and functional analysis.

Tumor progression and metastasis: Ras plus mutated genes disrupting epithelial polarity?

Epithelial to mesenchymal transitions (EMT) are essential during normal development, carcinoma progression and metastasis, and may be a hallmark of carcinoma stem cells. Oncogenic Ras plus TGFβ cause EMT and metastasis in the mammary epithelial cell model (EpH4/ EpRas), while a Ras mutant hyperactivating the PI3K pathway (EpC40 cells) induces a "scattering" phenotype upon TGFβ-addition and is tumorigenic, but not metastatic. We currently focus on 4 genes identified by polysome-bound mRNA expression profiling/protein studies, i.e. ILEI (interleukin-like EMT inducer), CREG (cellular repressor of E1A regulated genes, AnxA1 (Annexin-A1), and mScrib (mouse paralog of Drosophila Scribble). ILEI causes reversible EMT, slow

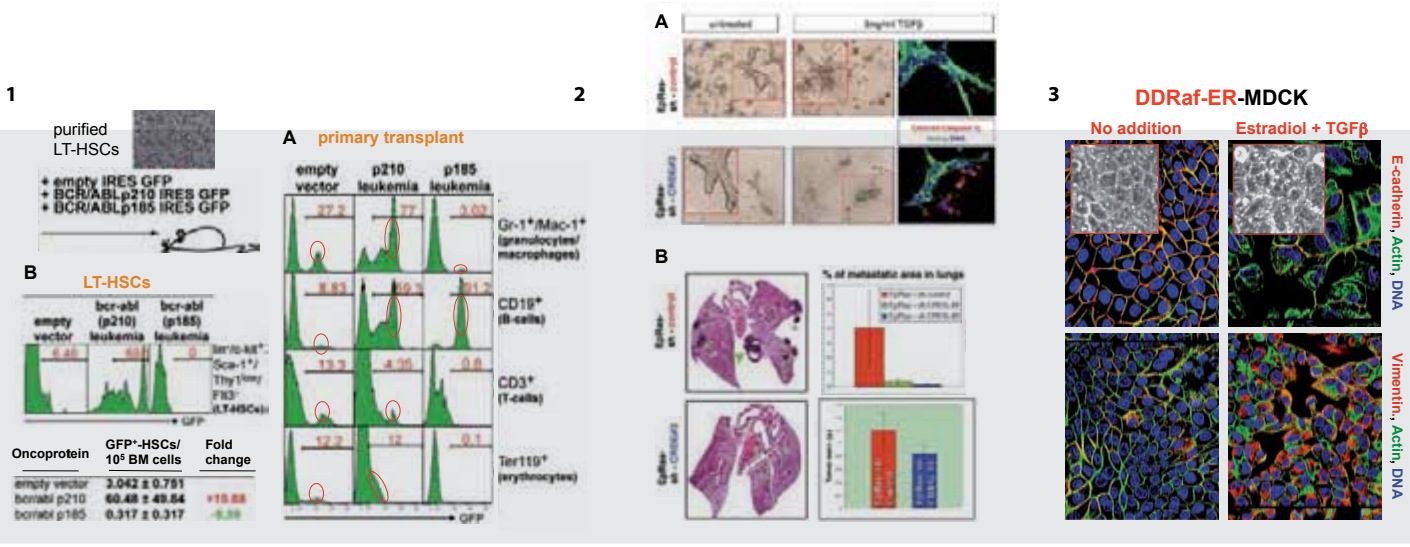

Figure 1: ***Oncogene-specific fate of LT-HSCs infected with p210-BCR-ABL and p185BCR-ABL.*** *Purified LT-HSCs were infected with retroviruses expressing the p210- and p185-BCR-ABL leukemia fusion oncogenes and transplanted into lethally irradiated mice (upper left). A. p210-BCR-ABL induced a multilineage leukemia (green cells in all lineages, marked by red ovals). These cells were also present in the BM of mice transplanted with empty GFP-vector infected LT-HSCs. P185-BCR-ABL, however, generated pure B-cell progenitors, which functioned as the LSC in secondary transplants. B. Sorting of cells with LT-HSC markers from the BM of leukemic mice revealed a strong increase in green LT-HSCs from p210-BCR-ABL mice with CML, but almost complete loss of such cells in p185-BCR-ABL mice with B-ALL.*

tumor growth and metastasis in oncogene-free EpH4 cells, but requires oncogenic Ras for these functions in cultured, *in vivo*-functional p19ARF-/- hepatocytes. ILEI thus resembles CREG, AnxA1 and mScrib in their requirement for oncogenic Ras to cause EMT and metastasis upon loss or gain of function.

Surprisingly, ILEI causes EMT by intracellular mechanisms, as shown by ILEI mutant proteins – neither secreted nor extracellularly cleaved by plasmin – that cause EMT. Secreted ILEI may, however, also perform extracellular functions: 1. Non-cleaved, secreted ILEI forms homodimers and is cleaved by plasmin after binding to ECM-fibronectin. 2. A highly secreted ILEI mutant protein lacking the propeptide removed by plasmin functions as a "super ILEI" in metastasis induction. Importantly, ILEI localizes to the trans-Golgi-network in normal cells, but is redistributed to vesicles in the entire cytoplasm upon EMT induction, explaining our earlier observation that cytoplasmic localization of ILEI is a strong predictor of metastasis in human breast cancer and melanoma. CREG, a secreted, mannose 6P-receptor binding, lysosomal protein, also caused EMT and metastasis in EpC40 cells, a feature shared by Drosophila and plant (Arabidopsis) CREG paralogs (Fig 2). Finally, RNAi-mediated knockdown of AnxA1 – a Ca++-binding, membrane-associated protein regulating multiple aspects of vesicle-trafficking – caused EMT and metastasis in EpC40 cells, but reversed EMT and abolished metastasis when overexpressed in dedifferentiated metastatic human mammary carcinoma cell lines. AnxA1 RNAi-induced EMT in EpC40 cells which required JAK/STAT3 signaling and Erk/MAPK activation. Therefore, cooperation of Ras with loss or gain of function in proteins from molecular machines essential for epithelial polarity causes EMT and metastasis, possibly by altering intracellular organelle-associated signal transduction occurring in MAPK, PI3K, TGFβR and STAT3 signaling. A variant of MDCK cells (the major cell model to study epithelial polarity) – able to undergo complete EMT in response to an estradiol-activated RafDD-ER protein (Fig. 3) – will be used to further investigate the above mentioned hypothesis.

Figure 2: ***Endogenous CREG is necessary for TGFβ-induced EMT in EpRas cells and for EpRas-induced lung metastasis after tail vein injection.*** *A. EpRas-clones infected with lentiviruses expressing si-RNA against mouse CREG (EpRas-sh-CREG #3) show suppression of TGFβ-induced EMT observed in control cells (EpRas-sh-control). Note CREG-siRNA-dependent reversion of distended, mesenchymal clusters (top middle) with cytoplasmic-or stress-fiber-localized β-actin (top right) to compact structures (bottom middle) with cortical β-actin (bottom right). Knockdown of endogenous CREG also abolished the Ras-dependent protection from TGFβ-dependent apoptosis (right panels; strong increase in cells expressing cleaved Caspase 3). B. Cells expressing or not expressing CREG-si-RNA-constructs were injected into the tail vein of nude mice. Lungs were retrieved when the control animals became moribund, were sectioned and stained with Giemsa. Note the almost complete absence of dark-violet metastatic nodules (EpRas-sh-control) in the lungs from EpRas-sh-CREG#3 mice (left panels), as confirmed by quantitative evaluation of the complete metastatic area in serial sections from these lungs (top right). Tumor formation by the same cells after fat-pad injection was almost unaffected by CREG-siRNA-induced loss of CREG in EpRas cells (bottom right)*

Figure 3: ***Activation of constitutively active DDRaf-ER fusion protein by estradiol plus TGFβ addition causes complete EMT in fully polarized MDCK-cells.*** *Untreated DDRaf-ER-MDCK cells form fully polarized, cobblestone-like monolayers (insets top left) on porous supports that express cell surface E-cadherin, cortical actin and hardly any vimentin (left panels) Treatment of the cells with estradiol plus TGFβ generated disorderly multilayers (insets top right) of cells lacking E-cadherin, showing cytoplasmic or stress-fiber-localized β-actin and abundant vimentin (right panels).*

MEINRAD BUSSLINGER

Stem cell commitment in hematopoiesis

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Tissue-restricted stem cells give rise to the different cell types of an organ by undergoing commitment to and subsequent differentiation along distinct lineages. By using a combination of mouse transgenic, cell biological and molecular approaches, we investigate the mechanisms by which transcription factors such as Pax5, E2A, Notch1 and GATA3 control the commitment of early hematopoietic progenitors to the lymphoid lineages.

B cell commitment

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 the transcription factors STAT5, E2A, EBF1 and Pax5. STAT5 is a downstream mediator of IL-7 signaling, which controls cell survival by upregulating the antiapoptotic gene *Mcl1* and suppresses premature immunoglobulin κ light-chain (*Igk*) gene rearrangements during pro-B cell development (Figure 1). E2A and EBF1 function as B cell specification factors by activating B-lymphoid genes. *Pax5* in turn controls the B cell commitment by restricting the developmental potential of hematopoietic progenitor cells to the B cell pathway. Surprisingly, conditional *Pax5* deletion allows mature B cells from peripheral lymphoid organs to dedifferentiate *in vivo* back to early uncommitted progenitors, which are subsequently able to develop into functional T cells. These experiments demonstrate that *Pax5* functions as a critical B cell identity factor to maintain B-lineage commitment from the pro-B to the mature B cell stage. We recently identified upstream regulators of *Pax5* by demonstrating that EBF1 is responsible for opening up the entire *Pax5* promoter region, whereas other transcription factors, such as PU.1, IRF4, IRF8 and NF-κB, control the B cell-specific activity of the *Pax5* enhancer in intron 5.

Molecular mechanism

Pax5 fulfils a dual role in B-lineage commitment by activating the expression of B cell-specific genes and by repressing the transcription of lineage-inappropriate genes. This reprogramming of gene expression restricts the broad signaling capacity of uncommitted progenitors to the B cell pathway, induces V_H-DJ_H recombination, facilitates (pre)B cell receptor signaling, regulates B lymphocyte adhesion and migration and promotes development to the mature B cell stage. Chromatin profiling by ChIP-chip and ChIP-sequencing analyses demonstrated that *Pax5* induces or eliminates active chromatin at promoters or enhancers of activated and repressed *Pax5* target genes, respectively (Figure 2).

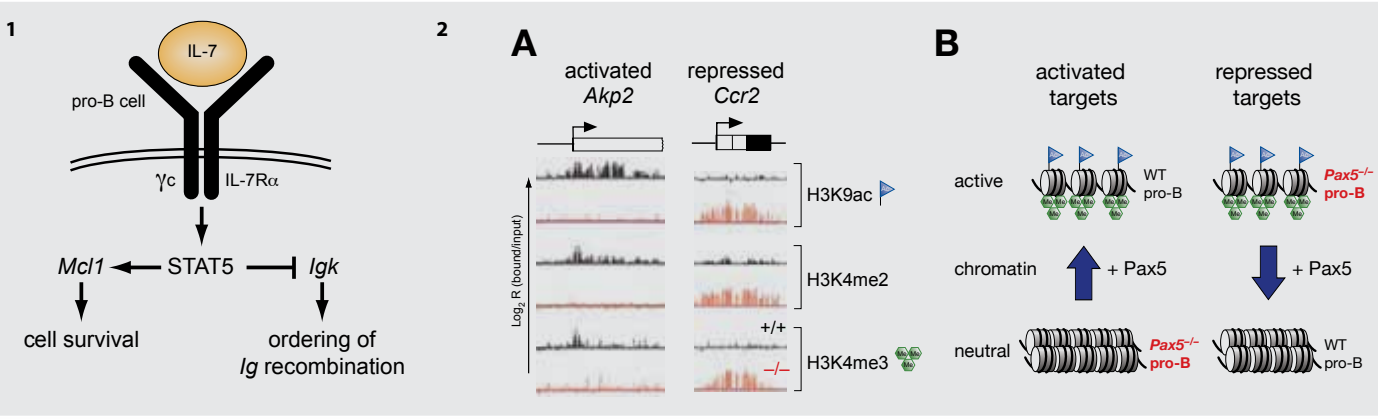

Figure 1: ***Role of STAT5 in early B cell development.***
As shown by genetic experiments, STAT5 and IL-7 signaling control the pro-survival gene Mcl1 and restrict Igk recombination during pro-B cell development.

Figure 2: ***Regulation of Pax5 target genes at the chromatin level.***
(A) ChIP-chip analysis of wild-type (+/+) and Pax5-deficient (-/-) pro-B cells was used to map the active histone modifications H3K4me2, H3K4me3 and H3K9ac at activated (Akp2) and repressed (Ccr2) Pax5 target genes. (B) Summary of the chromatin regulation at most Pax5 target genes.

T cell specification

Signaling through the Notch1 receptor is essential for the initiation of T cell development in the thymus. T cell specification also depends on the transcription factor *GATA3*. We previously demonstrated that Notch signaling induces the expression of Gata3. To investigate the molecular function of Notch1 and *GATA3* in early T-lymphopoiesis, we are identifying direct target genes of these two regulators by conditional mutagenesis, gene expression profiling and ChIP sequencing.

Spatial control 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 have a large size ranging from 0.67 (*Tcrb*) to 3 (*Igh* and *Igk*) megabases and exhibit a complex organization with the D, J and C segments constituting the proximal (3') domain of each locus. The largest part (> 80%) of the *Ig* and *Tcr* loci is devoted to the V gene cluster, which is separated from the proximal domain by a large distance on linear DNA. We have shown that the *Igh* and *Igk* loci undergo reversible contraction in pro-B cells and pre-B cells, respectively, whereas *Tcrb* and *Tcra/d* loci contract by looping in pro-T cells and pre-T cells, respectively. Reversible locus contraction by looping is thus a general mechanism that promotes the spatial communication between V genes and the proximal domain by juxtaposing distantly located V genes next to D or J segments, thus facilitating synapse formation and V gene recombination. We are currently elucidating the molecular mechanism by identifying *cis*-regulatory elements and *trans*-acting factors involved in *Igh* locus contraction.

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TIM CLAUSEN

Molecular mechanisms of protein quality control

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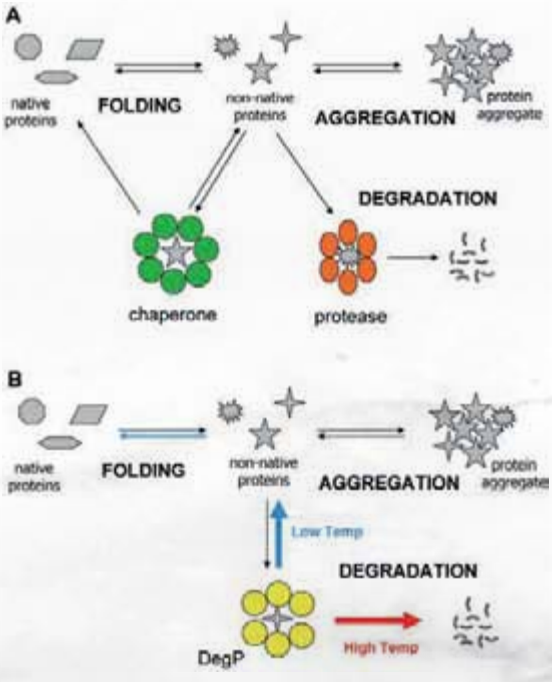
The misfolding and aggregation of protein molecules is a major threat to all living organisms. Cells have therefore evolved a sophisticated network of molecular chaperones and proteases to prevent protein aggregation (Fig. 1A). In addition, protein quality control and regulatory proteolysis are important 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

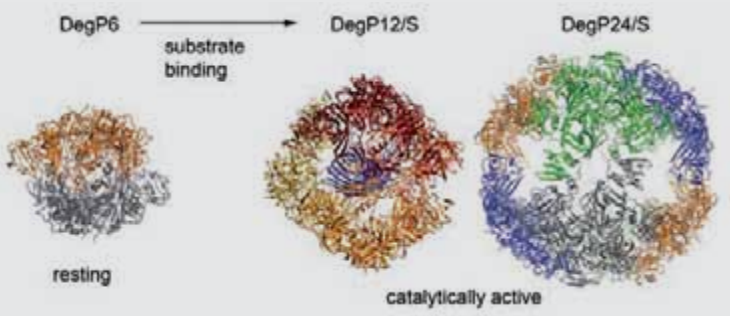
A combination of chaperone and protease function in a single protein could provide a direct and rapid response to protein folding problems. The heat shock protein DegP (HtrA) can switch between these dual functionalities in a temperature-dependent manner (Fig. 1B), and thus offers unique possibilities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

1



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.

2



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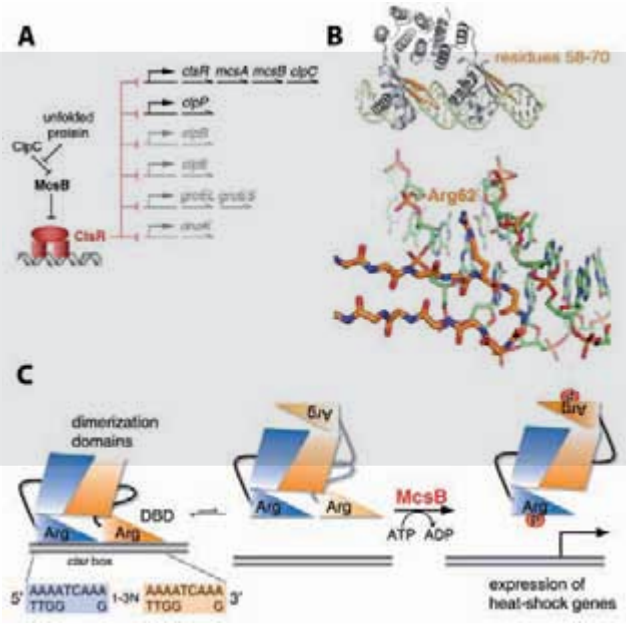

Figure 1: *Protein Quality Control (A) The scheme illustrates the different fates of misfolded non-native proteins. According to Gottesman and co-workers' "kinetic partitioning model", aggregate formation is counteracted by the action of proteases and chaperones. (B) DegP combines the antagonistic activities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

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 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

Cell polarity in development

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Cell polarity allows an enormous variety of specialised functions during development. Despite the range of contexts in which cell polarity is essential, the general steps of polarisation are the same: establishment, transduction, and segregation. We are investigating the molecular mechanisms of cell polarisation in one-cell C. elegans embryos.

