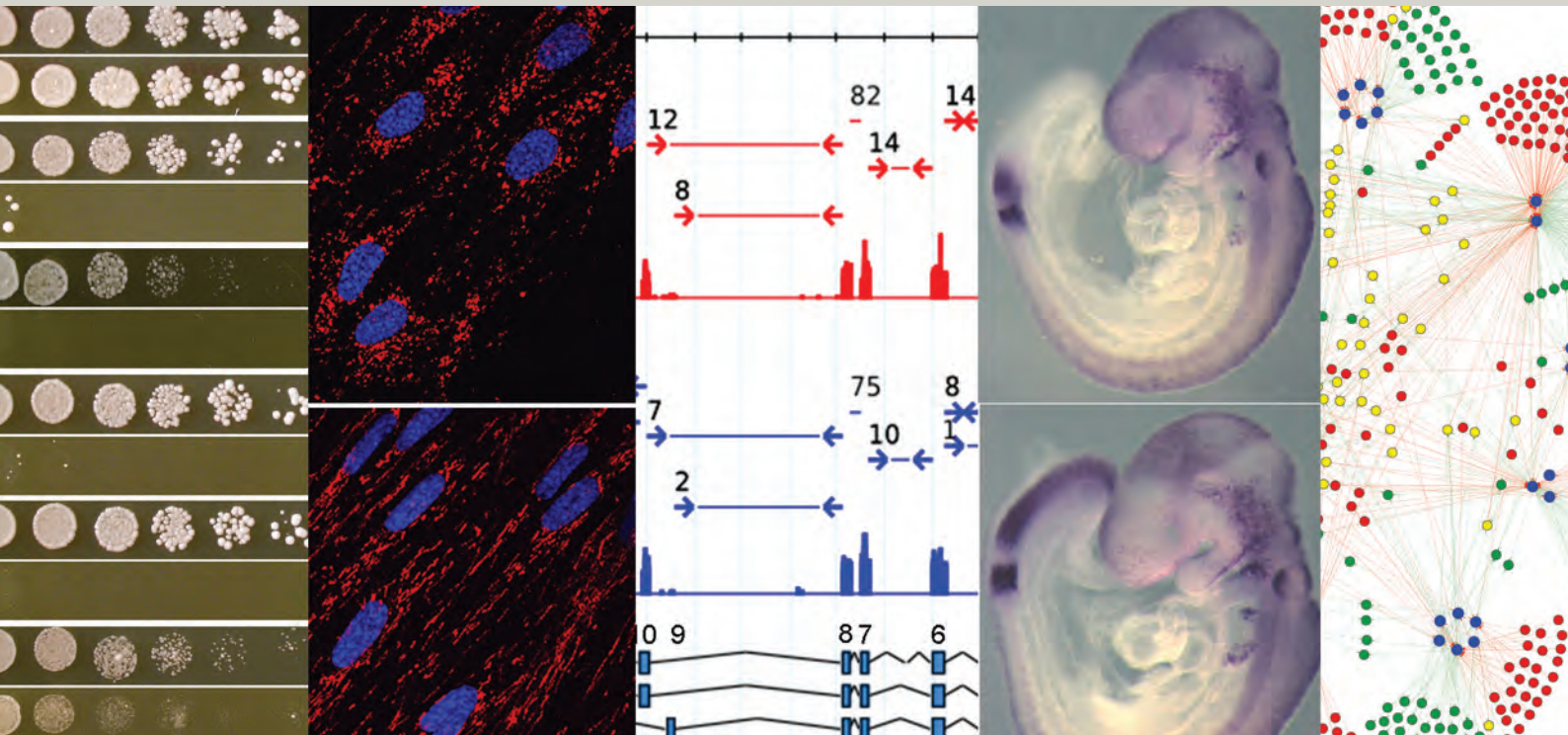
MPIMG

MAX-PLANCK-GESELLSCHAFT

Research Report 2009

Max Planck Institute for Molecular Genetics, Berlin



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Research Report 2009

Max Planck Institute for Molecular Genetics

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Berlin, December 2009

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In the publication lists of the departments ("General information about the whole Department"), department members are underlined.

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Organisational structure

The Max Planck Institute for Molecular Genetics

Mission

Research at the Max Planck Institute for Molecular Genetics (MPIMG) concentrates on genome analysis of humans and other organisms to elucidate cellular processes and genetic diseases. It is the overall goal of the combined efforts of all MPIMG groups to gain new insights into the development of diseases on a molecular level, thus contributing to the development of cause-related new medical treatments.

Development of the Institute

The Max Planck Institute for Molecular Genetics (MPIMG) was founded in 1964 with the appointment of Heinz-Günther Wittmann and Heinz Schuster as heads of department, followed by the appointment of Thomas Trautner in 1965. At this time, the research of the institute was focussing on DNA replication and gene regulation in bacteria, bacterial phage and fungi (departments Schuster and Trautner) and on the structure, function and evolution of ribosomes which were central to the work of H.-G. Wittmann. In 1970, the three departments, as well as four independent junior research groups (the future Otto Warburg Laboratories) moved into the new premises of the institute situated in the Ihnestraße, Berlin-Dahlem.

After the sudden death of H.-G. Wittmann in 1990 and the retirement of H. Schuster in 1995, the appointments of Hans Lehrach (1994, Dept. of Vertebrate Genomics), and Hans-Hilger Ropers (Dept. of Human Molecular Genetics, full-time since 1997) induced a major shift in the scientific orientation of the institute. Following the retirement of T. Trautner in 2000, Martin Vingron was appointed as head of the new Department for Computational Molecular Biology. At the same time, Stefan Mundlos was jointly appointed by the Humboldt University of Berlin as head of the Institute for Medical Genetics, now integrated in the Campus Virchow of the Charité - Universitätsmedizin Berlin, and by the Max Planck Society as head of an independent research group at the MPIMG. As most recent recruitment, Bernhard Herrmann was appointed as director and head of the Department of Developmental Genetics in 2003. Bernhard Herrmann was also jointly appointed by the Free University of Berlin as professor and head of the Institute of Medical Genetics (now part of the Campus Benjamin Franklin of the Charité).