Cell polarity is essential for development. Polarity establishes spatial information in a cell, allowing for different functions in different places. Cell polarity allows cells to migrate, to change shape, and to transport molecules in or out. Cell polarity also mediates the segregation of fate determinants, allowing for the development of different cell types. Bacteria, fungi, plants and animals all require cell polarity for morphogenesis and functional specialisation. Without cell polarity, cells would create homogenous masses, and indeed, the loss of cell polarity is an early event in tumour formation.

Our group is investigating how cell polarity is established, transduced, and segregated in *C. elegans* embryos. We use a combination of forward and reverse genetics, in vivo biochemistry, high-resolution time-lapse microscopy, ultrastructural reconstructions, automated quantitative analysis, and mechanical manipulations to answer the following questions:

How is cell polarity established?

In one-cell *C. elegans* embryos, cell polarity is established by the centrosome. Prior to polarisation, the centrosome floats in the cytoplasm, but coincident with polarity establishment, the centrosome moves to the cell cortex. The centrosome initiates a global reorganisation of the cortical acto-myosin network, which in turn promotes an asymmetric distribution of the highly conserved PAR proteins. We are investigating how the centrosome communicates with the cortex, including the mechanism of centrosome–cortex juxtaposition and the nature of the signal that initiates polarity.

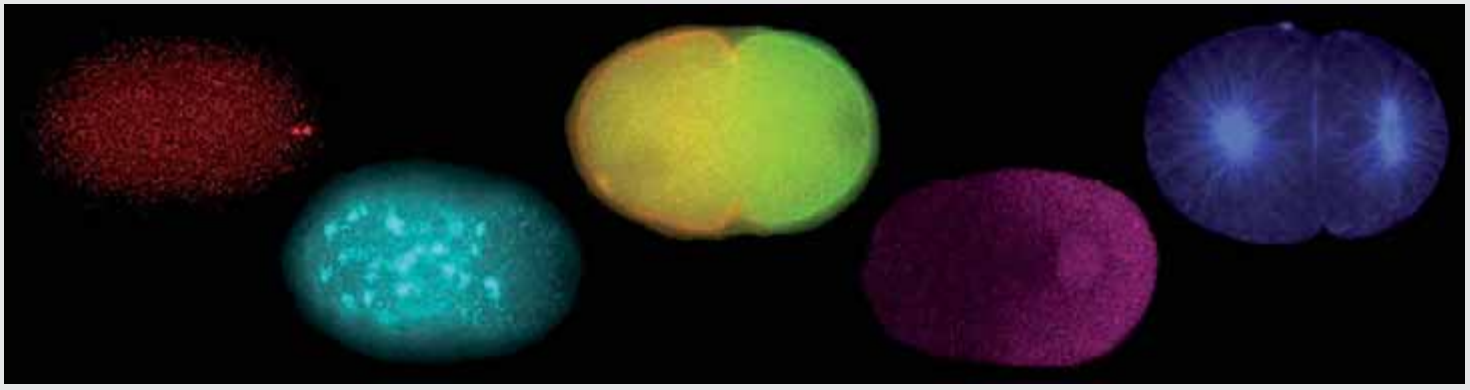

Figure: *Polarity establishment and segregation in C. elegans embryos. Centrosomes (red dots) move to the cortex to initiate polarization of the cortex. This leads to changes in the acto-myosin cytoskeleton (cyan) that underly the formation of anterior (red) and posterior (green) cortical domains, defined by PAR proteins. Cortical polarity controls the unequal segregation of fate determinants in the cytoplasm (magenta). During asymmetric division, the mitotic spindle (blue) repositions the boundary between the PAR domains, ensuring cell fate is inherited exclusively. This whole process takes approximately 25 minutes.*

How is cell polarity transduced into the cytoplasm and nucleus?

In one-cell *C. elegans* embryos, cell polarity is defined by complementary anterior and posterior cortical domains, which comprise distinct PAR proteins. Fate determinants, such as the germline-specific transcriptional repressor PIE-1, localise to the cytoplasm and nucleus. PIE-1 is distributed asymmetrically in the one-cell embryo, ensuring it is inherited only by the future germline blastomere. We are investigating how two-dimensional polarity at the cortex can organise the three-dimensional space of the cytoplasm to achieve asymmetric PIE-1 localisation.

How is cell polarity segregated during division?

In one-cell *C. elegans* embryos, the anterior and posterior cortical cell polarity domains are inherited by either the anterior or posterior blastomere. The boundary between the PAR domains is repositioned to match the site of cell division to achieve this exclusive segregation. The process of domain boundary correction operates through a size threshold that can, in extreme cases, lead to mis-segregation of cell polarity and cell fate transformation. We are investigating how the PAR domain boundary responds to the cytokinesis furrow, including the mechanism of domain size sensing and the furrow properties that signal boundary correction.

BARRY DICKSON

Neural circuits

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We are using molecular genetic techniques to study the function of neural circuits in Drosophila. We want to understand how the brain works. How does information processing in neural circuits generate complex animal behaviour? As a model system, we focus on the fly's mating behaviours. These behaviours are robust, adaptive, and particularly amenable to genetic analysis.

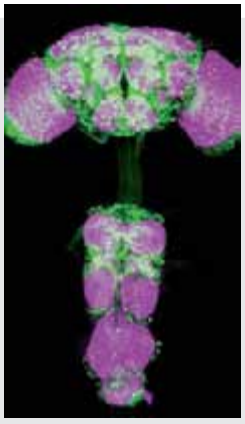
A major goal for neuroscience is to understand how information processing in neural circuits guides behavioural decisions. How, at any given moment, does an animal decide what to do? Somehow, the brain selects the best course of action by integrating information from multiple sources – sensory input, internal physiological states, and individual experience. We would like to find out how the brain does this.

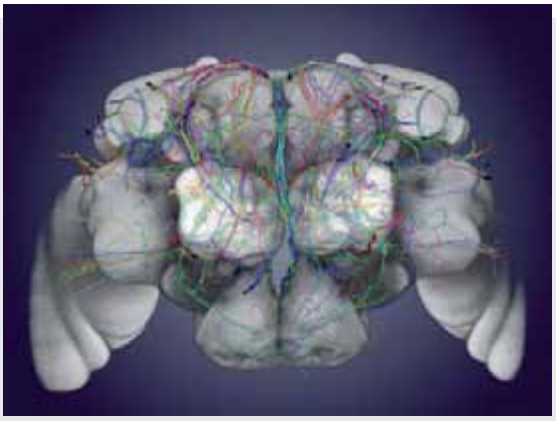
As a model, we have chosen to study the sex life of the fruit fly *Drosophila melanogaster* [1]. During mating, flies make decisions that are critical for their reproductive success, and hence their evolutionary fitness. Upon encountering another fly, the male estimates his chances of success and decides if it is worth investing time and energy in courtship. Whether or not mating actually occurs is a matter of female choice, based on how she assesses of her suitor's quality, as well as her own readiness to mate. 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 linking cellular biochemistry, circuit function, and animal behaviour.

The male brain

Remarkably, the different mating behaviour 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 are currently developing methods to gain genetic access to distinct subsets of *fru* neurons, so that we can study the anatomy and physiology of the *fru* circuit at single-cell resolution (Fig. 2). We would like to know if and how each of type of *fru* neuron contributes to courtship, what types of signal each neuron processes, and how and to what extent sexually dimorphic processing in these neurons leads to the distinct behaviours of males and females.





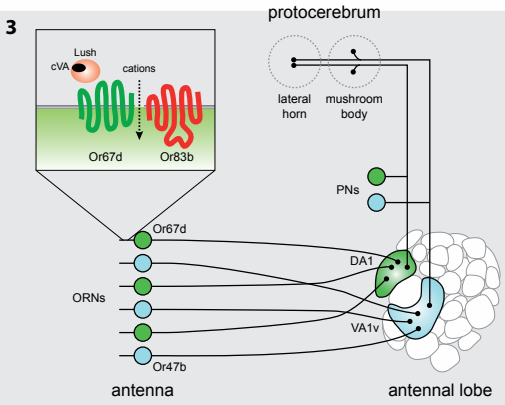


Figure 1: *fru⁺ neurons in the CNS, shown in green. Synapses are stained in magenta. This is a confocal image of the brain and ventral nerve cord of a fru^{GAL4} UAS-GFP male.*

Figure 2: *Genetic dissection of fru⁺ neurons in the central brain. Distinct neuronal cell types have been labelled, imaged, and traced in different colors. A synaptic counterstain was used to register individual samples onto common reference brain, creating a digital atlas of the fru circuit.*

Figure 3: *cVA detection and processing in the fru⁺ Or67d⁺ ORNs and DA1 PNs.*

One important class of *fru*⁺ neurons are the olfactory receptor neurons (ORNs) that detect pheromones. We have recently found that one subclass of *fru*⁺ ORNs expresses the odorant receptor Or67d and responds to a male sex pheromone. These neurons connect to second-order olfactory neurons in the antennal lobe of the brain (called DA1 projection neurons) (Fig. 3). The neurons are also *fru*⁺, and appear to make sexually dimorphic connections in higher brain centers. This may be a critical site for sex-specific processing of pheromone signals.

The female brain

The female decides whether to accept or reject the male based in part on her perception of his courtship song and pheromones. The biggest factor in the female's decision, however, is her own mating status. Virgin females are generally receptive to courting males, whereas females that have recently mated are not. This difference can largely be attributed to a small peptide, called the sex peptide (SP), that is present in the first male's seminal fluid. If females mate to males that lack SP, they remain receptive to other males. Conversely, direct injection of SP into virgin females renders them unreceptive. But how and where does SP act in the female to modulate her mating decision?

By screening our transgenic RNAi library, we have recently identified a molecular receptor for SP, a G-protein coupled receptor we call SPR (sex peptide receptor) [2]. SPR is broadly expressed in the nervous system, but we find that it is specifically required in a small subset of *fru*⁺ sensory neurons that innervate the reproductive tract and project axons to various regions in the central nervous system [3]. Our ongoing efforts are aimed at understanding how SP modulates the function of these neurons, and how this in turn impacts the functioning of circuits in the brain that assess male courtship signals and decide whether or not to allow mating.

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CHRISTINE HARTMANN

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 are determined as well as the positions of articulations (joints). Most skeletal elements are formed as cartilaginous templates and need to be remodeled into bony tissue to provide strength to the skeleton. This process is referred to as endochondral ossification. We use mouse and chick as model organisms to gain insight into how these different processes are regulated by Wnt-signaling pathways during embryonic and postnatal development.

Regulation of cell lineage differentiation

The different cell types of the appendicular skeleton, the chondrocytes, osteoblasts and cells contributing to the future synovial joints are of mesenchymal origin. In recent years we have demonstrated that the canonical Wnt-signaling pathway, which is mediated through β-catenin, plays an essential role in the development of the different lineages of the mouse skeleton (see Figure 1). In the absence of functional canonical β-catenin signaling, osteoblast precursors, the osteochondroprogenitor 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).

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. Interestingly, alterations in the process of chondrocyte maturation often lead to changes in the size of skeletal elements. Studying the Wnt9a knock-out animals we uncovered a very specific requirement for one of the Wnt-ligands, Wnt9a, during long-bone development: Wnt9a via β-catenin signaling controls the expression of the central regulator of chondrocyte maturation, Indian hedgehog, in a spatio-temporal manner (Spaeter et al., 2006). Thus sequential activation, or temporal recruitment of regulatory molecules, which like Wnt9a control a central regulator of chondrocyte maturation, are likely to be involved in fine-tuning the size.
We also uncovered a potential role for Calcium/Calmodulin-dependent kinase II (CaMKII) in the maturation process of chondrocytes. Activation of CaMKII results in premature maturation of the chondrocytes expressing the molecule and elongation of the skeletal element, while inhibition of the kinase by expression of a peptide inhibitor leads to a delay in chondrocyte maturation and shortening of the skeletal element (Taschner et al., 2008). (Figure 2)

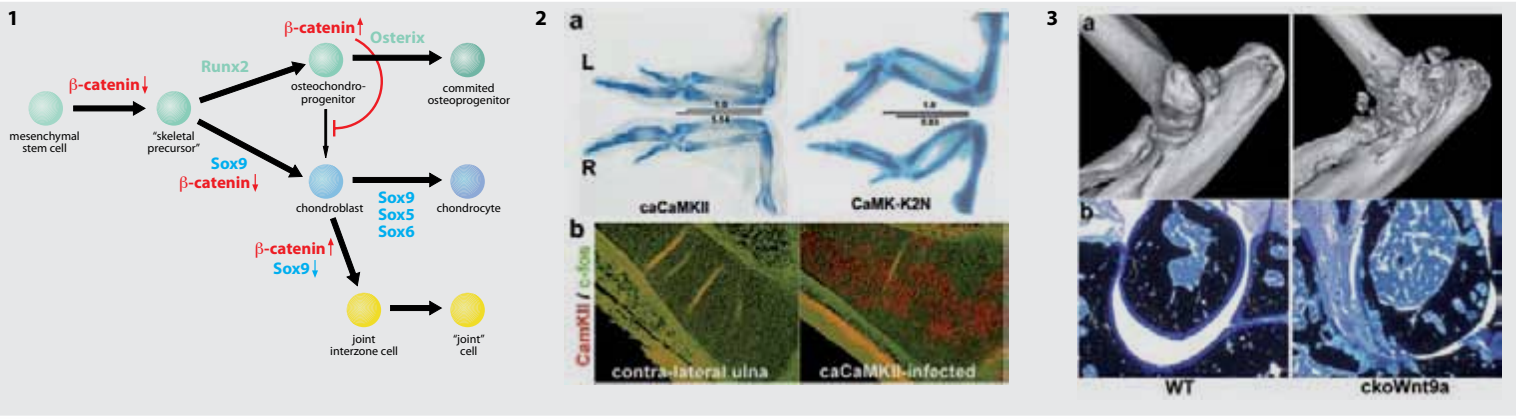

Figure 1: *β-catenin levels need to be modulated during skeletal lineage differentiation: only mesenchymal cells expressing low levels of β-catenin can differentiate into skeletal precursor which will give rise to osteoblasts, chondrocytes, and cells of the joint. Within the osteoblast and joint lineage β-catenin levels need to be increased to enable differentiation into osteoblasts and cells of the joint, respectively. 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) The lengthening is associated with the down-regulation of cfos expression (green) in the caCaMKII infected regions (red).*

Figure 3: *a) MicroCT of a wt elbow and an elbow of a limb lacking Wnt9a (cko Wnt9a), the latter showing ectopic ossifications. b) Histology of the elbow joints, showing loss of the articular cartilage (arrow in the wt elbow joint).*

Synovial joint development

In gain-of-function analysis in the chicken, Wnt9a was identified as a major player for the induction of synovial joint development (Hartmann and Tabin, 2001). In mouse Wnt9a is expressed in the early joint interzone and, after the formation of the synovial joint, in the articular cartilage and in the cells of the joint capsule. In mice lacking Wnt9a an ectopic cartilage nodule differentiates from synovial cells in the humeral-radial joint. In humans this phenotype is known as synovial chondroid metaplasia. Two other Wnt-genes are 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 to a complete fusion of all joints. Thus, the embryonic phenotypes point to a role for Wnts in maintaining joint integrity. Conditional deletion of Wnt9a specifically in the limb mesenchyme results in progressive changes in various joints, which show hallmarks of osteoarthritis. In addition these mice show ectopic mineralisation within joint ligaments (Figure 3).

In summary, our analysis has show that Wnt-signaling plays an important role during skeletal development, regulating differentiation of the various cell types and maturation of chondrocytes, and is important for maintaining the integrity of the developing and mature joint.

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The Molecular Basis of Migration

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

Neuronal Migration

Neuronal migration underlies the organisation of the mammalian brain. All neurons that are born in the proliferative ventricular zones (VZ) migrate to their final destination by extending their primary neurites and translocating their nuclei. This migration is crucial as it dictates not only the final destination of a neuron, but also determines the connections that it forms, and therefore the neuronal circuits in which it operates. How do the neurons know where they are going? and how do they get there?

The study of genetic diseases in which neuronal migration is impaired has provided some answers to these questions. Characterised by a lack of gyrations in the cortex, lissencephaly, is such a disorder. Mutations in DCX, reelin, LIS1, VLDLR have been shown to cause this disease. By studying the mouse mutant, *Jenna*, we have added the tubulin gene Tuba1a to this list (Keays, et al 2007). The vital role of tubulin in brain development is further evidenced by our most recent finding that mutations in TUBB2B (Jalgin et al, 2009), cause a rare neurdevelopmental disorder known as asymmetric polymicrogyria. We are continuing to investigate the role of the tubulin genes in the developing brain by employing molecular techniques such as *in situ* hybridisation in conjunction with transgenic mouse models.

Molecular basis of 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.

There are two theories that attempt to explain the mechanism that underlies it: (1) the radical pair theory of magnetoreception; and (2) the magnetite based theory of magnetoreception. The first theory holds that the earth's magnetic field influences the spin states of radical pairs in photosensitive molecules; and

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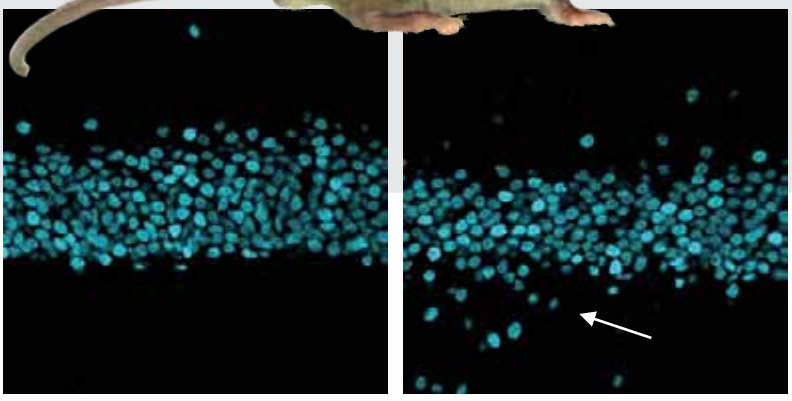

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Figure 1: *The Jenna mouse. Harbouring a S140G mutation in the TUBA1A gene, the Jenna mouse has a defect in neuronal migration. As a consequence it has anatomical abnormalities in the cortex and hippocampus, which results in hyperactivity and poor working memory.*

Figure 2: *PROX1 staining of granule cells in the adult hippocampus reveals cellular dispersion (shown with an arrow) in the Jenna mouse (right) when compared with littermate controls (left).*

Figure 3: *In this conditioning paradigm marked bees are trained to associate a food reward with the presence or absence of a magnetic anomaly*

the second is a mechanosensitive model that relies on intracellular magnetite crystals (Fe_3O_4). The second theory, which we are focusing on, has sprung from observations made in magnetotactic bacteria. These aquatic bacteria use the Earth's magnetic field to direct swimming towards growth-favouring regions in natural waters. It has been discovered that magnetotatic bacteria possess organelles called magnetosomes. Magnetosomes consist of membrane-enclosed magnetite crystals that twist into alignment with the Earth's magnetic field – thereby directing bacterial movement. The theory of magnetite based magnetoreception has been supported by the discovery of magnetite in a range of other organisms that detect and respond to magnetic fields; most notably birds, fish and bees. I am investigating the genes and molecules that are required for magnetite based magnetoreception simultaneously employing behavioural, anatomical and genetic tools in the honeybee *Apis mellifera* and the rock pigeon *Columbia livia*.

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Molecular Machines

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Molecular machines are essential to maintain life at the cellular level. We aim to understand the fundamental molecular design, assembly processes, and mechanistic details of such higher-order structures. At our laboratory we are specifically interested in protein translocation machineries that are essential for microbial infection.

Microbial Pathogenesis – the Type III Secretion System

One of the most exciting discoveries in the field of bacterial pathogenesis is the fact that many bacterial pathogens utilize supramolecular nanomachines to deliver bacterial proteins into eukaryotic cells. These proteins, which are collectively referred to as effectors, have the capacity to modulate a variety of cellular functions including cytoskeleton dynamics, vesicle traffic, cell cycle progression, and transcription. Probably one of the best understood machineries is the type III secretion system (TTSS), which is made up of more than 20 different proteins. Using Salmonella typhiumurium, we are investigating the molecular mechanisms and structural framework required to translocate effector proteins specifically and safely into eukaryotic cells.

The most prominent substructure of the TTSS is known as the "needle complex": a cylindrical, needle-shaped and membrane-embedded organelle protruding from the bacterial envelope (Figure 1). The needle complex is believed to serve as a conduit for safe transport of virulence factors from the bacterial cytoplasm through a number of natural barriers into eukaryotic cells. In Salmonella typhimurium, which serves as our model for bacterial delivery systems, this complex is formed by multiple copies of only five proteins: PrgH, PrgK, and InvG constitute the membrane-associated base structure, PrgJ the inner rod, and PrgI the needle filament extending into the extracellular environment (Figure 2).

In order to investigate the molecular mechanism of type III secretion, we first set out to determine structural components of the TTSS (Figure 3). We were challenged by the mega-dalton size of the complex, its natural composition (membrane and soluble proteins), and its limited availability. Nevertheless, we were able to purify sufficient quantities of the entire 'needle complex' and its precursor, the 'base', by a combination of detergent extraction and size separation by velocity gradient centrifugation. A detailed structural analysis by three-dimensional electron cryo-microscopy and single-particle analysis finally revealed a new structural component, the inner rod, which is located at the center of the needle complex. It extends the secretion path from the base into the needle filament and also serves as an anchor to stably connect the needle filament into the base. During assembly, the inner rod and the needle filament are added as new structural components to the base (Figure 2, 3). As a consequence,

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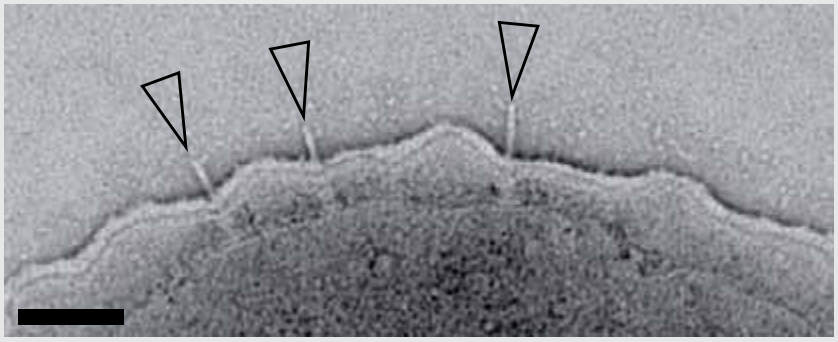

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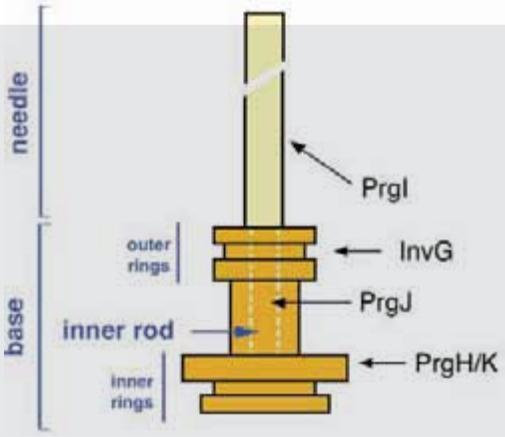

Figure 1: ***The needle complex is the core structure of the type III secretion system.*** *Needle-like structures that extend into the extracellular environment are visible on the surface of osmotically shocked S. typhimurium (bar 80nm)*

Figure 2: ***Schematic representation of the Salmonella needle complex and its components****. PrgH, PrgK, and InvG constitute the membrane-embedded base structure, whereas PrgI forms the helical filament protruding into the extracellular environment. The inner rod anchors the filament into the base.*

Figure 3: ***The structure of the needle complex*** *A) Surface renderings and projections of the ~30-nm-wide needle complex obtained from three-dimensional image reconstruction from vitrified needle complexes. B) Longitudinal sections of the base and the needle complex reveal the overall distribution of protein density within the complexes.*

it must undergo large conformational rearrangements which demonstrates the flexible but also stable qualities of the base. Functionally, this dynamic behavior is a crucial event in the assembly phase during which the secretion machine is reprogrammed to become competent for the secretion of virulence factors. Structurally, it underlines the importance of specific interaction epitopes critical for the assembly into a functional unit.

Recent crystallographic analyses of individual separated domains which are predicted to be periplasmically located, revealed a common structural motif organized in repeating modules. Attempts have been made to "dock" these protein domains into the needle complex structure, which resulted in different and mutually incompatible locations. We used a combination of methods, including bacterial genetics, biochemistry, mass spectrometry and cryo-electron microscopy/single-particle analysis, to experimentally determine which specific protein domains correspond to different substructures of the needle complex. In addition, we identified specific interaction sites among components of the needle complex, which are critical for stable assembly and consequently functional complex. In combination, this analysis provides the first experimentally validated topographic map of different components of the needle complex of the S. Typhimurium TTSS.

Although the design of the TTSS appears to be conceptually simple, several questions remain unanswered: What nucleates the assembly of the TTSS? How dynamic is the entire assembly process? And how are substrates recognized and translocated? We have started to address some of these questions. Understanding the molecular mechanism of TTSS-mediated protein transport should provide a basis for the development of novel therapeutic strategies to either inhibit its activity or modify the system for the purpose of achieving targeted drug delivery.

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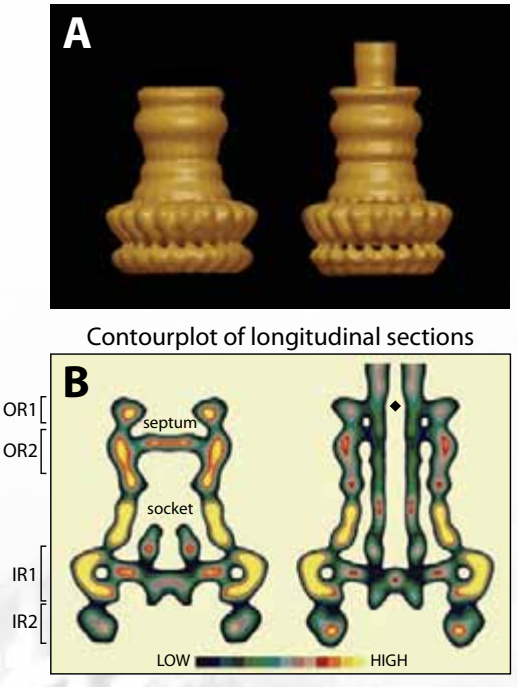

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Mitosis and chromosome biology

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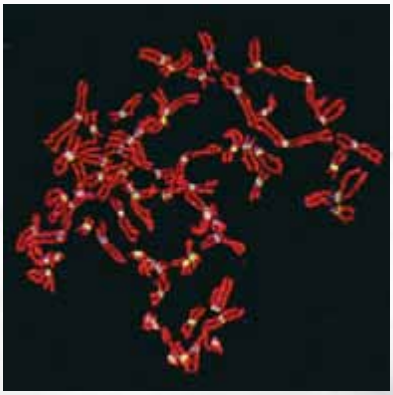

To pass the genome from one generation to the next, eukaryotic cells first replicate their DNA, then biorient chromosomes on the mitotic spindle, and finally separate their sister chromatids, thus enabling division of one cell into two genetically identical daughter cells. We are interested in understanding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

How is sister chromatid cohesion established during DNA replication?

Throughout interphase, numerous sites in mammalian genomes are bound by ring-shaped 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 DNA damage repair, but how cohesion is established is poorly understood. We discovered previously that the establishment of cohesion coincides with particularly stable binding of cohesin to DNA, and that this binding mode depends on a cohesin-associated protein known as sororin. The establishment of cohesion is also known to require acetylation of cohesin. We are therefore trying to understand how sororin and cohesin acetylation convert cohesin into a "cohesive" state during DNA replication.

How does cohesin control chromatin structure and gene regulation?

Although cohesin is best known for its role in mediating cohesion, we and others discovered that it also plays a significant role in gene regulation. We suspect that these functions are the reason why cohesin binds to chromatin even before cohesion is established, and why cohesin associates with DNA even in postmitotic cells, which will never establish cohesion. We found that cohesin co-localizes in mammalian genomes with the transcriptional insulator protein CTCF and showed that cohesin is required for gene regulation at the imprinted *H19-IGF2* locus. Gene expression at this locus is believed to be controlled by 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. Our future aims are to test whether cohesin plays a general role in forming chromatin loops, and to understand the mechanistic basis of this function and its relationship with cohesin's role in cohesion.

How is sister chromatid cohesion dissolved during mitosis?

Sister chromatid separation in anaphase depends on the removal of cohesin from chromosomes. Several years ago we discovered that this process depends on two mechanisms in vertebrate cells: the dissociation of cohesin from chromosome

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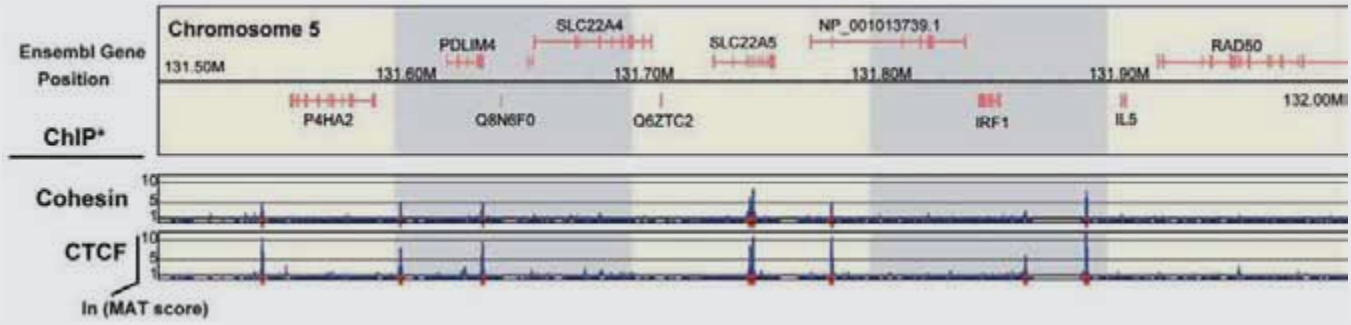

arms in prophase, and the proteolytic cleavage of cohesin at centromeres in metaphase. The prophase pathway depends on the cohesin-associated protein Wapl, whereas the metaphase pathway is mediated by the protease separase. Although the prophase pathway was identified several years ago, its function 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*.

How does the APC/C initiate anaphase?

In metaphase, when all chromosomes have been bioriented, the anaphase promoting complex/cyclosome (APC/C) is activated. The APC/C is a 1.5 MDa complex which assembles ubiquitin chains on securin and cyclin B. The subsequent destruction of these proteins by the 26S proteasome allows activation of separase, cleavage of centromeric cohesin, and sister chromatid separation. Until chromosome biorientation is complete, the APC/C is inhibited by the spindle assembly checkpoint (SAC). The SAC ensures that sister chromatids are only separated once chromosomes have become attached to both spindle poles. Despite the crucial importance of APC/C, it is poorly understood how this complex is inhibited by the SAC, how the inhibition is relieved in metaphase, and how active APC/C recruits and ubiquitylates its substrates. We are using biochemical assays and electron microscopic analyses of APC/C in different functional states to address these questions.

MitoCheck

Although mitosis has been studied for more than a century, our molecular understanding of this complicated process is far from complete. During the past five years, the MitoCheck consortium funded by the 6th framework program of the European Union has developed and applied genomic and proteomic approaches to study mitosis. The consortium has used RNA interference screens to identify proteins required for mitosis in human cells, tagging of genes in bacterial artificial chromosomes (BACs) to enable intracellular localization and affinity purification of these proteins and mass spectrometry to identify protein complexes and mitosis-specific phosphorylation sites on these. This work identified numerous protein complexes, many of which had previously escaped identification or had been poorly characterized. Importantly, the approaches developed by MitoCheck will generally be applicable to high-throughput analyses of other processes in mammalian cells. In the future we will develop quantitative assays for mitosis in a new project named MitoSys funded by the European Union.

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

Figure 2: *Co-localization of cohesin and CTCF on a region of human chromosome 5, as detected by chromatin immunoprecipitation-chip experiments. Courtesy of Kerstin Wendt and Katsuhiko Shirahige.*

Figure 3: *A 3D model of Xenopus APC/C obtained by angular reconstitution of negatively stained cryo-elctron microscopy preparations. Courtesy of Holger Stark.*

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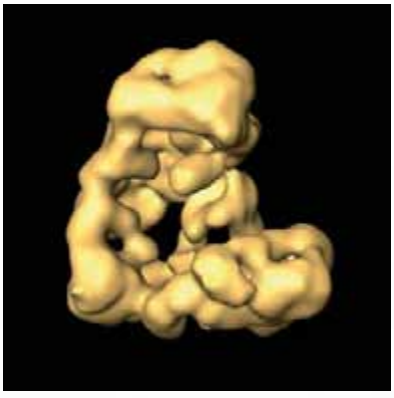

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Synaptic and circuit mechanisms of memory formation

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At our laboratory we are interested in the development, function and plasticity of neuronal circuits. More specifically, we would like to know how Man is able to store memories over long periods of time. This is fundamental in order to understand the human mind: memories of past experiences shape our personalities and influence our current perception.

Trafficking of synaptic receptors during memory formation

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. The molecular mechanisms underlying synaptic plasticity have been largely studied in cultured neurons. Synaptic plasticity is thought to be expressed, at least in part, through molecular changes that lead to the addition or removal of synaptic AMPA receptors. In particular, synaptic addition of AMPA receptors containing the subunit GluR1 has been identified to be crucial for increases in synaptic strength. However, the processes actually occurring in the brain during the formation of a memory trace are still poorly understood.

In order to delineate the role of specific candidate molecules and learn how memories are stored at the circuit level, we use molecular tools in the rodent in the context of memory-related behavior. Pavlovian conditioning is a simple and robust behavioral model of learning and memory formation, in which animals associate a tone with a mild foot-shock and subsequently "freeze" when presented with the tone alone. Plasticity in the lateral amygdala was shown to play an essential role in this behavior.
We recently tested the hypothesis that synaptic addition of the GluR1 subunit containing AMPA-type glutamate receptors mediates adaptation of synaptic strength in the lateral amygdala, which underlies the formation of memory in the tone-shock association. We find that, during Pavlovian conditioning, about a third of amygdala neurons undergo synaptic plasticity by incorporation of GluR1-containing receptors. This suggests that the memory of fear is encoded by many neurons and has a widely distributed nature. Surprisingly, we find that the blockage of synaptic delivery of GluR1-containing receptors in no more than 10-20% of amygdala neurons is sufficient to disrupt the formation of memory. This indicates low redundancy in memory coding and suggests that successful memory retrieval requires participation of the vast majority of coding neurons.