In the years 2004 - 2005 all three independent junior research group leaders of the Otto Warburg Laboratory obtained new positions and left the institute. Ann Ehrenhofer-Murray and Andrea Vortkamp were appointed as full professors by German universities, Adam Antebi joined the faculty of Baylor College in Houston, Texas, U.S.A. In the meantime, Adam Antebi has been appointed as member of the Max Planck Society and head of a research department. The externally funded fourth group leader, Edda Klipp, was appointed in 2008 as full professor at the Humboldt University of Berlin. In 2004 a new independent junior research group in Bioinformatics, headed by Michael Lappe, took up its work, and in 2007 another group working on molecular interaction networks was recruited, which is headed by Ulrich Stelzl. More recently, the Otto Warburg Laboratory was brought up to four groups, two of them, headed by Sylvia Krobitsch and Sascha Sauer, funded by external sources.

In 2006, the International Max Planck Research School for Computational Biology and Scientific Computing was founded together with the Free University of Berlin. In addition the institute is involved in the Berlin School for Regenerative Therapies, housed at Humboldt University, and in teaching of medical student in several curricula. In 2008, an internal PhD programme for students was started at the MPIMG.

Research Concept

Genome research, the systematic study of genes and genomes, has changed the way in which research in molecular genetics is pursued. The focus and composition of the MPI for Molecular Genetics reflects this development. Large scale genome research (Dept. Lehrach), applying a variety of technologies, generates data on genome sequences, genes, and their function, which are then used to build predictive models of biological networks in human diseases and development. Human molecular genetics (Dept. Ropers) employs complementary approaches to search for novel disease genes and their biological function in a systematic manner, as a strategy for identifying genetic risk factors and pathogenetic pathways for common and rare diseases. Computational molecular biology (Dept. Vingron) exploits genomic data to better understand gene regulation and evolution. The Developmental Genetics Department (Dept. Herrmann) uses systematic functional analysis for understanding developmental mechanisms.

Recently, new sequencing technologies (massively parallel sequencing) have opened up new approaches for genome-wide analyses of gene expression (transcriptome) and epigenetic control (analyses of methylated DNA regions and chromatin modifications). It also provides more sensitive ways for the detection of chromosomal rearrangements, copy number differences and other genetic alterations involved in disease, and it promises to yield novel tools for their diagnosis. Massively parallel sequencing has become in-dispensable for the research of all departments.

Almost half of the institute's research budget is obtained as grant money from external sources, such as the German Ministry of Education and Research, the European Union, the German Research Foundation (DFG) and others. The institute is - as sole German member - involved in the 1000 Genomes Project, a follow-up of the international Human Genome Project and HapMap Project. Several groups of the institute coordinate or participate in consortia involved in the German National Genome Research Network (NGFN), which focuses on basic and translational research of human disease. Major topics comprise cancer research and mental retardation. Other prominent projects include a number of EU projects, as well as DFG Collaborative Research Centres ("Sonderforschungsbereiche").

With its involvement in national and international research projects and by virtue of the research output of the institute, the MPIMG is perceived internationally as a stronghold of genome and genetics research in Germany. Maintaining this status in the future will require continuing technological innovation and close cooperation with the universities. Integration between genome research and genetics, as well as between experimental and computational biological research are central in this effort.

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(Established: 11/2003)

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Introduction

The major focus of the Department of Developmental Genetics is on understanding the regulatory networks controlling tissue formation and organogenesis during embryonic development. We investigate patterning, induction and differentiation processes in the trunk. Tightly linked to this major goal is the molecular analysis of tumour formation. In recent years it has become apparent that tumours can develop from cancer stem cells triggering aberrant tissue formation. Cancer stem cells derive from adult stem cells required for tissue regeneration in the adult organism, and follow the principles of embryonic development. Thus, processes of embryonic development, tissue regeneration and cancer are closely related. There is also compelling evidence that tumour spreading to other sites in the body also requires genetic programs regulating cellular shape changes, which are shared between embryos and tumours. This switch in cellular behaviour, called epithelial-mesenchymal transition (EMT), is a very old evolutionary event and the basis for the evolution of complex organisms with many tissues and organs. Organogenesis is not possible without EMT, and thus it is also a central process of trunk development.

* externally funded

Finally, we have started to investigate the effects of genetic variability on disease development. Individual genetic differences have an important impact on whether we are likely to get, for example cancer, where one in three will be affected, or not. We have set out to look for modifiers influencing intestinal tumour formation in the mouse. In addition, we are investigating the evolution and action of modifiers, which trigger male infertility in the mouse and, in combination with a mutant gene, *Tcr*, contribute to non-Mendelian inheritance.

Scientific overview

Regulatory networks controlling trunk development

Trunk formation comprises many processes, such as patterning, endowment of cells with position information, EMT, induction and maintenance of differentiation programs, which are controlled by complex regulatory networks. In principle, three different cell types are involved; stem cells, their descendants, and organizer cells which provide instructive signals. Trunk formation takes place in a growth zone located at the rear (caudal) end of the embryo, called the primitive streak (ps) and, at a later stage, the tail bud (tb). Knowledge about the organization of the ps/tb is still fragmentary. The organizer(s) and stem cells have been localized only roughly, the genetic programs controlling their identities are far from being understood.

In recent years we have put large efforts into developing cell type specific markers, methods and tools for a systematic molecular investigation of tissue differentiation and organ development *in vitro* and *in vivo*. We have performed and completed a large-scale gene expression analysis in E8.5-E11.5 mouse embryos in order to identify the important regulators of differentiation programs and cell specific markers. We have investigated the transcriptome of five regions dissected from E8.5 mouse embryos, providing high-resolution sequence data on all known, and many novel transcription units, including non-coding genes, splice variants, and alternative promoters, to name a few. We have worked out a method for conditional inactivation of gene function based on miRNA-mediated knock-down, allowing the functional analysis of genes involved in embryonic processes. We have introduced and improved the ChIP-seq technique for application on small amounts of tissue typically obtained from embryos, permitting genome-wide analysis of epigenetic modifications. Another advance is the genome-wide localization of chromatin-bound regulators (such as transcription factors and co-factors, chromatin remodelling proteins) by ChIP-seq, enabling the identification of target genes of such regulators. Another important breakthrough in the department was the establishment of a protocol allowing the *in vitro* differentiation of mesodermal cell types, which will be invaluable in analysing and understanding the early events of differentiation from stem cells to a committed cell type. Finally, all these new developments would not deliver interpretable results without the appropriate bioinformatic evaluation tools, which have been developed and setup in our department.

Genetic variability and disease

One important aspect of regulatory networks controlling any process in a multicellular organism is the fact that they differ from individual to individual. This statement becomes quite apparent when we simply consider the differences between human individuals, for instance in size, looks and skills. Individual differences are due to differences in the genome and the regulatory networks expressed

by the genome. Genetic differences not only determine our capabilities, they also have impact on health and disease, e.g. if one develops cardiovascular disease or cancer.
We follow two different approaches to identify modifier genes having an impact on a particular phenotype. One is placed in the field of tumourigenesis, the other in non-Mendelian inheritance.

Modifiers of intestinal tumour formation and progression

In this project we search for modifier genes exerting a global effect on intestinal tumour formation and progression. It is based on the finding that individual genetic differences have a strong impact on susceptibility to disease. It is completely unclear which differences are important, and which are not. It is known that early tumour formation is correlated with changes in the DNA methylation patterns (hypo- or hyper-methylation) of genes, which influences gene expression (up- or down-regulation). Therefore we set out to search for factors affecting gene methylation, gene expression, and tumour number or phenotype. We assume that there is a good chance to find genes controlling all three aspects. The system we are using to introduce genetic variability, influencing tumour formation and gene control, into the genome is the so-called chromosome substitution (consomic) strain system developed by J. Forejt in Prague. It consists of 27 mouse strains in which a single chromosome or chromosomal sub-region of a *Mus musculus* strain (PWD) was introduced into the *Mus domesticus* inbred strain C57BL/6. In essence, all strains differ by only one chromosome or less from the C57BL/6 strain. We use a mouse model of colon cancer, the APC^{Min} mouse, which has a pure C57BL/6 genetic background, to ask if any of the PWD-derived chromosomes carried by the consomic strains expresses a modifier(s) influencing the formation or phenotype of tumours in the APC^{Min} mouse. This, we have found, is indeed the case. Now we can investigate what these factors are and if they also influence epigenetic modifications and gene expression. This project has the potential to reveal a pathway or network controlling susceptibility to tumour formation and progression in the mouse, which may become important for developing anticancer drugs in the future.

Modifiers of non-Mendelian inheritance and male fertility

Details of the system and approaches can be obtained in the research report of the Transmission ratio distortion group. Here I want to point out the principal important aspects of the project in terms of understanding the evolution of modifiers and how they can trigger a disease phenotype. We have isolated four modifiers having a strong impact on the motility of sperm. These modifiers have co-evolved to produce a strong phenotype, almost exclusive transmission of a particular chromosomal region, the so-called t-haplotype. This phenotype depends on the activity of another important gene, the t-complex-responder (Tcr), which can rescue a sperm cell from the negative action of the modifiers. However, in the absence of Tcr the combined action of several (probably six) such modifiers can trigger a disease phenotype, male sterility. Each modifier alone contributes only approximately 10-15% to the phenotype, and sperm cells can tolerate modifier activity to some degree. Too much activity, however, leads to imbalance and triggers the disease.
The four modifier loci we have isolated evolved by different genetic alterations; gene amplification, switch between alternative promoters leading to up-and down-regulation of alternative transcripts, gene inactivation and a point mutation resulting in a dominant negative protein. The modifiers act on two signalling pathways, one activating and one inhibitory, controlling parameters of sperm motility. It is

quite striking how they interact to produce this phenotype. The inhibitory pathway is down-regulated, while the activating pathway is up-regulated by the modifiers, thus reinforcing each other.
It is conceivable that similar mechanisms may be involved in triggering multifactorial disorders in humans. Thus, studying the modifiers of non-Mendelian inheritance and male fertility can teach us a lesson about the molecular basis of multi-factorial disease.

Cooperations within the Institute

Dept. of Vertebrate Genomics

- Jörn Glökler: *SELEX for binding sites of transcription factors required for murine axis development*
- Christina Grimm: *NGFNplus Consortium: Modifiers; Establishment of primary cell lines from consomic mouse strains*
- Christina Grimm, Silke Sperling: *Screen for genes expressed in the heart, and OPT of mutant mouse hearts*
- Heinz Himmelbauer, Florian Mertes, T. Nolden: *Analysis of gene expression in the mouse embryo*
- Zoltán Konthur: *Selection of phage displayed antibodies for specific detection of Smok/ Tcr; Selection of Aptamers/Intrabodies affecting signal transduction pathways* (together with S. Krobitsch, Otto Warburg Laboratory)
- Bodo Lange, together with Ulrike Korf, DKFZ: *NGFNplus Consortium: Mutanom*
- Hans Lehrach: *NGFNplus Consortia: Modifiers; Mutanom. Individualized medicine on cancer patients*
- Markus Ralser: *Generation of mutant ES cell lines by gene targeting*
- Christoph Wierling: *Computer model of the segmentation clock in the mouse*

Dept. of Human Molecular Genetics

- Andreas Kuss, Lars Jensen: *Analysis of gene expression*

Dept. of Comput. Molecular Biology

- Alexander Schliep, Ruben Schilling: *Development of standard 3D models of mouse embryos for automated annotation of morphology and gene expression via 3D registration of volume data (OPT)*

Research Group Development & Disease

- Mateusz Kolanczyk: *OPT of mouse NF1 mutant cartilage and bone, and of Nox mutant fetuses*
- Georg Schwabe, Daniel Birker: *OPT of Dsh (Shh hypomorphic) mutant mouse embryos*
- Sigmar Stricker: *OPF of chick organs over-expressing muscle regulatory genes*

Otto Warburg Laboratory

- Sylvia Krobitsch: *Yeast-two-hybrid screening*
- Ulrich Stelzl: *Yeast-2-Hybrid Screen for factors interacting with regulators of embryonic development or factors involved in non-Mendelian inheritance; Interactions of t-haplotype molecules; Identification of protein-protein-interactions*

Next Generation Sequencing Group

- Bernd Timmermann: *Transcriptome analysis of the t-haplotype*.