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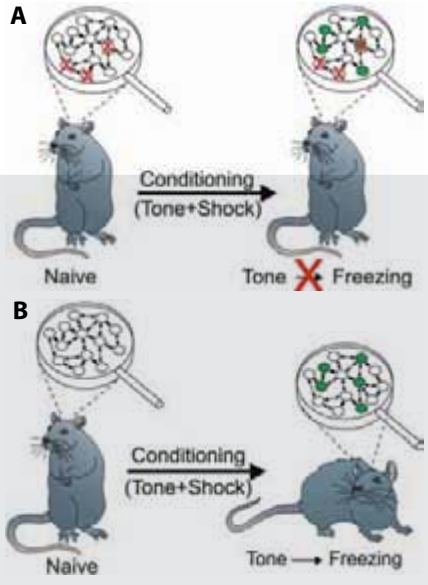

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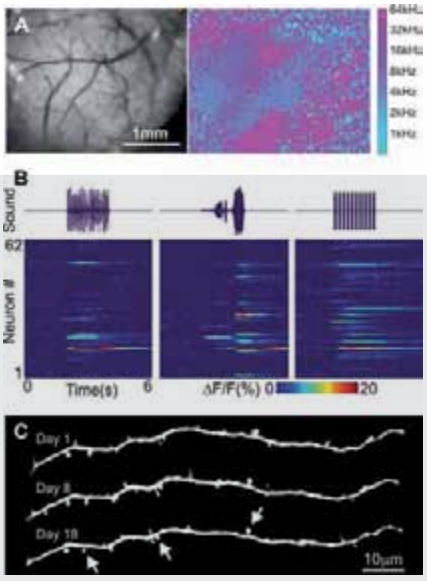

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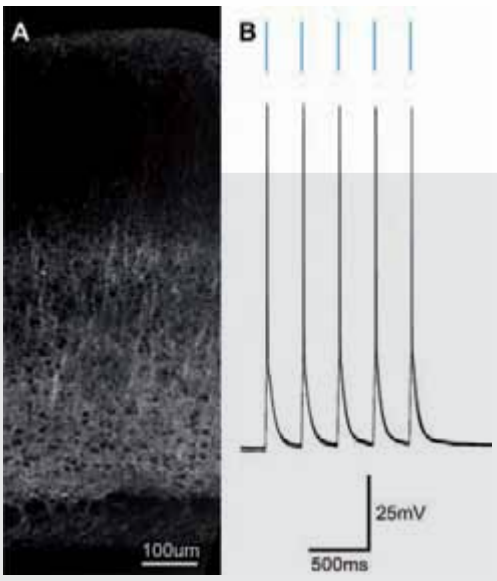

Figure 1: *Memory traces in the lateral amygdala appear to be distributed, but are highly sensitive to perturbations of synaptic plasticity. (A) Rats show freezing behavior when presented with a tone that was previously paired with a shock during a conditioning session. This behavior indicates that an association is formed between the two stimuli, and successfully stored. About a third of neurons in the amygdala undergo plastic changes during conditioning and show synaptic incorporation of GluR1-type AMPA receptors (green circles). (B) Blocking GluR1-type AMPA-receptor-mediated plasticity in only 10-20% of neurons (red crosses) is sufficient to impair the formation of memory.*

Figure 2: *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 3: *Using light to control neurons. (A) Cross-section of the auditory cortex after infection with a virus coding for the light-activated ion channel Channelrhodopsin. Massive expression of the construct is seen in layers V and VI. (B) Patch-clamp whole-cell recording of the membrane potential of a cortex neuron in an acute brain slice taken from an infected animal expressing Channelrhodopsin. A series of action potentials is elicited by a burst of brief light pulses (indicated by blue bars; overlay of five repetitions).*

Peering into the brain

How can we continuously store novel memories in a given neuronal circuit without corrupting previously stored memories? In order to obtain information about mechanisms by which multiple memory traces are coordinated, we are currently applying chronic in vivo imaging techniques to the auditory cortex of mice. In addition to the amygdala, auditory areas of the cortex have been shown to undergo learning-induced plasticity during auditory cued fear conditioning. Two-photon laser scanning microscopy in transgenic animals expressing green fluorescent protein only in a small subset of cells allows the same neurons and even the same individual synapses to be revisited day after day. This is truly remarkable, as we estimate that the brain contains about 10 trillion (10^{13}) synapses. In the future we plan to investigate the impact of auditory cued fear conditioning on the dynamics of a given set of synapses and neurons in the auditory cortex. We hope this approach will pave the way for a series of novel experiments addressing the storage of information in living neuronal networks - a field of research largely confined to theoretical neuroscience thus far.

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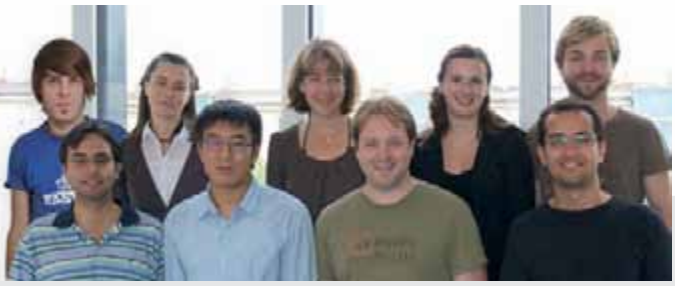

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Systems biology of regulatory motifs and networks – towards understanding gene expression from the DNA sequence

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The regulation of gene expression in response to developmental or environmental stimuli is central to 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. We use both bioinformatics and molecular biology methods to gain a systematic understanding of enhancer structure and function. Our goal is to "crack" the regulatory code, predict enhancer activity from the DNA sequence, and to understand how transcriptional networks define cellular and developmental programs.

The regulatory code, gene regulatory motifs and regulatory networks

The regulation of gene expression is central to the development of all organisms. In higher eukaryotes, genes are expressed dynamically in complex spatial patterns and mis-expression often results in developmental failures and diseases such as cancer. Tissue specific gene expression is determined by regulatory programs and in turn defines the different animal cell types and their characteristics. Given their central role, core regulatory circuits or kernels have been found to be conserved between animals as diverged as flies and mammals.

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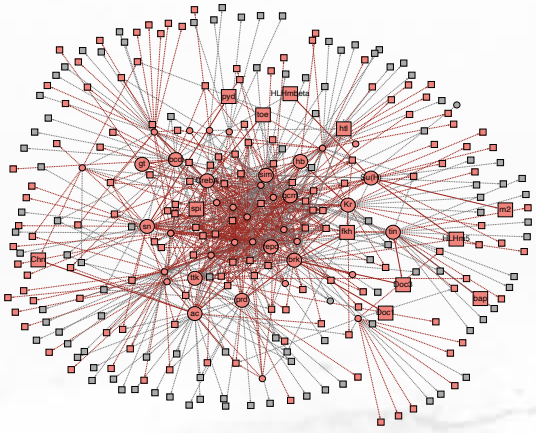
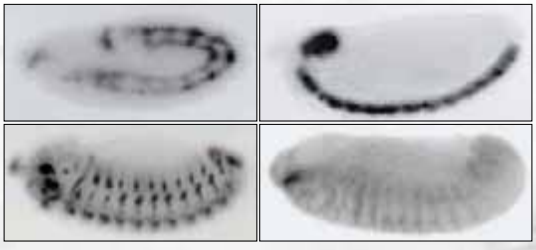
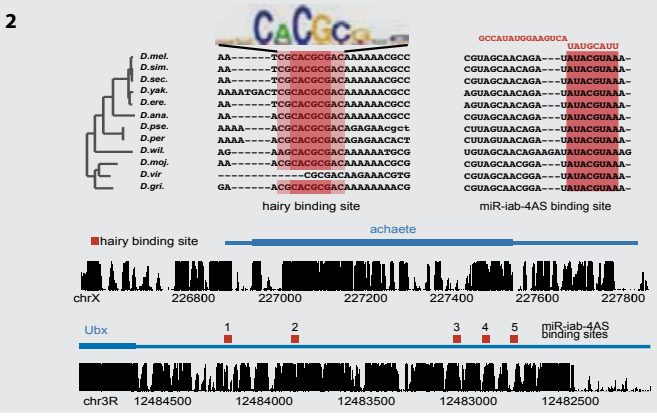




A major challenge in molecular biology is to define these circuits and to decipher how the cell utilizes the regulatory information present in the DNA. This is currently hampered by the lack of a regulatory code that– analogously to the genetic code for protein-coding sequences – would allow us to predict spatio-temporal enhancer activity from the DNA sequence. Our group uses both, bioinformatics and molecular biology methods to study enhancer structures, characterize tissue- and cell-type specific expression, and to predict and validate regulatory targets of transcription factors. We focus on the different cell types and organs in *Drosophila* and aim to explain their expression programs using the regulatory connections of transcription factors.

We use ChIP-Seq experiments to identify tissue-specific targets of transcription factors, and sequence determinants (e.g. other motifs, factors and their combinations) that mediate this tissue-specificity.

We are currently determining the spatio-temporal activity patterns of a large collection of putative enhancers in embryos, and systematically test candidate promoters and enhancers in specific cell-types. Sequence analyses of enhancers with similar activities will allow us to determine the sequence features underlying enhancer function, which we plan to integrate with qualitative and quantitative information about transcription factor expression.



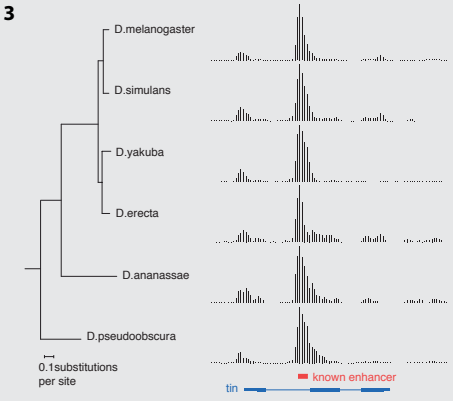


Figure 1: *Regulatory network and tissue-specific expression patterns in Drosophila embryos. See Kheradpour et al., 2007 and Stark et al., 2005 for details.*

Figure 2: *Conservation of transcription factor and microRNA binding sites in 12 Drosophila genomes and their genomic context. See Kheradpour et al., 2007 and Stark et al., 2008 for details.*

Figure 3: *Conserved Twist binding at a functional enhancer in the tinman locus. D. melanogaster and 5 other Drosophila species at increasing phylogenetic distances display highly significant ChIP-Seq tag enrichments indicative of positionally conserved binding.*

Experimental and computational comparative genomics

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*. We find that transcription factor binding is highly conserved in species as distant from *D. melanogaster* as platypus or chicken from human. Conservation of binding is strongly correlated with conservation of the corresponding transcription factor (sequence) motifs. We anticipate that these comparative data will allow a detailed dissection of enhancer structure and grammar.

We have developed computational methods to score motif conservation in 12 *Drosophila* genomes. We discovered novel motif types, identified functional targets of many transcription factors and microRNAs with high confidence, and found that they can help to understand and refine experimental ChIP data. Comparative genomics and related bioinformatics approaches will allow us to integrate our data and knowledge to predict developmental enhancers, regulatory targets for transcription factors, and the expression patterns of genes. They also allow us to integrate microRNA-mediated regulation into regulatory networks and to understand their role in tissue-specific expression programs.

Regulation of gene expression and genome stability by novel classes of small RNAs Novel high-throughput sequencing technology reveals a myriad of novel small RNAs from different functional classes. These are for example involved in regulating gene expression by the microRNA and siRNA pathways and in the control of mobile genetic elements through related silencing pathways involving the PIWI-clade of Argonaute proteins. We are collaborating extensively with experimental labs in the analyses of small RNA and their functional characterization.

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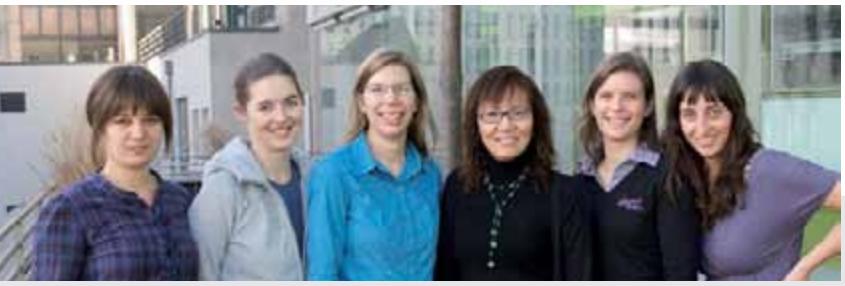

PEGGY STOLT-BERGNER

Transfer of Signals and 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.

Substrate Transport

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.

Currently, we are focusing on the three-dimensional structural 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. In addition, due to the small number of available structures, it is likely that some transport mechanisms remain uncharacterized, as the available structures do show diversity (Fig. 1). Additional structures at higher resolution and structures of different conformational states of the transport cycle will help to address these questions.

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.

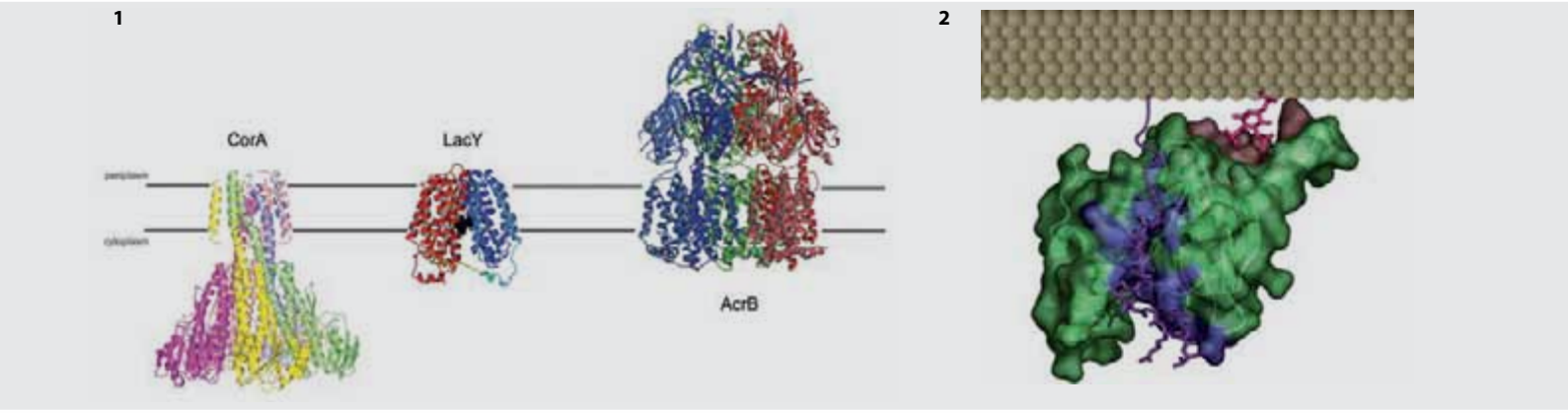

Figure 1: ***Transporter structures reveal diverse types of transport mechanisms***
Recent structural work has uncovered evidence for diverse transport mechanisms. The E. coli lactose permease LacY adopts the alternating access model, which most likely involves large conformational changes (figure adapted from Abramson et al, 2004 Curr Opin Struc Biol 14:413). In contrast, the pentameric structure of the CorA divalent metal transporter from T. maritima suggests that its mechanism of transport is similar to that of a gated channel (figure adapted from Eshaghi et al, 2006 Science 313:354). The trimeric E. coli multidrug efflux pump AcrB utilizes an alternating binding site mechanism, which involves more subtle conformational changes (Seeger et al, 2006 Science 313:1295; figure adapted from Murakami et al, 2002 Nature 419:587).

Figure 2: ***Crystal structure of the Disabled-1 PTB domain bound to peptide and phosphoinositide ligands***
The Dab1 phosphotyrosine binding (PTB) domain interacts with the NPxY motif of ApoER2 through a peptide binding groove on one face of the domain, and with the phosphatidylinositol-4,5-bisphosphate headgroup through a group of positively charged residues on the opposite side of the domain. Interaction with both ligands is necessary for transduction of the Reelin signal. The Dab1 molecular surface is shown in green, while the ligands are shown in ball-and-stick representation (Stolt et al, 2003 Structure 11:569).

Signal Transduction

Signal transduction from the external environment to the interior of the cell is essential in controlling diverse processes such as cell growth and proliferation, metabolism, and cell migration. Many transmembrane receptors transduce signals by clustering upon binding of an extracellular ligand. However, the mechanisms by which receptor clustering leads to downstream signal transduction are in many cases unclear. Reelin signaling is a pathway that regulates the migration of newly formed neurons during brain development, and also plays a less well-characterized role in learning and memory formation in the adult brain. Binding of the extracellular ligand Reelin to the lipoprotein receptors ApoER2 and VLDLR triggers receptor clustering, and this leads to phosphorylation of the Disabled-1 (Dab1) adaptor protein by Src family kinases, which in turn leads to further downstream signaling. My previous work in this field focused on structural and biochemical characterization of the Dab1 adaptor protein and its interaction with the Reelin receptors and phosphoinositides in the cell membrane (Fig. 2). We are now using the Reelin signaling pathway as a model system to understand the molecular mechanisms by which receptor clustering triggers downstream signaling, using both biochemical and structural approaches.

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STEFAN WESTERMANN

Molecular Mechanisms of Kinetochore Function

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First described by cytologists more than a century ago, the kinetochore, the protein complex that connects chromosomes to microtubules during mitotic and meiotic divisions, lies at the heart of a fundamental process for life. Our lab studies kinetochore function using a combination of in vivo and in vitro approaches with the aim of gaining insights into how this molecular machine assembles, interacts with dynamic microtubules, signals attachment errors, and enables the generation of force.