Electron Microscopy Group

- Rudi Lurz: *Localization of the Tcr-protein and transcript in testis and sperm;* Electron microscopic analysis of mouse tissues

Transmission ratio distortion

(Established: 11/2003)

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Scientific overview

Current state of research and scientific findings

A considerable proportion of wild mice carry two variant forms of chromosome17, the wild type and the *t*-form. Males heterozygous for the *t*-haplotype transmit this chromosome at a high ratio to their offspring, at the expense of the wild type chromosome. This is in opposition to Mendel's laws, according to which two homologous chromosomes are transmitted at equal rates to the offspring. Thus trans-

* externally funded

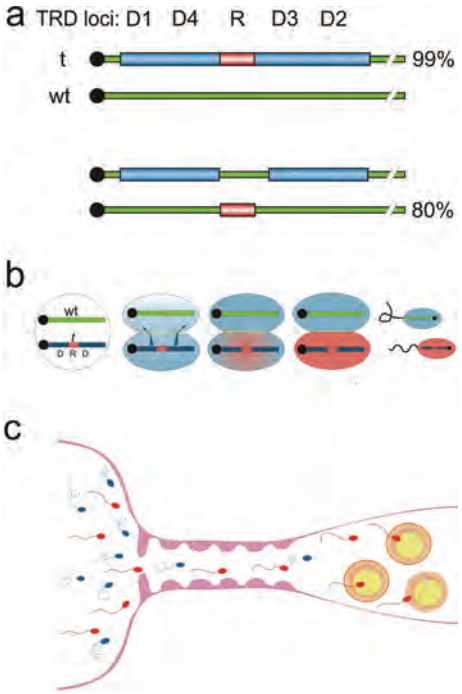

Figure 1: Genetics of the mouse t-complex and a model of cellular processes during transmission ration distortion (TRD). ***(a)*** *Genetics of the t-complex. The transmission rate of the chromosome carrying the t-complex-responder (R) is enhanced by the t-complex-distorters (D). The Distorters can be located on the same chromosome as the Responder (natural condition, upper part) or on a different chromosome (experimental set up, lower part).* ***(b)*** *Proposed cellular mechanism of TRD. Harmful products (blue) of the Distorter genes are distributed between t- and wild type meiotic partners across cytoplasmic bridges. Therefore both, t- and wild type meiotic partners are compromised. In contrast, the function of the Responder gene (red), which counteracts the harmful Distorter effect, is restricted to t-sperm and not shared. Consequently only t-sperm are rescued. They show a more progressive swimming behaviour as compared to their wild type competitors and have an advantage in fertilizing the egg cells* ***(c)****.*

mission ratio distortion (TRD) by the *t*-haplotype is a paradigm for non-Mendelian inheritance in mammals. Mouse geneticists have identified several mutant loci involved in this phenomenon. The central factor is the *t-complex responder* (*Tcr*). Transmission of the chromosome carrying *Tcr*, is promoted by *t-complex distorters* (*Tcd1 - 4*). The latter act as quantitative trait loci. Cumulative action of all *Tcds* may enhance the transmission of *Tcr* to 99% (Fig. 1a).

A model explains these genetic observations: Tcds are distributed to all sperm during meiosis and exert a harmful effect on both, *t*- and wild type meiotic partners. Tcr is able to rescue the harmful effect of Tcds but remains restricted to the cells carrying the gene, the future *t*-sperm. Wild type sperm thus remain compromised while *t*-sperm is rescued (Fig. 1b). This leads to preferential fertilization of the egg cells by *t*-sperm and prevalence of *t*-animals in the next generation (Fig. 1c).

Work in our group is focused on understanding the molecular mechanisms of TRD. A first step towards this goal was the cloning of *Tcr*, which encodes a dominant negative variant of a novel Ser/Thr protein kinase, termed *Smok1*, and is expressed in spermatids (Herrmann et al., Nature 402:141 - 146, 1999). Central to the model of TRD is the generation of phenotypically different sperm by Tcr – an exception to the general principle of equality of sperm due to gene product sharing between meiotic partners. We have recently shown the basis for cellularly restricted action of Tcr (Véron et al., Genes & Development 2009).

Defining the molecular nature of the *Distorter* genes is crucial for a detailed understanding of the system and represents a particular challenge: Four large inversions prevent recombination with the wild type chromosome, thus the genes can be located only coarsely to very large genetic intervals. Despite this we were able to clone the first *Distorter* gene *Tcd1*, encoding Tagap1, a GTPase activating protein (GAP) for small G-proteins of the Rho subfamily (Bauer et al., Nature Genetics 2005). Rho small G-proteins cycle between an active, GTP bound state and an inactive conformation in which they have bound GDP. GAPs enhance GTP hydrolysis to GDP and thus inactivate G-proteins. We found the *t*-allele of *Tagap1* to be a hypermorph. In contrast, previous genetic studies have shown that a deletion allele of *Tcd1* phenocopies *Distorter* action. Thus, we have postulated additional *Distorter* activity in the *Tcd1* region, acting upstream or epistatically to *Tagap1*. We have now isolated a candidate gene in the *Tcd1* region fulfilling this prediction. Transgenic animals expressing an extra dose of this gene show decreased *t*-haplotype transmission. We therefore propose that this gene encodes *Tdc1b* (Fig 2, Charron et al., unpublished).

In addition, we identified a positive regulator of Rho proteins as a transmission ratio distorter in the *Tcd2* region. This gene, *Fgd2* (*faciogenital dysplasia 2*) encodes a GEF (guanosine nucleotide exchange factor). GEFs activate Rho proteins by promoting the exchange of GDP for GTP (Bauer et al., Genes & Development, 2007).
The *t*-forms of *Tagap1* and *Fgd2* represent hypermorphic alleles, and both promote the transmission ratio of the *t*-haplotype. Since the two factors have contrary effects on their respective G-protein, but act additively, we conclude that they act on two different G-proteins, which exert opposite effects on Smok1 (Fig 2, Bauer et al., Genes & Development 2007).
We have also shown that *Tcd2* similar to *Tcd1* is composed of more than one gene. Analysis of a second candidate gene revealed its activity as a transmission ratio distorter (*Tcd2b*). Unlike *Tagap1* and *Fgd2*, the *t*-form of *Tcd2b* is phenocopied by a knock-out allele suggesting, that the *t*-allele encodes a hypomorph. We are currently analyzing whether this gene acts as a negative regulator of the activating (Fgd2-) pathway (Fig 2, Bauer et al., unpublished).
Since spermatozoa derived from *t*/+ males show impaired flagellar function, we suggest that Tcd2b, along with, Tagap1 (Tcd1a), Tcd1b and Fgd2 act in signalling pathways controlling sperm motility. We propose a model, in which the *Distorters* hyperactivate Smok1, compromising sperm function. Tcr acts as an "antidote", bringing the signalling pathway back to a level favourable for normal flagellar function (Fig. 2).

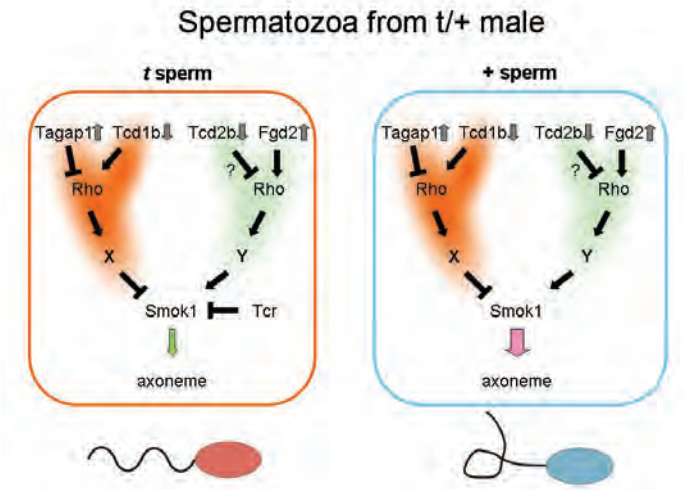

Figure 2: Signaling events in Transmission ratio distortion. Distorters hyper-activate Smok1 via two distinct pathways: They reduce the activity of a Smok1 inhibitory pathway (left branch, red shade), and promote activity of a Smok1 activating pathway (right branch, green shade). Synergistic hyperactivation of Smok1 by the two pathways leads to compromised sperm function in all cells since Distorter molecules are distributed between t-*spermatogenic cells and their meiotic partners (see Fig 1b). Tcr, a dominant negative version of the Smok1 kinase counterbalances hyperactivated Smok1 and thus rescues specifically* t-*sperm (left) to which its gene products are restricted (see figure 1). Hypermorphic and hypomorphic Tcds are symbolized with grey upward and downward arrows respectively. Three of the four distorters known to date (Tcd1b, Fgd2, Tcd2b) have been characterized in the period 2006 – 2009.*

General information

Selected publications

Verón N., Bauer H., Weisse A., Lüder G., Werber M. & Herrmann B.G. (2009) *Retention of gene products in syncytial spermatids promotes non-Mendelian inheritance as revealed by the t-complex-responder.* Genes & Development 23:2705-2710

Bauer H., Veron N., Willert J. & Herrmann B.G. (2007). *The t-complex-encoded guanine nucleotide exchange factor Fgd2 reveals that two opposing signaling pathways promote transmission ratio distortion in the mouse*. Genes & Development 21, 143-7.

Bauer H., Willert J., Koschorz B. & Herrmann B.G. (2005). *The t-complex-encoded GTPase-activating protein Tagap1 acts as a transmission ratio distorter in mice.* Nature Genetics 37, 969-73.

Work as scientific referee

Hermann Bauer serves as scientific referee for the journal Development Genes and Evolution.

Patent

Herrmann B.G., Bauer, H. *Isolation of the t-complex distorters and applications thereof*. No: PCT/EP 2006/007977, August 2005.

External funding

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Regulatory Networks of Mesoderm Formation & Somitogenesis

(Established: 11/2003)

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Introduction

The formation of complex organisms from a single cell requires tight control of multiple reiterative steps of cell proliferation, patterning and differentiation, comprising frequent cell interactions and changes of the cellular readout. Trunk development involves a large repertoire of cellular responses controlled by competing signaling pathways, which employ different sets of transcriptional regulators forcing cells into various differentiation pathways. Developmental geneticists in general use mutagenesis tools to remove or alter the function of single genes to be able to analyse their roles in embryogenesis (provided a phenotype is observed). Often the knowledge gained from such analyses ends at the description of the phenotype. However, advances in genomics research have set the stage for gaining deeper insight into the genetic control of developmental processes, even of such complexity as trunk formation. To reach a deeper understanding of the regulatory networks controlling complex cellular interactions and responses leading to tissue and organ development, we have set up a number of techniques allowing the faster generation and analysis of mutants, derive genome-wide datasets of epigenetic gene control and gene expression, and analyse differentiation programs and regulatory networks *in vivo* and *in vitro*.

* externally funded

Scientific overview

We have recently completed a large-scale expression screen in mouse embryos investigating the temporal and spatial control of several thousand genes in day 8.5 – 11.5 mouse embryos. The data are publicly available in the MAMEP database. This herculean task was undertaken to determine the molecular anatomy of the mouse embryo and thereby identify the genes controlling patterning, differentiation and organogenesis. The data are invaluable in deciphering regulatory networks controlling these processes.

The database on individual gene expression patterns was recently complemented by and extended to whole transcriptome data on five tissues (head, heart, somites, spinal cord, presomitic mesoderm) dissected from E8.5 mouse embryos. A genome wide sampling of transcriptional activity was obtained by deep sequencing of RNA on an Illumina GAIIx station (RNA-seq). Bioinformatics analysis enabled annotations of all known and many novel transcription units, including non-coding genes, splice variants, alternative promoters and novel long range spanning RNAs. Moreover, we complemented this transcriptome dataset by genome-wide histone modification landscape data obtained by deep sequencing of immunoprecipitated chromatin (ChIP-seq). We obtained an unmatched catalog of genome activity on the expression and chromatin level in the developing murine embryo. Throughout the analysis and evaluation of RNA-seq and ChIP-seq data we developed many important bioinformatics tools and established computational workflows that are crucial for effective utilisation of these large scale data sets.

In addition to the above, we have also developed a vector system allowing manipulation of cultured cells or embryos by conditional (spatially and temporally controlled) expression of wild-type or mutant genes from transgene constructs integrated as single copy genes into a defined recipient locus. Another technical advance is the development of a vector system for conditional down-regulation of gene transcripts using miRNA-mediated gene knock-down. These methods are complemented by advanced embryological techniques allowing the production of chimera, which are almost exclusively derived from ES cells. This technique permits the immediate functional analysis of the consequences of genetic alterations, introduced into ES cells in vitro, in chimaeric embryos derived from such ES cells, thus avoiding time consuming establishment of transgenic mouse lines.