Building a kinetochore in the test tube

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

Building a kinetochore in the cell

To define functional modules within the complex kinetochore architecture we have taken a reductional approach to design simple kinetochores *in vivo*. By artificially recruiting individual kinetochore components to engineered binding sites on circular plasmids and native yeast chromosomes, we were able to demonstrate that the Dam1 complex is not only required, 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 course of the cell cycle. The basic signals that couple cell cycle progression with the regulation of kinetochore function have remained elusive thus far. Combining time-resolved analysis of post-translational modifications with yeast genetics should enable us to identify general principles of regulation.

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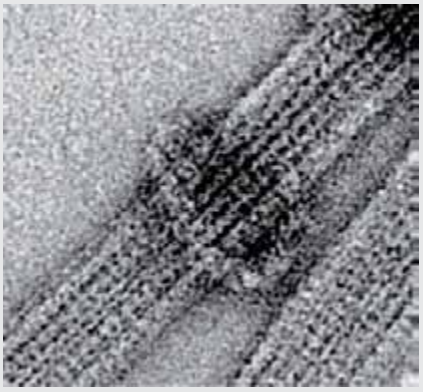

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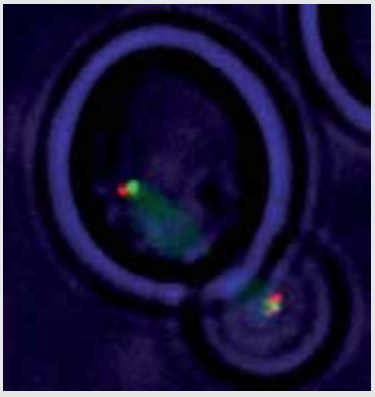

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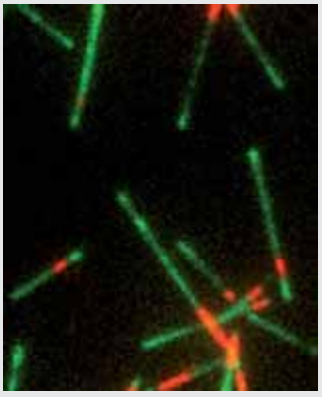

Figure 1: *The 10-protein Dam1 complex oligomerizes into a ring around the microtubule in vitro. Negative stain electron microscopy of a Dam1 ring decorating a taxol-stabilized microtubule.*

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 trace 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 execute this remarkable task? What features enable it to follow a polymerizing microtubule end and also stay connected during disassembly? How does the kinetochore modulate microtubule dynamics? To analyze this process we reconstituted dynamic microtubules *in vitro* and visualized the interaction of individual kinetochore components using total internal reflection fluorescence (TIRF) microscopy. This technique permits the observation of individual kinetochore complexes and microtubule-binding proteins with single-molecule sensitivity in order to reveal their mode of interaction with dynamic plus-ends. Our initial analysis was focused on the yeast EB1 protein Bim1p (Figure 3). We were able to show 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 regulates the interaction of Bim1p with microtubules and critically regulates the amount 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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ANTON WUTZ

Mammalian X-chromosome inactivation

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For successful development, the information stored in the genome needs to be precisely regulated. During differentiation, each individual cell uses an ever-changing repertoire of epigenetic mechanisms to achieve proper control of gene expression. Our research focuses on the regulated formation of heterochromatin during the process of X inactivation.

X inactivation is the process that mammals use to compensate for the dosage difference in X-linked genes between the sexes. This is achieved by transcriptional silencing of one of the two X-chromosomes in female cells (Wutz and Gribnau, 2007). The long non-coding *Xist* RNA is critical for initiation of this silencing process and associates physically along the entire chromosome (Figure 1).

The function of Polycomb group proteins at the initiation of X inactivation

Recruitment of Polycomb group (PcG) complexes by *Xist* can be observed at an early stage in X inactivation. Polycomb repressor complex 1 (PRC1) and PRC2 mediate chromosome-wide modifications of histones. We have shown that recruitment of both PRCs is strictly dependent on *Xist* RNA at all stages of differentiation. Nonetheless, the function of PcG complexes remains elusive. *Xist* can initiate chromosome-wide silencing in ES cells lacking PRC2 activity by a mutation in *Eed*. Notably, in *Eed*-deficient ES cells PRC1 can be recruited by *Xist* and mediates ubiquitination of histone H2A. In order to investigate the function of PRC1 in X inactivation we have disrupted *Ring1B*. In *Ring1B*-deficient ES cells several PRC1 proteins are lost. We observe derepression of developmental control genes and aberrant differentiation of ES cells lacking PRC1 function (Figure 2). Notably, *Xist* can still recruit PRC2, which mediates chromosome-wide tri-methylation of histone H3 lysine 27 (Figure 3). Despite the disruption of the Polycomb system we find that chromosome-wide silencing by *Xist* is unaffected. Thus, neither PRC1 nor PRC2 are essential for X inactivation in embryonic cells. Since PRC1 and PRC2 both have essential roles in embryogenesis and can be recruited by *Xist* independently of each other, we propose that they might act redundantly during X inactivation. To address this question we have established ES cell lines which are deficient in both PRC1 and PRC2. Current work aims to characterise these cells to define Polycomb function in X inactivation and stem cell self-renewal. Recent work indicates that PRCs can act together or individually on distinct classes of genes (Shibata et al., 2008). It will be important to identify the mode of Polycomb regulation genome wide by analysing gene expression patterns.

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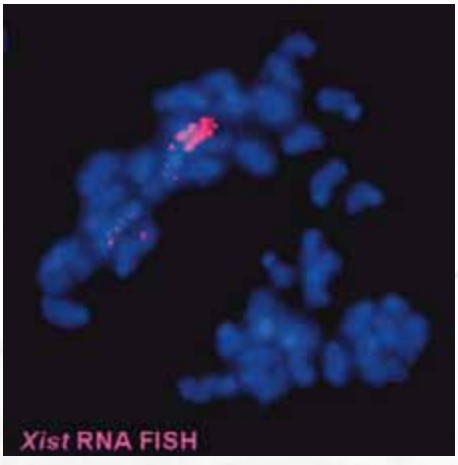


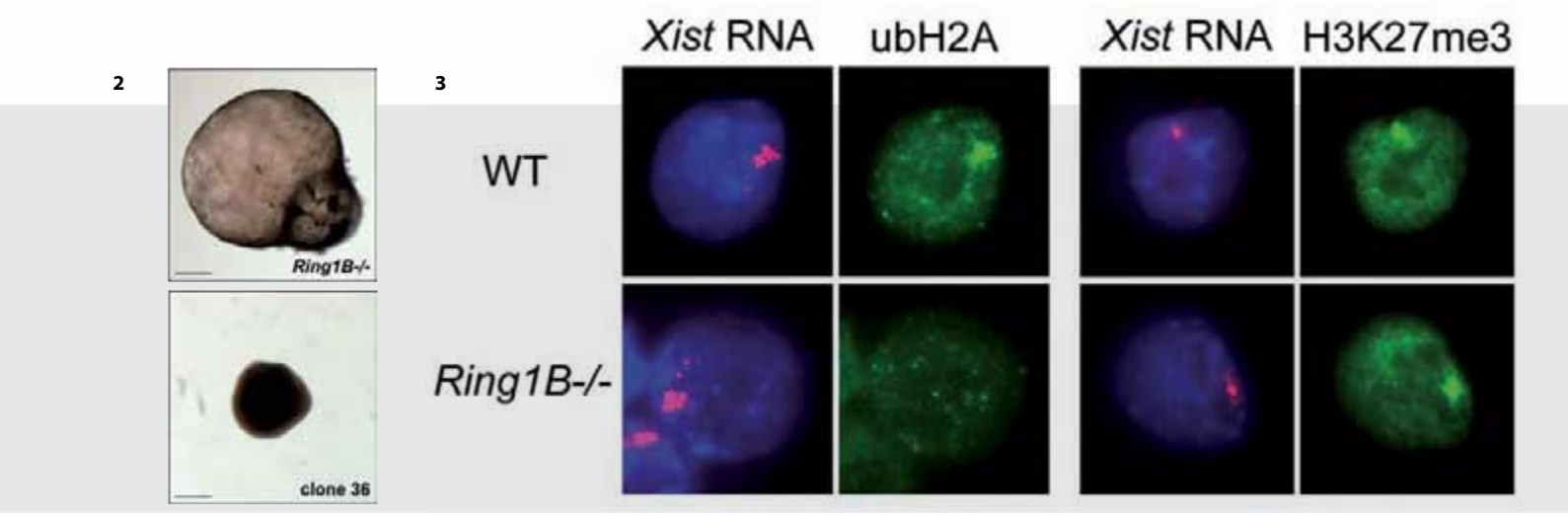


Figure 1: *Xist RNA association with chromatin revealed by RNA FISH. Mouse chromosome spreads were prepared from ES cells expressing Xist from a transgene on chromosome 11 and hybridised with a fluorescent probe detecting Xist RNA (red). DNA was counter-stained with DAPI (blue).*

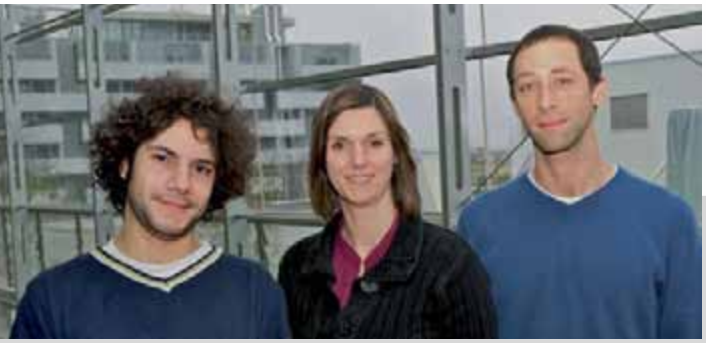
Figure 2: *Aberrant differentiation of ES cells lacking Ring1B. Embryoid bodies derived from Ring1B-deficient ES cells show striking hollow sphere morphology compared to the compact spherical structures formed by control ES cells (WT).*

Figure 3: *Recruitment of Polycomb group proteins and histone modifications by Xist. Xist expression recruits PRC1 and PRC2, which mediate chromosome-wide ubiquitination of histone H2A (ubH2A) and tri-methylation of histone H3 lysine 27 (H3K27me3). In Ring1B-deficient ES cells ubH2A is not triggered by Xist expression, whilst H3K27me3 can still be observed.*

Developmental control of the gene silencing function of *Xist*

Xist expression is the trigger for the inactivation of one of the two female X chromosomes early in embryogenesis. As differentiation progresses, *Xist* becomes dispensable for the maintenance of the inactive X. Conversely, its function for initiation of silencing is lost in differentiated cells. Thus, initiation of silencing is restricted to a specific time interval at the onset of cellular differentiation. How *Xist* mediates gene repression remains an open question. Using an inducible *Xist* allele in mice we defined the potential for *Xist* to initiate silencing during embryogenesis. In adult male mice, ectopic expression of *Xist* causes an anaemia that can eventually lead to lethality. *Xist* has the ability to initiate silencing in immature blood precursor cells. In contrast, stem cells and mature blood cells are unable to initiate ectopic X inactivation. This indicates that critical pathways for silencing are transiently activated in hematopoietic differentiation. We have identified a mouse tumour model, which contains cells that have the appropriate context for gene silencing by *Xist*. Current approaches are directed towards understanding the molecular basis for this epigenetic difference and the identification of pathways that are crucial for *Xist*-mediated silencing.

Functional studies of X inactivation will provide insight into the epigenetic regulation of gene expression in mammals. It appears that *Xist*-mediated silencing is a paradigm for a powerful epigenetic system that is capable of hetero-chromatinising an entire chromosome and determining its specific nuclear localisation. It is expected that similar interactions underlie the regulation of other genes – however, with less dramatic consequences. Regulation of chromatin by non-coding RNAs is the focus of contemporary epigenetic research, and might pinpoint a general process by which transcription feeds back onto the chromatin structure.



KATRIN HEINZE

Fluorescence tools operating on a single-molecule scale

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Discoveries in biosciences are frequently stimulated by the invention of new scientific tools. We like to push fluorescence techniques beyond the classic spatial and temporal resolution limits. Such low-invasive approaches offer the fascinating prospect of observing biomolecules in their native environment and understanding how they act in concert.

Our group focuses on the measurement and manipulation of inter- and intramolecular dynamics in a cellular setting. Tools for this purpose may be based on devices or (fluorescent) probes. A *device-based* approach we would like to highlight this year is a promising superlens-microscope design. An ongoing *probe-based* approach at our laboratory addresses the disputed question as to how protein binding may be affected by laser illumination itself and how these effects could be controlled in fluorescence imaging or even used as a tool.

A superlens assisted fluorescence microscope for surface imaging of biomolecules

The concept of a "perfect" lens that is capable of resolving features smaller than the diffraction limit by amplifying the near-field was suggested nearly a decade ago (Pendry, 2000) and has intrigued the scientific community ever since. Obviously, such lenses would have far-reaching applications, particularly in fields such as nano-lithography, optical storage, and bio-imaging.

However, the production of materials or metamaterials with suitable properties and of suitable design has been hindered by practical problems such as material loss and surface roughness. Recent advances in the field have altered the situation: the production and application of a superlens-based microscope has become feasible in terms of technology. One design that may reduce some of these undesirable effects is the stacked metal-dielectric superlens. We are exploring the imaging ability of such a design for the specific purpose of imaging Green Fluorescent Protein (GFP) and other FP's in the vicinity of the superlens surface. We recently found that a metallic/dielectric/metallic superlens may be suitable for imaging such fluorescent molecules with a deep sub-diffraction limit resolution (see Fig. 1). We are now working on fine tuning the parameters of the metallic and dielectric layers for imaging specific fluorophores. The proposed lens could be incorporated in a microscope setup and permit ultra-fast super-resolution surface imaging.

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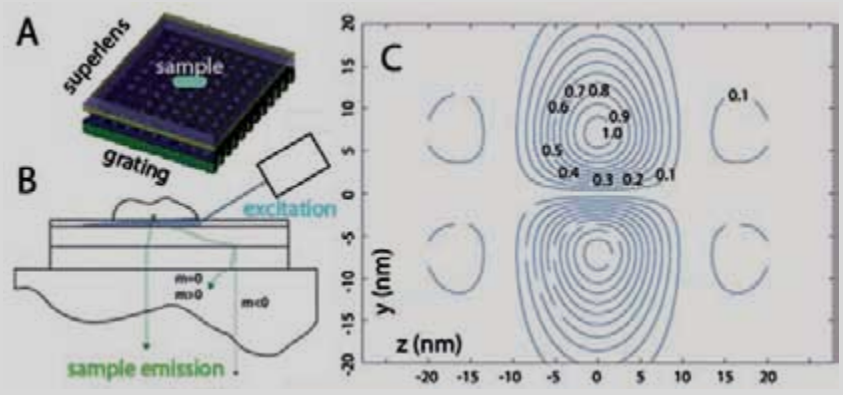

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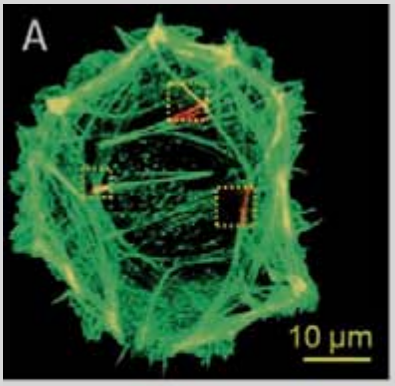

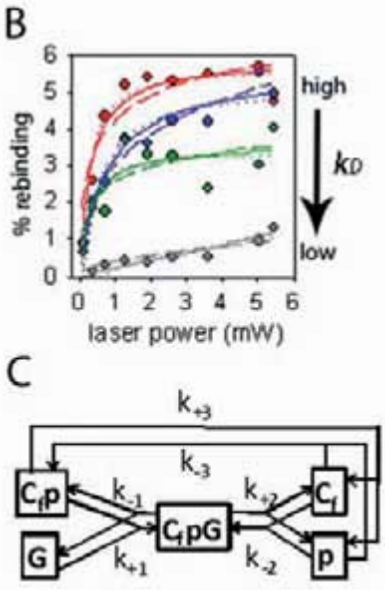

Figure 1: ***Towards a far-field superlens microscope.*** *A: The superlens design is based on a coverglass coated with two composite silver-based metallic layers of 15 nm thickness sandwiching a high-κ dielectric filler layer. We have shown that $Ag_{1-x}Si_x$ (x~0.15) metallic-layers and SiC dielectric-layers are suitable for the GFP-like dipole emission spectrum. A sub-wavelength pitch grating behind the superlens can be used to transfer the image to the far-field. B: The sample, e.g. cells, can be deposited or grown onto the superlens-coverglass for microscopy. C: Electric field profile behind the superlens from a fluorescent molecule placed 15 nm above the lens.*

Figure 2: ***A: Photo-unbinding and rebinding of labeled phalloidin (Alexa488 - Alexa647) from actin filaments in a human fibrosarcoma cell.*** *Unbinding/rebinding patches (red) after laser illumination at 800 nm (two-photon excitation) are framed in yellow; left: 14 mW (10.7 mJ/µm²); top: 20 mW (15.4 mJ/µm²); right: 24 mW (18.4 mJ/µm²). B: Calmodulin-Alexa 647 rebinding at various laser powers for peptides CKII(290-312) (grey symbols), CKII(292-312) (green symbols), CKII(293-312) (blue symbols), and CKII(294-312) (red symbols); the data fits shown in solid lines are based on the "two-step" model diagram in panel C: Photo-unbinding primarily occurs through a pathway (k_1 and k_2) that is distinct from that of conventional dissociation (k_3). In this case the labeled CaM-peptide pair (CfP) forms an intermediate complex (CfPG) following illumination by the laser light. The intensity of the illumination is related to the effective abundance of G.*

Towards the mechanism of photo-unbinding

Fluorescent probes are commonly used in biological experiments. Despite their great success story over the last century, it is known that fluorescent conjugates can also influence the properties of the molecules under study. Our recent studies have shown that fluorescently labeled antibodies can be dissociated from their antigen by illumination with laser light; the same has been observed for protein-peptide binding, including toxins (see Fig. 2). This year, we succeeded in gaining insight into the mechanism of photounbinding by studying labeled calmodulin (CaM) and a set of CaM-binding peptides with different affinities to CaM. Our findings suggest that photounbinding is linked to photobleaching and a 'radiative' process requiring a fluorescent label. Interestingly, the photounbinding effect becomes stronger with increasing binding affinity, but does not induce breakage of covalent bonds. Our model is based on the assumption that an intermediate (transitional) complex is formed before the unbinding occurs. This is consistent with the labeled protein undergoing a conformational change which in turn is responsible for the unbinding.

We believe that knowledge of the involved molecular processes would not only lead to the systematic improvement of quantitative fluorescent studies, but also pave the way for inducing or inhibiting molecular interactions by light.

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VIENNA DROSOPHILA RNAi CENTER (VDRC)

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RNAi library maintenance and development

Reinhard Klug / Stock Maintenance Supervisor
Thomas Micheler / Software Developer
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Martin Kinberg / Technical Assistant
Andreas Gansch / Technical Assistant
Judith Utner[1] / Technical Assistant
Alisher Tashpulatov / Technical Assistant
Kaiser René / Technical Assistant
Irina Kolarov / Technical Assistant
Michaela Eckmann / Technical Assistant
Kristina Belogradova / Technical Assistant
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Sonja Lang[1] / Technical Assistant
Florence Maxwell / Technical Assistant
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Michaela Fellner / Technical Assistant
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Sandor Urmosi – Incze / Technical Assistant
Elena Popowich / Technical Assistant
Stefanie Wandl / Technical Assistant
Zsuzsanna Portik Dobos / Technical Assistant

Keleman research group members

Sebastian Krüttner / PhD Student
Barbara Stepien / PhD Student
Katharina Jandrasits / Technical Assistant

[1] 50%

Genome-wide RNAi and memory formation

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 our own research group, we use transgenic RNAi and other methods to understand how the fly forms memories that shape its mating behaviour.

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. The VDRC, maintains, further develops and distributes this library.

Recently, we have embarked on the construction of a new RNAi library (the KK library) that exploits site-specific transgene integration and to overcome some of the problems associated with random insertion of the P element transgenes of the original collection (GD library). In the new KK library, all RNAi transgenes are targeted to the VIE-260b site, selected on the basis of its low basal expression and high levels of GAL4-induced expression. An additional attractive feature of our new collection is that it targets a different gene fragments than our first library. We have completed 10,000 RNAi lines, which were made available to researchers world-wide in March this year.

Since the VDRC opened two and a half years ago, we have already delivered over 200,000 lines to more than 1,500 registered users world-wide. This has been made possible in part through core funding provided by the city of Vienna and the federal government, with the rest of the costs covered by user fees. The VDRC also provides such support for researchers in house, having delivered over 80,000 lines to IMBA and IMP groups.

Learning and memory

The evolutionary mission of a male fly is to father as many offspring as possible. With an almost unlimited supply of sperm, his success depends largely on his ability to discriminate receptive virgins females from unreceptive females. If he is too promiscuous, he may waste a lot of time and energy in futile courtship; if he is too choosey, he will miss out on excellent mating opportunities. The right balance appears not to be hard-wired into the fly's brain, but is something he learns by trial-and-error during his first few courtship experiences (Figure 2). His memory of these first sexual encounters can shape the male's mating strategy for several days – a long time in the life of a fly.

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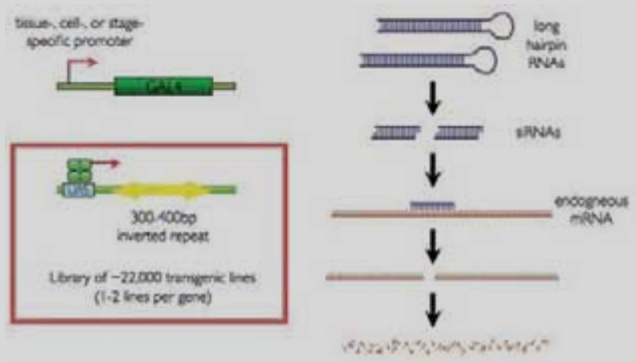

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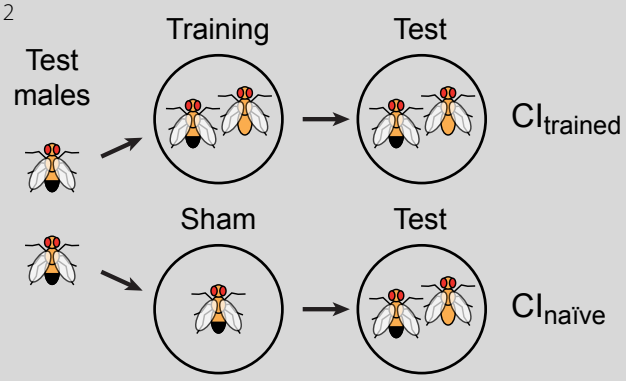

Figure 1: *RNAi in Drosophila.*

Figure 2: *Courtship conditioning. When tested with unreceptive mated females, males previously exposed to mated females court less than naïve (sham-trained) males (CI = courtship index; CItrained < CInaïve)*

Figure 3: *Mushroom body neurons in the fly's brain – Orb2 is required in these neurons for courtship conditioning.*

We are trying to understand the molecular and cellular mechanisms that underlie this robust and powerful form of long-term memory. We have recently demonstrated that the CPEB protein Orb2 – a regulator of mRNA translation – plays a critical role in this process [2]. We found that Orb2 function is required in a specific set of mushroom body neurons during or shortly after training (Figure 3). Without Orb2, or more specifically without its intriguing glutamine-rich domain, a memory initially forms but decays within just a few hours.

To learn more how Orb2 functions in *Drosophila* long-term memory, we are dissecting its structural and functional requirements using both genetic and biochemical approaches. We are also planning to identify Orb2-interacting proteins and target mRNAs, and to assess their roles in long-term memory. Meanwhile, we are also using the transgenic RNAi library in unbiased approaches to find other factors involved in long-term memory function and dysfunction.

We are also trying to find out exactly what the *Drosophila* male learns during courtship conditioning. What are the specific cues – probably pheromones – that the male learns to discriminate? And, as the ultimate goal, we would like to define the specific molecular and cellular changes in the brain that underlie this form of learning.

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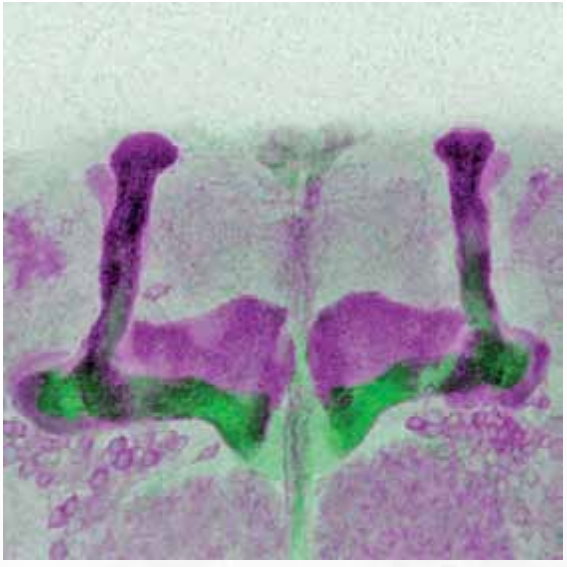

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BIOOPTICS FACILITY

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Karin Aumayr / Head of BioOptics

Pawel Pasierbek / Microscopy
Gabriele Stengl / Flow Cytometry/Microscopy
Gerald Schmauss / Flow Cytometry/Image Analysis

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

Flow Cytometry

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

Microscopy

The BioOptics Facility currently manages more than twenty microcopy systems, covering widefield microscopy, confocal laser scanning microscopy (CLSM), two-photon (2P) microscopy, total internal reflection (TIRF) microscopy techniques, and an automated slide scanner for samples with or without fluorescence. Most of the systems are motorized, thus providing automation for higher throughput, and are suitable for both fixed samples and live cell experiments. The facility provides assisted use and training on all instrumentation and consultation concerning all microscopy-related subjects, including project planning, staining, microscope selection, etc.

Image Processing and Analysis

Five state-of-the-art computer workstations are available at the BioOptics Facility, operating most of the common commercial image processing and visualization software. For the deconvolution of microscopy images, a server solution with a web-based interface has been set up in order to provide 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. Several image analysis algorithms are available, such as object tracking and tracing, determination of measurement parameters like intensity, distance, area, volume and co-localization. For advanced image analysis and automated object recognition, customized classification and measuring algorithms are developed within the facility.

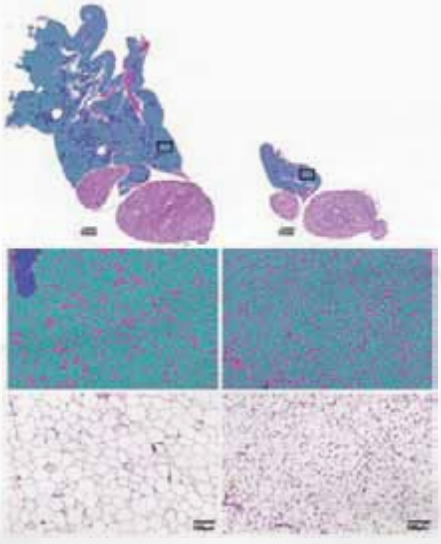

Figure: *Morphometric analysis of adipose tissue, performed on complete HE stained sections scanned with the MIRAX scanner. Panel left: WT section. Panel right: KO section. Adipocytes in blue. Automatic image analysis with the application of the internally developed Definiens algorithm permits analysis of cell size and number throughout entire sections (more than 35,000 individual cells from control animals and more than 10,000 cells for knockout animals).*

ELECTRON MICROSCOPY

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The Electron Microscopy Facility provides a wide variety 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 can be provided via an external collaboration.

Guenter Resch / Head of Electron Microscopy Facility

Marlene Brandstetter / Technical Assistant
Nicole Fellner / Technical Assistant

Specimen Preparation

Know-how, training and instrumentation for a wide variety of preparation techniques for visualization of ultrastructure in tissues or cells, and biomolecules by transmission electron microscopy (TEM), are being offered by the Electron Microscopy Facility. Techniques routinely used at the facility include the production of support films, negative staining, rotary shadowing of sprayed molecules, chemical and physical fixation, resin embedding in epoxy- and acrylic resins, and ultrathin sectioning of resin-embedded or frozen samples. In 2009, high-pressure freezing was established to produce optimally preserved specimens in conjunction with freeze substitution.

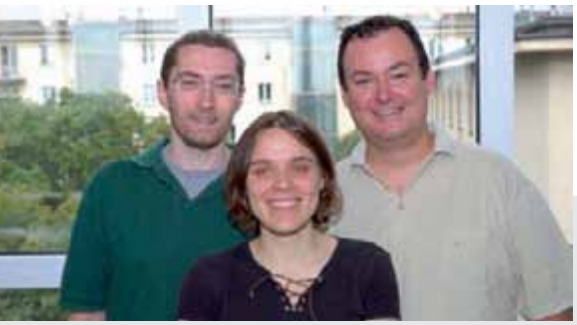
Microscopy

The FEI Morgagni is a robust and easy-to-use 100 kV TEM equipped with a 11 megapixel CCD camera. It is tailored for routine applications in the multiuser environment of the facility. 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 status monitoring of the microscope were developed on the Polara. Workstations and training are provided for image processing of EM data, especially those obtained from electron tomography.

A: z-Sections from a three-dimensional reconstruction of the endothelial lining in a blood vessel by electron tomography.
B: Negatively stained rotavirus-like particles (micrograph courtesy of Cornelia Gänger, Ringrose Group, IMBA)
C: Glycerol sprayed and rotary shadowed α-actinin molecules
D. Dam1-decorated microtubules visualised by cryo-electron microscopy (sample: Fabienne Lampert, Westermann Group)
E: Myelin sheath of a neuron in cross-section (sample: Toshikatsu Hanada, Penninger Group, IMBA).
F: Wild type Drosophila visualised by scanning electron microscopy.



BIOINFORMATICS

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

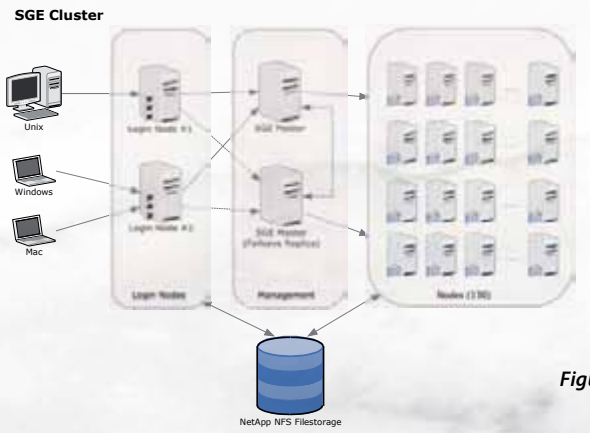
The Bioinformatics unit assists research groups in molecular-biology-related fields by providing sequence analysis services, scientific data mining, hardware and software infrastructure, and training in bioinformatics.

Sequence analysis

The main expertise of the IMP-IMBA Bioinformatics unit lies in the field of 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 being provided for widely used scientific applications related to protein motif analysis, similarity searching (BLAST, PSI-BLAST, FASTA), whole-genome viewing (GBrowse), transcription factor analysis (Transfac), 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 the 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.
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 Grid Engine (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, NAMD), 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. Attendees of the course learn the basic principles and limitations of sequence analysis and data integration.

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

GENOMICS

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Martin Radolf / Engineer
Harald Scheuch / Engineer
Andreas Sommer / Engineer
Markus Sonntagbauer / Technical Assistant *(part-time)*

The Genomics Department comprises the microarray services, the cDNA clone repository, liquid handling robotics and next-generation sequencing. Major projects and accomplishments in 2009:

Microarray:

After re-arraying the RIKEN (FANTOM I to III) library in 2008, we hybridized more than 200 microarrays. Over 22,500 genes per array were analyzed and processed with an internal programmed fully automated analysis software tool. We offer Spotfire from the summer of 2009 onward. Spotfire is a powerful tool to visualize and analyze microarray data. We now use the IBD extension for Spotfire, which is a tool for visualization and analysis of microarray data. An additional technology we have established in cooperation with MFPL is the hybridization of Agilent arrays. We set up the entire equipment for hybridization at the Genomics Department; scanning is performed at MFPL (special thanks to Walter Glaser). Although more expensive, Agilent arrays are of better quality and equipped with more features than our cDNA arrays. With Agilent we can also offer microarrays to analyze DNA Methylation, microRNAs, and custom-made microarrays for various model organisms.

Robotics:

A further noteworthy aspect of our work at the Genomics Department is the use of robotics in the lab. We are currently working with a Tecan TeMO provided with 96 channels, and a Biotek Precison XS for 8-channel and single pipetting. In the near future we will also have the XIRIL 100 which will assist us in one of our main projects, namely automated genomic DNA isolation for genotyping and automated plasmid mini-preparation.

Next-generation Sequencing:

The capacity of next-generation sequencing has been increased by the addition of two genome analyzers to the facility at the beginning of 2009. Each is equipped with a paired-end module which permits the sequencing of both ends of DNA fragments. The superior software and hardware of the instruments led to a significant (>30%) increase in sequence yield. Due to improved sequencing reagents, we are now able to standardly sequence 72 base-pair read lengths.
Next-generation sequencing offers a wide range of applications and new ones are being consistently added. So far we have successfully adopted protocols for ChIP-Seq, mRNA-Seq, ncRNA-Seq and whole genome/SNP sequencing.

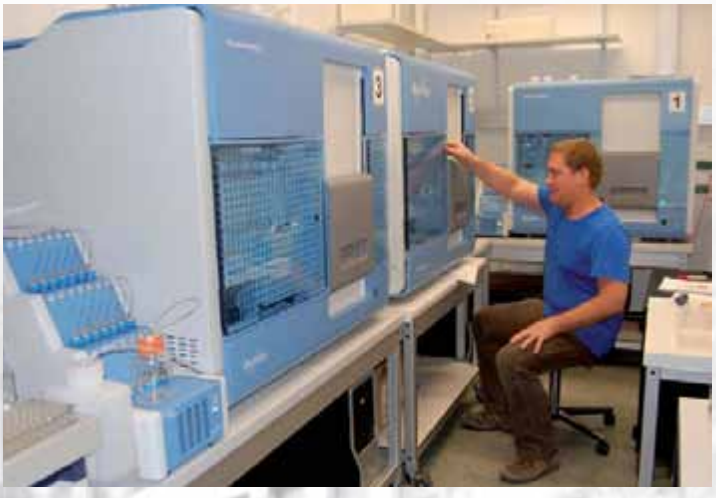

Figure: *Illumina Genome Analyzer II*

PROTEIN CHEMISTRY FACILITY

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Köcher Thomas / Postdoc
Großteßner-Hain Karin / PhD Student
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Hudecz Otto / Technical Assistant
Krssakova Gabriela / Technical Assistant
Madalinski Mathias / Technical Assistant
Mazanek Michael / Technical Assistant
Opravil Susanne[1] / Technical Assistant
Roitinger Elisabeth / Technical Assistant
Schutzbier Michael / Technical Assistant
Steinmacher Ines / Technical Assistant

[1] joint appointment with Stefan Westermann

Absolute Quantification of Proteins and Determination of Protein Complex Stoichiometry

We developed a novel method that permits accurate and cost-effective absolute quantification and determination of the stoichiometry of protein complexes. Using our method, which we call Equimolarity through Equalizer Peptide (EtEP), we verified the 1:1 stoichiometry of the MP1-p14 scaffolding complex with great accuracy and precision. Importantly, EtEP is compatible with state-of-the-art analytical techniques and protein complex isolation methods such as affinity purifications. (Lit. Holzmann et al.)

iTRAQ-based Relative Quantitation of Proteins

While the demand for mass spectrometry data in biological research has been steadily increasing in recent years, quantitative analysis of proteomic samples is also used extensively. We developed an iTRAQ (isobaric tags for relative and absolute quantification)-based method for the LTQ-Orbitrap, the instrumentation of choice in most proteomics laboratories, which dramatically increases the number of identified and quantified peptides. Simultaneously, the analytical precision of quantitation was improved significantly.
We reported the evidence obtained from our principal experiments, and applied the method to protein expression profiling experiments in an in vivo cardiovascular disease mouse model. (Lit. Koecher et al.)