Finally, we have invested in the development of an *in vitro* differentiation system, which allows the mass production of different cell types from embryonic stem cells in the culture dish. This technology is essential for providing sufficient cellular material of early stages of differentiation, which in the mouse embryo occur in very few cells. The combined advances in technology introduced into our approaches now permit us to manipulate embryonic stem cells, differentiate them *in vitro* to particular cell types and study the genetic/epigenetic events controlling the differentiation program, or use the cells to produce embryos and study the differentiation program *in vivo*. We can manipulate the processes by changing gene expression and gene function, and analyse the genetic/epigenetic effects of these alterations *in vitro* and in the embryo. Such analyses will allow us to unravel the regulatory networks controlling stemness, induction, commitment and differentiation into various cell types.

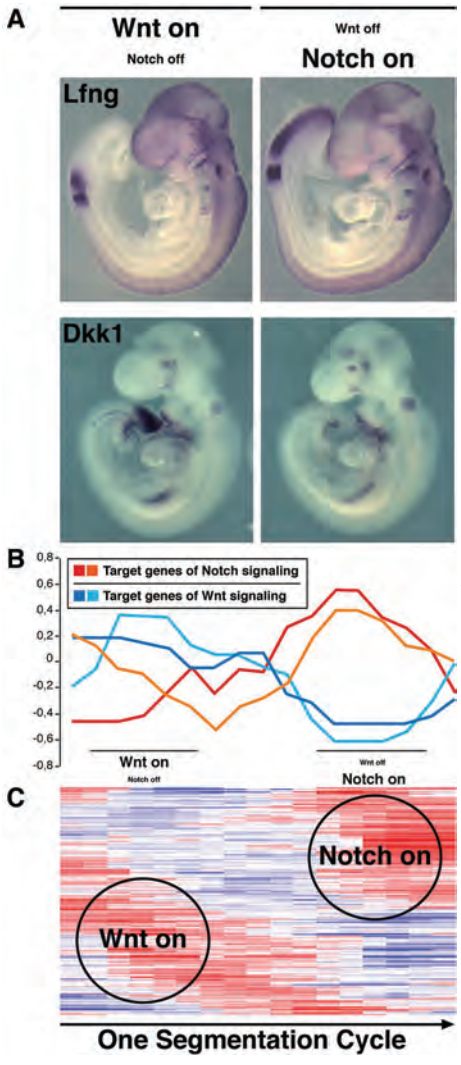
Trunk development involves patterning processes leading to the formation of several transient stem cell types: preneural cells, presomitic, lateral and intermediate mesoderm, and endoderm from epiblast stem cells. The patterning process is controlled by two main signaling cascades, WNT and BMP signaling. Within hours the transient stem cell types become committed to differentiation into neural tissue (spinal cord), somites (precursors of skeletal muscle, cartilage and bones of the axial skeleton), somatopleure and splanchnopleure, gonads and kidneys, and

the midgut and hindgut. These early patterning events set the stage for subsequent differentiation processes, in a strictly hierarchical manner. Therefore, understanding the early patterning events during trunk formation will provide essential knowledge about the control of tissue and organ development in general.

Presomitic mesoderm formation and patterning

Our primary focus so far was on understanding the formation and segmentation of presomitic mesoderm (psm). This is a highly integrated process controlled by the WNT signal cascade. We have analysed the hierarchy of several transcriptional regulators, which play an essential role in psm formation, and showed that signal molecule Wnt3a controls this process in cooperation with the downstream transcriptional regulators T, Tbx6 and Msgn1. Of course, many more genes are involved in the regulatory networks controlled by these factors. For a detailed analysis of this differentiation process we use a combination of target gene identification and chromatin analyses using ChIP-seq, RNA-seq analyses of wild-type and mutant tissue as well as *in situ* gene expression data.

Somitogenesis divides the presomitic mesoderm into a regular array of segments, cellblocks, which are aligned on either side of the neural tube and undergo pairwise fusion to form vertebrae. Tight spatial and temporal control of psm segmentation is essential for the formation of a well shaped vertebral column. We have shown that the segmentation process is also controlled by WNT signaling, and that psm development and segmentation are highly integrated processes. Wnt3a plays a double role in the segmentation process: it forms a morphogen gradient and drives an oscillator, which together define where the position of the segment boundary, the separation between neighboring cellblocks, is set. The oscillator is defined by the cyclic activity of three interacting signal pathways, WNT, FGF and Notch. Though many components of and interactions between these pathways have been described, there is still debate about the molecular mechanisms controlling the segmentation process. It is not known what is the pacemaker of the oscillator nor what keeps neighboring cells synchronized. We work on these fundamental questions. We have cooperated with Christoph Wierling and Ralf Herwig from the Lehrach department to model the oscillator in the computer, further improving our understanding of the crosstalk between signaling cascades involved in the oscillator.



*Cyclic gene expression in the presomitic mesoderm as readout of the segmentation clock. **(A)** Whole mount in situ hybridization analysis of mouse embryos at 9.5 days of development with probes for Lunatic fringe (Lfng) and Dickkopf related 1 (Dkk1). The expression of the Notch dependent gene Lfng and of the Wnt dependent gene Dkk1 show dynamic and alternating activity in the presomitic mesoderm. **(B)** Expression levels of Wnt (red and orange line) and Notch target genes (blue lines) demonstrate that both signalling pathways establish alternating oscillations of target gene activity over one segmentation cycle. **(C)** Cluster analysis of the expression profiles of 17 staged tissue samples comprising one segmentation cycle; "Wnt-on" and "Notch-on" phases of the segmentation cycle become apparent, thus allowing the identification of novel components of the oscillator.*

General information

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EW Deutsch, CA Ball, JJ Berman, GS Bova, A Brazma, RE Bumgarner, D Campbell, HC Causton, J Christiansen, F Daian, D Dauga, DR Davidson, G Gimenez, YA Goo, S Grimmond, T Henrich, BG Herrmann, MH Johnson, M Korb, JC Mills, AJ Oudes, HE Parkinson, LE Pascal, N Pollet, J Quackenbush, M Ramialison, M Ringwald, D Salgado, SA Sansone, G Sherlock, CJ Stoeckert, Jr., J Swedlow, RC Taylor, L Walashek, A Warford, DG Wilkinson, Y Zhou, LI Zon, AY Liu, LD True (2008). *Minimum Information Specification For In Situ Hybridization and Immunohistochemistry Experiments (MISFISHIE)*. Nature Biotechnology 26, 305-312

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Selected memberships

B.G. Herrmann is elected member of EMBO

Work as scientific referee

B.G. Herrmann serves as referee for the following journals: Biomed Central Developmental Cell, Developmental Dynamics, Development Genes and Evolution, FEBS Journal, Genes & Development, Genetical Research Cambridge, Genome Biology, Mechanisms of Development, MoD Gene Expression Patterns, Nucleic Acids Research.