Detection of Arginine-Phosphorylation

In cooperation with Tim Clausen's group (IMP), we identified a rarely described protein modification using mass spectrometry, namely the N-phosphorylation of arginine. The modified protein, the bacterial transcription factor CtsR, is impaired in its DNA-binding abilities after phosphorylation. This might be an indication of the importance of arginine phosphorylation for processes that involve protein-DNA binding. We developed a novel enrichment procedure for this chemically labile modification, followed by a specialized mass spectrometry analysis to potentially identify other arginine-phosphorylated proteins. (Lit. Fuhrmann et al.)

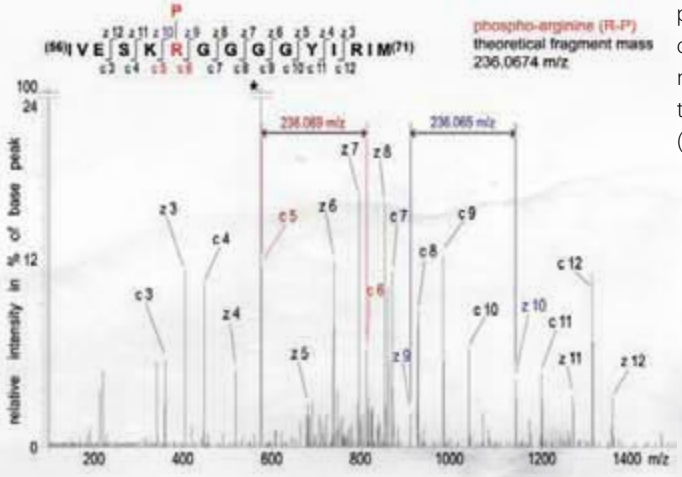

Figure: *ECD-MS/MS spectrum of the arginine-phosphorylated peptide IVESK(pR)GGGGYIRIM. The mass spectrum exhibits fragment ions for a phosphorylated arginine (ΔM = 236.067 Da) in both investigated ion series (c- and z- ions).*

SERVICE DEPARTMENT

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The Service Department offers a variety of high-quality rapid services to IMP, IMBA and GMI scientists. Our work mainly consists of DNA sequencing, fly food production, and the preparation of various media and solutions.

Our Media Kitchen and Fly Food staff prepare substantial quantities of reagent quality solutions and media for cell culture, flies (more than 1,700,000 tubes and bottles per year), and other organisms. The Fly Food facilities are fully accommodated at the new premises within the IMP building. These rooms provide daylight and much more space to create better and more convenient working conditions for preparing fly food and storing all the goods we need. We also prepare selected reagents such as DNA molecular weight markers, enzymes, a variety of transformation-competent E.coli strains, and maintain a stock of cloning vectors, sequencing primers and other reagents.

Gotthold Schaffner / Head of Facility

Ivan Botto / Technical Assistant
Markus Hohl / Technical Assistant
Shahryar Taghybeeglu / Technical Assistant
Gabriele Botto / Media Kitchen
Christa Detz-Jaderny / Media Kitchen
Ulrike Windholz / Media Kitchen
Sabine Jungwirth / Fly Food Preparation
Franziska Stransky / Fly Food Preparation
Oliver Botto / Help Fly Food Preparation
Thomas Haydn / Help Fly Food Preparation
Anna Windholz / Help Fly Food Preparation

Production of antibodies

The production and isolation of several monoclonal antibodies in hybridomas in collaboration with IMP group members, and organizing antibody production in rabbits with an external company, occupy a part of our work capacity in terms of time.

Sequencing and DNA isolation

The ABI 3730 DNA Genetic Analyzer with 48 capillaries is the only work horse. The 16 capillaries ABI 3100 Genetic Analyzer is solely used as a back-up sequencer for emergencies.
We sequenced approximately 73,000 samples during the first 9 months of this year. The demand has again increased substantially due to screening projects, the new fly library and new groups at IMBA and IMP plus the GMI, who joined our services this summer.
We mainly use the 3730 DNA Analyzer because of its sensitivity and of lower running costs in terms of consumables. The standard read-length is 850-900 bases for good DNA samples .

DNA sample quality and concentration, even when prepared with sophisticated Qiagen Kits such as Midi-, Maxi- or Minipreps is a problem. The same is true for inappropriate primer sets or insufficiently documented plasmid constructs from external sources. Sequencing is done faster and more easily than analyzing the samples by restriction digests or running them on an agarose gel. The clean-up protocol with Sephadex G50 superfine columns on 96-well microtiter plate format with optimized sephadex consistency and centrifugation conditions is now transferred to a BioTek benchtop minirobot. The results tell us that there is no difference between the plates prepared by the robot and those prepared manually, and no "dye blobs" arise when DNA samples of good quality are used.

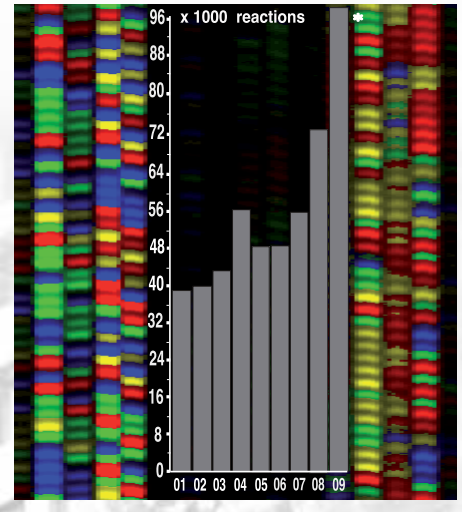

Figure: *A sequencing run on an ABI 377 PRISM and numbers of reactions analyzed on ABI 3100 (from 2001 onward) and ABI 3730 (from June 2004 onward) with dye deoxy terminators (v3.0 from 2001 onward) from 2001 to 2009 (scale 0 to 96,000).*
**calculated from data collected between January 2009 and September 2009*

HISTOLOGY DEPARTMENT

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Vukoslav Komnenovic / Head of Facility

Mihaela Zeba / Technical Assistant

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).

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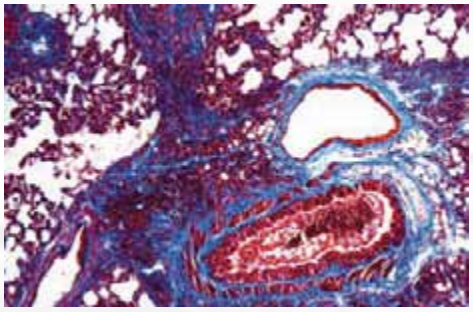

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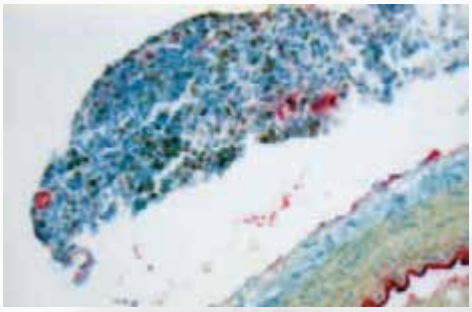

Figure 1: *Trichrom blue staining. The picture shows 4 different signals, light blue for Lung fibrosis, red for Mast cells and Neutrophils, pink for Myeloiud cells and dark blue for the Nuclei.*

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

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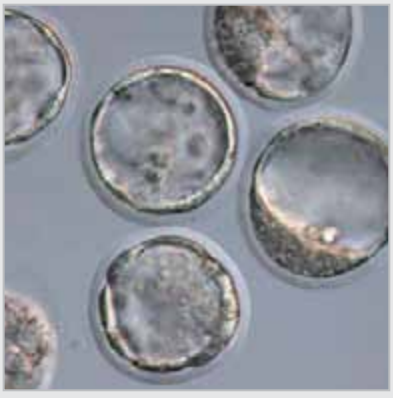

ANIMAL HOUSE

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MOUSE SERVICE

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Animal House

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.

Animal House 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.

The Mouse Service Department *was set up at the beginning of 1998 to cope with the increasing demand for mouse studies and generation of transgenics. The Mouse Service Department services are shared by the IMP and IMBA.*

The main duties of this service unit are the injection of ES cells into blastocysts (also tetraploid and 8-cell) and of DNA into the pronucleus of fertilized mouse eggs. This service also provides for the transfer of 'clean' embryos into our Animal House, the freezing of embryos for the preservation of specified mouse strains and the teaching of basic embryological techniques to the IMP and IMBA staff.
Many different ES cell clones and DNA/BAC constructs are being injected per year. The activities of this department are overseen by an Animal User Committee, which meets bimonthly to set priorities and to coordinate the duties. At present, it is chaired by Meinrad Busslinger.

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

Figure 2: *Mouse blastocysts.*

Publications

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CLAUSEN

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PROTEIN CHEMISTRY FACILITY

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HISTOLOGY

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MOUSE SERVICE

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IMP Awards & Honors 2009

Meinrad Busslinger

Elected to the Austrian Academy of Sciences as Full Member (May)

Johann Holzmann

Research Award of the Austrian Association of Molecular Life Sciences and Biotechnology 2009 (September)

Research Award of the Sanofi-Aventis Foundation 2009 (December)

Eva Kiermaier

Kirsten Peter Rabitsch Award (October)

Fabienne Lampert

FASEB Mitosis Spindle Assembly Young Investigator Award (September)

Martin Leeb

VBC PhD Award (November)

Alex Stark

ERC Starting grant by the European Research Council (October)

Stefan Westermann

Elected to the Austrian Academy of Sciences as "Mitglied der jungen Kurie" (April)

Jai Yu

VBC PhD Award (November)

Seminar Speakers

JANUARY

07.01.09 Svante Pääbo
Max Planck Institute for Evolutionary Anthropology
A Neandertal Perspective on Human Origins

13.01.09 W. Judson Hervey, IV
Oak Ridge National Laboratory, USA
Evaluation of Affinity-Tagged Protein Expression Strategies using Local and Global Isotope Ratio Measurements

19.01.09 David Glover
Dept. of Genetics, University of Cambridge
Control of Polo and Plk4 in centrosome maturation and centriole duplication

22.01.09 Laura Attardi
Stanford University School of Medicine
Using Mouse Models to Understand Mechanisms of p53 Action in vivo

23.01.09 Mark Huebener
MPI of Neurobiology, Munich
Structural correlates of functional plasticity in mouse visual cortex

29.01.09 Andreas Strasser
The Walter and Eliza Hall Institute of Medical Research
The many roles of apoptosis in tumour development and cancer therapy

30.01.09 Marcos Malumbres
CNIO Madrid
Functional analysis of mitotic kinases and the APC/C proteolytic machinery in mammals: from the cell cycle to human disease

FEBRUARY

12.02.09 Daniel Finley
Harvard Medical School
Regulation of Proteasome Activity

13.02.09 Anthony Holtmaat
University of Geneva
Experience-dependent structural plasticity in the mouse barrel cortex

17.02.09 Charles J. Weitz
Harvard University
Circadian clocks in mammals

19.02.09 Ivan Dikic
Goethe University Medical School
Ubiquitin signaling networks - from structures to functions

23.02.09 Robin Allshire
University of Edinburgh
Synthetic heterochromatin, CENP-A chromatin and kinetochore assembly

25.02.09 Gad Asher
University of Geneva
NAD+ as a metabolic regulator of circadian rhythms

26.02.09 Elaine Fuchs
Howard Hughes Medical Institute, New York
Stem Cells: The Privileged Few

MARCH

02.03.09 Magdalena Götz
GSF-Institut für Stammzellforschung
Neurogenesis from glial cells - new insights into neural stem cells and repair

05.03.09 Thomas Langer
Universität zu Köln
AAA proteases, mitochondrial membrane dynamics, and neurodegeneration

11.03.09 Rafael Carazo Salas
ETH Zurich
Systemic regulation of microtubule organization and its role in the establishment of cellular coordinates

12.03.09 Tim Lebestky
California Institute of Technology
Dopaminergic modulation of arousal in Drosophila

16.03.09 Jeroen Dobbelaere
Wellcome Trust/Cancer Research UK
Towards a molecular understanding of the Drosophila centrosome

25.03.09 Jonas Frisen
Karolinska Institute, Stockholm
Stem cells and neurogenesis in the adult central nervous system

APRIL

02.04.09 Matthias Peter
Institute of Biochemistry, ETH
Regulation and function of cullin-based E3-ligases

08.04.09 Manuel Mayer
King's College, University of London
New insights from an –omic approach to cardiovascular disease

15.04.09 Michael Orger
Harvard University
Neural circuits underlying innate visually guided behaviors in zebrafish

16.04.09 Roland Lill
Philipps-University, Marburg
Biogenesis of cellular iron-sulfur proteins: The essential and minimal function of mitochondria.

22.04.09 François Spitz
EMBL
Deciphering the regulatory architecture of mammalian genomes with chromosomal engineering

MAY

06.05.09 Venki Ramakrishnan
MRC Laboratory of Molecular Biology, Cambridge
Induced fit: A common strategy used by the ribosome in decoding, peptide bond formation and termination

07.05.09 Nenad Ban
ETH Zuerich
Springs, tethers, hinges, latches and switchblades: mechanistic insights into the functioning of large macromolecular assemblies

15.05.09 Martha Bulyk
Harvard Medical School
Genomic Analysis of Transcription Factors and cis Regulatory Elements: Regulatory Codes in DNA

25.05.09 Gwyneth Card
California Institute of Technology
The escape responses of Drosophila as a model for the neural basis of decision-making

28.05.09 Hans Wolf-Watz
University of Umea
Virulence factors as drug targets; What can be expected?

JUNE

02.06.09 Sebastian Herzog
Max-Planck Institute of Immunobiology, Freiburg
Molecular switches - How adaptor proteins regulate proliferation and differentiation in developing cells

03.06.09 Tim Hunt
Cancer Research UK
Getting in and out of mitosis

04.06.09 Maria Carmo-Fonseca
University of Lisbon
In vivo tools to visualize co-transcriptional RNA processing

16.06.09 Pavel Tomancak
Max Planck Institute of Molecular Cell Biology and Genetics
Molecular anatomy of the fly embryo

18.06.09 Jan Karlseder
SALK Institute for Biological Studies
Telomere dynamics in mammals and nematodes: C. elegans as a cancer model?

19.06.09 Megan Carey
Harvard Medical School
Instructive signals for learning and associative synaptic plasticity

22.06.09 Hugo Sanabria
University of Texas Medical School at Houston
Fluorescent spectroscopy to study Ca2+ dependent signaling

25.06.09 Roger Y. Tsien
HHMI, Dept. of Pharmacology, UCSD
Breeding and building molecules to spy on cells and tumors

JULY

01.07.09 Marc Kirschner
Harvard Medical School
Cell protrusion and actin assembly

02.07.09 Stephen Baylin
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
Epigenetic Gene Silencing - Embryonic Stem Cells, Induced Pluripotent Stem Cells and Cancer

03.07.09 Andrew Alpert
PolyLC Inc. Columbia
Selective Isolation of Phosphopeptides and Sialylated Glycopeptides by ERLIC

16.07.09 Frauke Melchior
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23.07.09 Alex Schier
Harvard University
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AUGUST

05.08.09 Eva Nogales
HHMI, University of California
Structural Bases of microtubule dynamics and its coupling to chromosome movement

06.08.09 Vadim Bolshakov
McLean Hospital, Harvard University
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13.08.09 Nick Barker
Hubrecht Institute, Utrecht
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24.08.09 Alan Colman
Institute of Medical Biology, Singapore
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27.08.09 Sir David Lane
University of Dundee
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27.08.09 Müzeyyen Sevim
German Cancer Research Center, Heidlberg, Germany
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Duke University Medical Center
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04.09.09 Jonathan Fritz
University of Maryland
The quest for the neural basis of auditory attention

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University of California, Howard Hughes Medical Institute
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Whitehead Institute, Cambridge
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17.11.09 Thijn Brummelkamp
Whitehead Institute, Cambridge
Haploid Genetic Screens in Human Cells Identify Host Factors used by Viruses and Bacteria

23.11.09 Artyom Kopp
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Wellcome Trust Centre for Stem Cell Research
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Centre de Regulació Genòmica (CRG)
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15.12.09 Eran Segal
Weizmann Institute
Reading the genome: from DNA sequence to expression

18.12.09 Michael Kühl
University of Ulm
Molecular mechanisms of cardiogenesis: Lessions learnt from Xenopus

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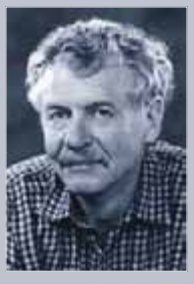

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In order to maintain the highest standard of research, the IMP has installed a process of review and feedback: the Scientific Advisory Board (SAB), consisting of internationally recognized scientists. The Board meets yearly at the IMP, and, together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

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The 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 PR agency, 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 1000 people from 40 different nations currently work at the Campus VBC. Campus members enjoy a scientifically and socially stimulating environment and take advantage of shared facilities such as the Max Perutz Library at the IMP and the Vienna Biocenter International PhD Program. A number of events, including seminars and lectures, are open to all.

Vienna – a City of Many Facets

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

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

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

YOUR CAREER AT THE IMP

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

Researchers of all backgrounds will find intellectual stimulation at the IMP. The weekly VBC Thursday seminar series invites renowned scientists to present their discoveries; everyone is encouraged to interact with these guests. Additionally, the IMP has initiated a monthly IMP seminar series, named after the IMP´s founding director Max Birnstiel, to highlight outstanding scientific researchers from around the world. 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 Friday 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/training*

MAX BIRNSTIEL LECTURE: ANDREAS STRASSER

In his lecture "The many roles of apoptosis in tumour development and cancer therapy", Andreas Strasser (The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia) discussed the key findings that the control of cell death is crucial for normal development as well as for cancer formation, autoimmunity and other diseases. His group identified novel important pro- and anti-apoptic regulators and deciphered a molecular rationale for how they control the balance between life and death of a cell. He presented the first clinical data indicating that inhibitors of the pro-survival protein Bcl2 may be successfully used for the eradication of B cell lymphoma in patients.
Lecture hosted by Meinrad Busslinger

VBC PHD RETREAT

This year's PhD Retreat took place from June 4 to 5 in the green hills of Semmering in Lower Austria. The two days were fruitfully spent with talks on science, discussions of careers, strengthening relationships with colleagues, and generally having fun. The absolute highlight of the event was Tony Hyman's brilliant teamwork. Tony Hyman was an invited speaker. The Nobel Prize laureate Tim Hunt joined the retreat spontaneously after delivering a scientific lecture at the VBC the previous day. Together, they shared their experiences with the students and gave them valuable advice. "Choose your mentor wisely", "Work on something you are really interested in" and "Interact with good people" were some of their salient messages. Tim Hunt's talk titled "How to win a Noble Prize" was especially fascinating; he spoke about the scientific and social stepladder that led him to the Nobel Prize.
The next morning was reserved for discussions on how a PhD program could be made more attractive, based on the results of a recent survey conducted among PhD students on the campus. Despite the active program the attendees had time to relax and enjoy the beautiful scenery of Semmering.

01/09

MAX BIRNSTIEL LECTURE: SVANTE PÄÄBO

Svante Pääbo is a founder of the field of paleogenomics, a field that attempts to trace human origins through the recovery and sequencing of ancient DNA. He began this work in 1985 with the isolation of DNA from a 2,400 year old egyptian mummy, and in 1997 reported the first DNA sequences from the Neandertal mitochondrial genome. In January 2009, Svante came to the IMP for a Max Birnstiel lecture to report on his most astonishing acheivement to date. Thanks to better preserved Neandertal specimens, improved methods of DNA recovery, and deep sequencing technology, he could now report the completion of a ~1X coverage of the entire nuclear genome. This sequence provides the strongest evidence to date that Neanderthals did not interbreed with modern humans. Moreover, by comparison to other primate genomes, the Neanderthal sequence is a boon in identifying those regions of the genome underwent positive selection in recent human evolution. One gene that Svante focused on in his talk was FoxP2, which has been implicated in language development. According to Svante, his Max Birnstiel seminar was the first public presentation of the Neandertal genome sequence – a prelude to his plenary talk at the AAAS meeting in Chicago the following month. A major scientific development was unveiled for the first time in a Max Birnstiel lecture!
Lecture hosted by Barry Dickson

06/09

DRAGON BOAT CUP, 3RD EDITION

On June 27 the IMPerfectos participated again in the Austrian Dragon Boat Cup. Competing with more than fifty teams in a 250-meter race on the Alte Donau, Vienna, the IMPerfecto team secured the third position.

MAX BIRNSTIEL LECTURE: TIM HUNT

On the 3rd of June Tim Hunt, who is currently working at the Cancer Research UK in London, gave an early afternoon Max Birnstiel lecture with the title "In an Out of Mitosis". In 2001 Tim Hunt was awarded the Nobel Prize in Physiology or Medicine together with Leland Hartwell and Paul Nurse for their discoveries about cell cycle regulation and cyclin-dependent kinases. His talk about the "Nobel Prize winner" experiments and about his recent work on the role of phosphatases in cell cycle regulation was not only interesting but also entertaining.
Lecture hosted by Borbala Gerle

IMP SUMMER PARTY

The weather smiled: plenty of sunshine and a mild wind during the IMP summer games which were held for the second time on July 24 this year. Following the impressive inaugural ceremony headed by the senior scientist Meinrad Busslinger, who carried the IMP flame through the arena to ignite the eternal torch, the IMP Summer Games were declared open. As the invitations and posters during the run-up had been quite vague, everybody was eager to discover what games like "Into the dark", "Tower of Pisa" or "Davis Cup" meant. It was astonishing to find out that "Hot Couture" was a t-shirt designing contest, "Regatta" was an air mattress race 5 times around the buoy and back, and "Bondage" was a tricky game: pairs of runners tied to each other at their legs had to cover an obstacle course. It proved to be a hilarious afternoon for all. The Gold Medal winners, a team known as IMPink won a book voucher, champagne, and the honor of organizing next year's games. We already look forward to the occasion…..

MAX BIRNSTIEL LECTURE: EVA NOGALES

Eva Nogales from the University of California, Berkeley, presented the molecular details, dynamics and organization of the microtubuli building blocks and their interaction with kinetochore complexes. Date: August 5, 2009 Titel: "Structural Bases of microtubule dynamics and its coupling to chromosome movement" Lecture hosted by Thomas Marlovits

07/09

08/09

MAX BIRNSTIEL LECTURE: MARC KIRSCHNER

The Birnstiel lecture in July was presented by Marc Kirschner, chairman of the Department of Systems Biology at Harvard Medical School, Boston. Marc has given numerous previous lectures at the IMP, remarkably each on a different topic. This is a reflection of Marc's broad interest in biology, ranging from cell division to signaling, cytoskeleton, development and evolution. In all of these areas, Marc has made outstanding discoveries and has trained generations of scientists. In his Birnstiel lecture Marc talked about regulation of actin polymerization during cell motility. He presented a series of elegant biochemical experiments which revealed how integration of different signals by the WAVE complex leads to actin assembly and cell protrusion.
Lecture hosted by Jan Peters

VBC SUMMER CONCERT

The VBC is fortunate to have music as a part of its cultural program. The solo program of this year's summer concert featured music by Mozart, Debussy and Bach, two Turkish love songs, and readings of poetry.
In the second part of the concert the MolBioOrchestra took center stage. The twenty-piece orchestra featuring students and staff from all over the campus presented an extensive program which included piano concertos by Mozart and Beethoven, "Water Music" by Handel, a waltz composed by the resident pianist Klemens Kunz and, as a final highlight, an arrangement of "Bohemian Rhapsody" by Queen.

MAX BIRNSTIEL LECTURE: GIACOMO RIZZOLATTI

On the 2nd of September we had a pleasure to host Giacomo Rizzolatti for the Max Birnstiel lecture series. Giacomo Rizzolatti is a senior scientist of the research team that discovered mirror neurons in the pre-motor cortex of the macaque monkey. Mirror neurons are believed to mediate an understanding of the behaviour of another animal, as they fire both when an animal acts and when the animal observes the same action performed by another animal, especially of the same species. These neurons have been directly observed in primates and are believed to exist in humans and other species. In humans it is thought that mirror neurons may be important for understanding of actions and intentions of other people, and for learning new skills by imitation. The discovery of mirror neurons is considered one of the most significant discoveries in neuroscience of the last decade.
Lecture hosted by Krystyna Keleman

POSTDOC RETREAT

This year's Postdoc Retreat took place from August 18 to 20 in the Salzkammergut region in Upper Austria. An old monastery was chosen as the location: it now hosts the newly founded "International Academy of Traunkirchen". This institution aims to support top-level Austrian research and fosters young scientists who wish to widen their horizons. The scientific highlights included a talk by Anton Zeilinger, the physicist who demonstrated that particles may exist simultaneously in two places at once, thus proving that teleportation was more science than fiction. This was followed by a lesson in science ethics, as virologist Tim Skern from the MFPL presented a detailed political perspective on the HIV/AIDS situation in Africa. Finally, Michael Kiebler from the Medical University of Vienna delivered a talk on neuronal cell biology. The official part of the retreat was followed by a walk along the beautiful Traunsee lake and a visit to the historic salt mines of Hallstatt.

RECESS

From September 30 to October 2, 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, were once more impressed by the scientific performance and high standards of the research presented. The IMP would like to thank all its SAB members for their commitment and advice. Sincere thanks go to Prof. Cornelia Bargmann (Rockefeller University, New York, USA) who took over the chair this year.
IMP SAB members: page 55 in this booklet

09/09

10/09

MAX BIRNSTIEL LECTURE: ROBERT TJIAN

The Birnstiel lecture on September 7th was given by Robert Tjian from the University of California at Berkeley, Howard Hughes Investigator and co-founder of the company Tularik. Bob's name is legend in the transcription field. No one else has used activity based protein purification to identify and characterize as many components of the eukaryotic transcription machinery, and has used as many of these in reconstitution experiments of mind boggling complexity. In his Birnstiel lecture Bob added a new dimension to this work by asking if different cell types utilize the same or different transcriptional mechanisms. Strikingly, Bob presented evidence that different cell types do not only express different sequence specific transcription factors but also use alternative forms of core promoter recognition complexes.
Lecture hosted by Jan Peters

EUROPEAN RESEARCHERS' NIGHT 2009

"Research is art" was the motto of the VBC-ERN which took place on September 25 at the Campus Vienna Biocenter. ERN stands for the "European Researchers' Night" - a multinational public science event sponsored by the European Commission. It consisted of a one-night event offering "edutainment" activities which provided an opportunity for the attendees to improve their knowledge about researchers and research.
On the day of the event, approximately 2500 visitors came to witness science in action. More than 100 VBC colleagues gave demonstrations of their current research and lent a helping hand to visitors who were eager to try out an experiment for themselves. From IMP and IMBA, the Dickson and Knoblich Groups and the Histology Unit were represented.
Apart from research demonstrations the visitors could enjoy an enactment of DiNA on the Road, take part in the quiz "Spot the Scientist", and play the VBC game. The Walk of Fame ceremony highlighted the merits of the VBC's founding fathers, including the first IMP Director Max Birnstiel who had travelled a long distance from Switzerland to grace the occasion. One of the highlights was the Paint your PhD contest. Fifteen scientists were placed on an impressive scaffolding construction, equipped with paints and brushes, and given one hour to translate the topics of their dissertation into a piece of art. The IMP and IMBA were well represented by Lucia Aronica, Arabella Meixner, Mark Palfreyman (first prize, postdocs), Anne Philipsborn and Ivana Primorac (first prize, students). The paintings were auctioned and the money earned - 3000 Euros in total – was donated to St. Anna Children's Cancer Research Institute (CCRI).

MAX BIRNSTIEL LECTURE: POUL NISSEN

Poul Nissen is a world leader in the field of structural biology, focusing mainly on eukaryotic membrane proteins. As a postdoc in Tom Steitz' lab at Yale University, he made essential contributions to the structural understanding of the large subunit of the ribosome, work that directly contributed to the Nobel Prize in Chemistry for Tom Steitz, Venki Ramakrishnan, and Ada Yonath, which was awarded on the day of Dr. Nissen's visit to the IMP! Dr. Nissen is currently Professor of Protein Biochemistry at the University of Aarhus and Director of the Center for Membrane Pumps in Cells and Disease. His work over the last 10 years has revealed phenomenal insights into how P-type ATPases convert the chemical energy of ATP into electrochemical gradients to drive all other cellular transport processes. These proteins are of fundamental biological importance and provide fascinating insights into the structural mechanisms of complex molecular machines. His Max Birnstiel Lecture described his recent work on characterizing the first structure of the Sodium-Potassium-ATPase, the first structure of the plasma membrane Proton-ATPase, and the structure of the Calcium-ATPase in a previously uncharacterized functional state.
Lecture hosted by Peggy Stolt-Bergner

MAX BIRNSTIEL LECTURE: JOHN KURIYAN

John Kuriyan is currently the Chancellor's Professor of Biochemistry and Molecular Biology at the University of California-Berkeley and is a Howard Hughes Investigator. His lab is performing leading research in understanding the molecular mechanisms underlying signal transduction and DNA replication. Work from his lab has led to fundamental insights into the regulation of kinases and other enzymes regulating cellular signaling, including Src and Abl tyrosine kinases, the GTP-binding protein Ras, and the epidermal growth factor receptor (EGFR). Additionally work from his laboratory has contributed to understanding the molecular mechanism of DNA recognition by the replication machinery, through structural and functional studies on clamp loading complexes. Some of his most recent work has revealed important insights into the mechanism of EGF receptor activation by dimerization, the topic of his Max Birnstiel Lecture.
Lecture hosted by Peggy Stolt-Bergner

11/09

12/09

2009 NOBEL PRIZE FOR CHEMISTRY GOES TO IMP SAB MEMBER

On October 7 Dr. Venki Ramakrishnan of the Medical Research Council Laboratory of Molecular Biology won the 2009 Nobel Prize in Chemistry for his investigation of the structure and function of the ribosome. Dr. Venki Ramakrishnan is a member of the IMP Scientific Advisory Board since 2008. IMP congratulates him on this occasion. He shares the prize with two other scientists, namely Thomas A. Steitz and Ada E. Yonath.

VBC-PHD SYMPOSIUM "ANDROID AND EVE"

The topic of this year's PhD Symposium from November 12th to 13th was dedicated to the advancing fusion of the human and the computer world. We can no longer envisage our daily lives without the computer. The aim of the symposium was to bring together leading scientists in the fields of neuronal interfaces, artificial organs, nanobiotechnology and biological microdevices.
About 190 interested students, 70 off-campus guests from all over Europe, and a large number of journalists took part in this event. The scientific highlights were Carlo Montemagno who gave fascinating insights into his work about bio-machines, Miguel Nicolelis and Andrew Schwartz who explained how the human brain could be connected to computers which move roboter devices, Jackie Ying on smart synthetic materials, and Niels Bierbaumer about his work on stroke and locked-in patients. The most controversial talk was Kevin Warwick's: he presented his vision of the Cyborg world in which all humans will, sooner or later, become Cyborgs by incorporation of microchip implants, as predicted in the film "Matrix".

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Hans Krist (pp: 2, 22)
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point of view (pp: 26, 30, 32, 34, 45, 59)
Dialog Gentechnik (p: 62)

Cover Illustration

The fruitless neuronal network in the Drosophila brain. To understand the organisation this network, distinct neuronal cell types have been labeled, imaged, and traced in different colours. A synaptic counterstain was used to register individual samples onto a common reference brain to creating a digital atlas.
Courtesy of Jai Yu

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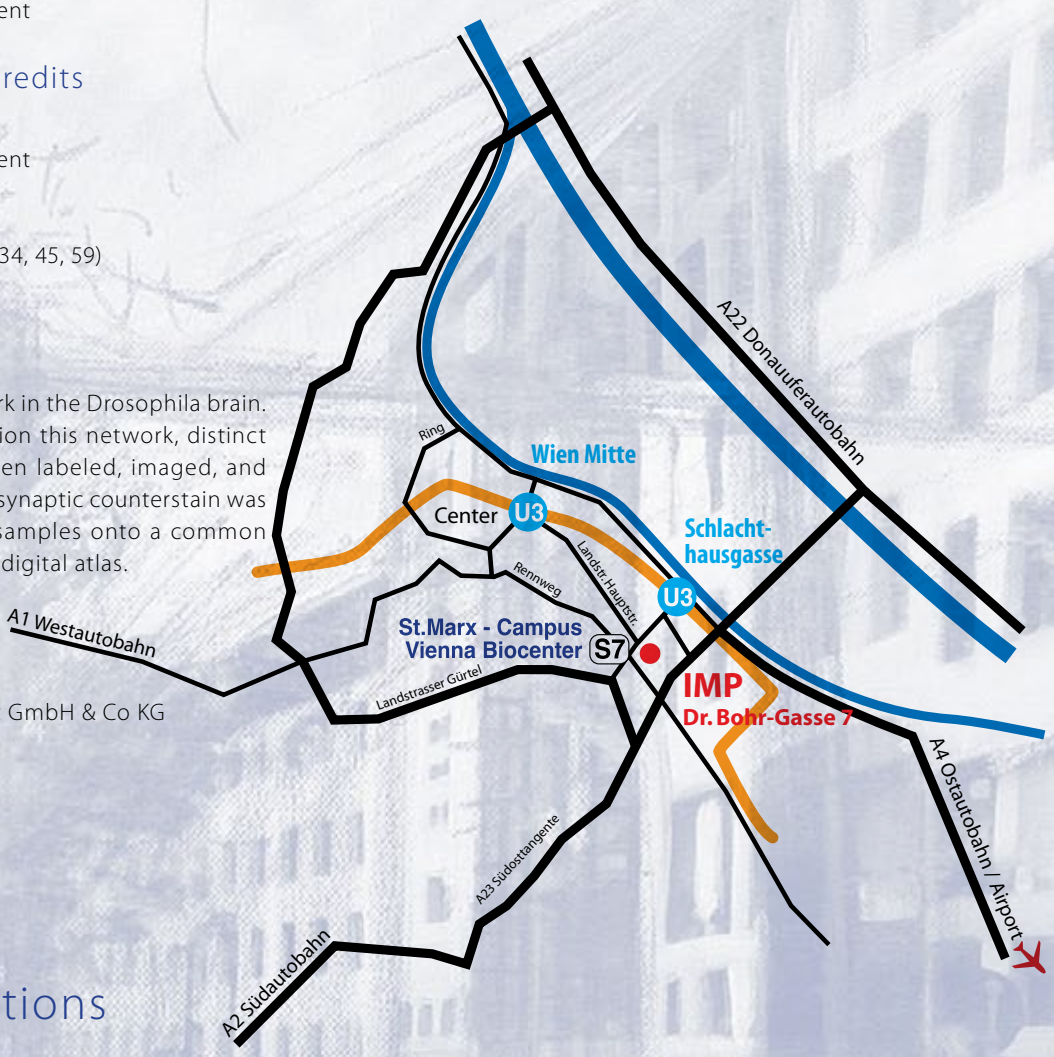

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