Membership in journal editorial boards

- Development Genes & Evolution (co-editor until 2009)
- GENES (Open Access Journal)

Service to scientific community

B.G. Herrmann serves as referee for the following Science Organizations and Institutions: Deutsche Forschungsgemeinschaft (DFG), EMBO, Eidgenössische Technische Hochschule Zürich (ETH), GEN-AU (Austrian Genome Research Program), RIKEN, Swiss National Science Foundation, The Wellcome Trust

Spin-offs

B.G. Herrmann is Co-Founder of ALACRIS Pharmaceutical GmbH (founded in Berlin 2008)

External Funding

BMBF, Rare Diseases: *CURE-Net,* 02/2009-01/2012 (Wittler/Herrmann)

BMBF, NGFNplus: *Modifier SP2,* 06/2008 - 05/2011 (Herrmann)

BMBF, NGFN-2: *SMP-RNAi*, 01/2005 -07/2008 (Herrmann)

BMBF, NGFN-2: *SMP-DNA*, 11/2004-05/2008 (Herrmann)

BMBF, NGFN-2: *SMP-RNA,* 11/2004 -05/2008 (Herrmann)

Organisation of scientific events

Day of science, MPI for Molecular Genetics: Grote, Schwartz

Public relations

Bernhard Herrmann: interviews and expert information for Deutschlandfunk, Süddeutsche Zeitung, Wirtschaftswoche, interviews for TV

Lange Nacht der Wissenschaft (Long Night of Sciences): Introduction to vertebrate embryology, organized by Wittler and Spörle, supported by many members of the department; since 2005

Hosting the artist Gaby Schulze collaborating with the MPIMG (with Dr. Urban), Spörle 2005-2009

Advanced Training for Teachers: MNU Congress 2009, Berlin (talk by H. Bauer)

Hosting School Classes: *A Day in the Lab*; Bauer, Wittler, Schrewe 2007, 2008, 2009

Signal Transduction in Embryogenesis and Tumour Progression

(Established: 05/2005)

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Phillip Grote* (since 10/08)
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PhD Students
Marc Leushacke* (since 11/05)
Joana Vidigal* (since 02/07)
Benedikt Schwartz* (since 10/08)

Introduction

In embryonic development, signalling networks interact in a controlled manner to specify cell fates. During tumour formation and progression, oncogenic mutations perturb such signals. As a consequence, the balance between stemness, proliferation, differentiation and apoptosis is lost in the tumour. The mouse is an excellent model to study vertebrate development and disease, due to the availability of inbred and consomic strains, a complete and annotated genome sequence, and the ability to modify the genome by transgenic techniques. In our projects, we expand the toolkit of mouse genetics and genomics and apply innovative methods to study tumour initiation and progression in the mouse.

Scientific Overview

Inducible and tissue-specific transgenesis and RNAi in the mouse

In this project, we have developed an integrated system for mouse transgenesis, consisting of a tetracycline-dependent on/off switch and a cassette for recombinase-mediated cassette exchange (RMCE) in a single genomic locus. We have employed the system for the integration of transgene and RNA interference cassettes, which subsequently can be expressed under the control of doxycycline. We have

* externally funded

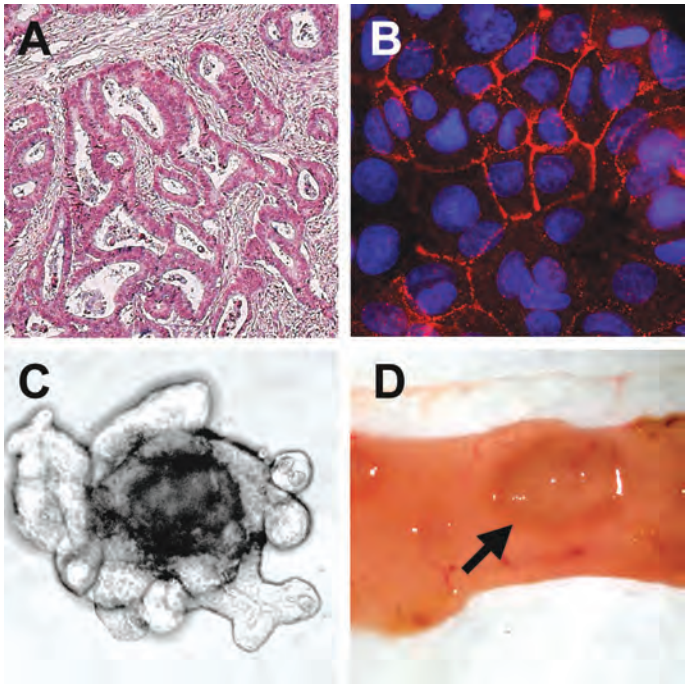
generated mice from embryonic stem cells carrying the transgene system and demonstrated inducibility and efficacy of RNA interference *in vivo*, with minimal off-target effects.
Importantly, the modular nature of the single-locus RMCE transgene system will allow a wide array of applications, including general or tissue-specific transgene overexpression and RNA interference in the embryo and in the adult mouse. Mouse models based on this integrated vector system have several advantages over conventional transgenics, such as transgene insertion lines or conditional knock-out mouse lines: the use of tissue-specific promoters and/or an inducible tet-system allows a high degree of control of transgene expression. In addition, transgenic mice can be directly derived from transgenic embryonic stem cells, providing mouse models with a uniform genetic background. Finally, crossing of transgenic mice will generate offspring with a high percentage (50%) of transgenic animals, unlike other complex genetic systems that rely on several transgenes in different genomic locations.

Systematic analysis of oncogenes in the mouse (Mutanom)

High-throughput sequencing approaches have recently isolated a plethora of mutations in tumour genomes. Functional studies however have mainly focussed on a few of these mutations up to now. The NGFNplus project Mutanom aims at the systematic functional characterisation of somatic mutations in breast, prostate and intestinal cancer cells.
We complement this systematic approach in cancer cell biology by analysing key mutations in tumour-prone mice, using the RMCE transgene system for the expression of mutated oncogenes in the intestine and the mammary gland. The possibility of systematic *in vivo* analysis of the roles of selected oncogenic mutations in the mouse will likely unravel important aspects of tumour biology that cannot be assessed in cell culture or in retrospective analysis of human tumour samples.

Modifiers of intestinal tumour formation and progression

The individual genetic background is known to have a major effect on the lifetime risk of developing cancer, and on cancer progression. The genetics of cancer susceptibility are however complex (polygenic), and thus it is almost impossible to assess the influence of the individual genetic background of humans on the lifetime risk of developing this disease.
We use two powerful new tools for genetic analysis, B6/PWD chromosome substitution strains and ultrahigh-throughput sequencing (Solexa) to isolate genetic traits that affect intestinal cancer initiation and progression in the APC^{Min} mouse, which is an established model for human intestinal cancer. In the first phase of the project, we have



Experimental options for the analysis of intestinal tumour initiation and progression. ***(A)*** *Retrospective analysis of tumour tissue. A section of human metastatic colon carcinoma is shown;* ***(B)*** *Cell culture. Photo shows immunofluorescence of the cell adhesion molecule E-cadherin in SW480 colon carcinoma cells.* ***(C)*** *Intestinal crypt culture established from untransformed mouse intestine.* ***(D)*** *Mouse tumour models. Arrow points to adenoma in the intestine of a* APC^{Min} *mouse.*

found that PWD genomic sequences protect APC^{Min} mice from intestinal cancer. We have subsequently mapped multiple tumour modifiers to individual mouse chromosomes and sub-chromosomal regions. In co-operation with the Department of Vertebrate Genomics (Lehrach) the intestinal tumour transcriptome and epigenome of mice carrying modifiers is analysed. Our newly developed system for RMCE will facilitate the generation of mouse models with an uniform genetic background for validation of modifier candidate genes. Ultimately, such research will allow to define genetic networks of tumour susceptibility and progression, and thus, to define high- and low-risk groups among colon cancer patients.

A loss of function phenotypic screen for EMT and metastasis genes in intestinal tumour cells

Key signalling pathways, such as Wnt, Fgf and Tgf-beta signalling pathways instruct cells in the embryo to execute specific developmental programs and aquire appropriate phenotypes. One key phenotypic switch during embryonic development is epithelial-to-mesenchymal transition (EMT): cells that undergo EMT lose apical-basal polarity, re-organize their cytoskeleton, down-regulate cell adhesion, and become motile. Tumour progression to metastasis involves EMT-like changes in tumour cell morphology and involves similar signalling pathways and key molecules.

We have utilized a loss of function phenotypic screen to assess the effect of approx. 400 EMT candidate genes from the embryo on SW480 intestinal tumour cells. Our esiRNA-based approach allowed for the first time to screen a large number of EMT candidate genes in a simple functional assay. Multiple genes involved in tumour progression were isolated, highlighting the similarity between EMT in embryogenesis and tumour metastasis. We currently focus on the role of Fgf signals in intestinal cell biology and have delineated signalling cascades and downstream target genes of Fgf in the intestine, using tumour cell lines and primary organ culture. We have found an essential role for Fgf signals for the maintenance of a mesenchmal, motile phenotype in intestinal tumour cells.

Perspectives

Our work focusses on intestinal tumour initiation and progression, combining modern high throughput methods with innovative mouse models. Over the last years, we have established or gained access to a wide array of resources, such as *in vivo* RNAi and tissue-specific transgene systems in the mouse, an exhaustive library of oncogenes, second generation sequencing methods, and intestinal crypt culture. We are therefore well equipped to elucidate signalling networks that control cell homeostasis, proliferation and differentiation in the normal and transformed intestine.

General Information

Selected publications

Fritzmann J., Morkel M., Besser D., Budczies J., Kosel F., Brembeck F.H., Stein U., Fichtner I., Schlag P.M., Birchmeier W.: *A colorectal cancer expression profile that includes transforming growth factor beta inhibitor BAMBI predicts metastatic potential*. Gastroenterology 2009 Jul; 137(1):165-175. (M. Morkel is equally contributing principal author)

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Morkel M., Huelsken J., Wakamiya M., Ding J., van de Wetering M., Clevers H., Taketo M.M., Behringer R.R., Shen M.M., Birchmeier W.: *Beta-catenin regulates Cripto- and Wnt3-dependent gene expression programs in mouse axis and mesoderm formation*. Development 2003 Dec;130(25):6283-6289.

Work as scientific referee

M. Morkel serves as scientific referee for the International Journal of Cancer.

External funding

BMBF, NGFNplus: *Modifier SP 5,* 06/2008 - 05/2011 (Morkel)

BMBF, NGFNplus: *Mutanom,* 06/2008-05/2011 (Morkel/Herrmann)

Fundacao para a Ciencia e Tecnologia (PhD grant to Joana de Campos Alves Vidigal)

Organogenesis

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Pedro Veliça (Marie Curie Fellow, University of Birmingham, UK; 10/2006 – 4/2007)
Sebastiano Battaglia (Marie Curie Fellow, University of Birmingham, UK; 7/2008 – 4/2009)

Technician
Manuela Scholze*

Scientific overview

Organogenesis is the process by which complex and highly specialized structures develop from a small population of undifferentiated embryonic cells. The understanding of the role of signaling pathways, specific and general transcriptional regulators, and cellular interactions in cell proliferation, cell differentiation, cell migration, and development of organs is important for understanding tissue regeneration and repair, and ultimately for growing organs in culture.

Functional analysis of Slit-like 2 (Slitl2) in organogenesis

The Slitl2 protein is a typical single-pass type I transmembrane protein. Its extracellular amino-terminal end contains a putative hydrophobic signal peptide, one leucine-rich repeat (LRR) region comprising ten leucine-rich repeats flanked by an amino- and a carboxy-terminal LRR motif, one epidermal growth factor (EGF) repeat, and a fibronectin type III (FNIII) domain. It shows structural similarities to Slit, a secreted cell guidance molecule, isolated and characterized first in *Drosophila*. It was shown that the human Slitl2-homologue Vasorin can bind to TGF-β1 and negatively modulate TGF-β signaling. We generated a comprehensive temporal and spatial expression pattern by describing not only endogenous *Slitl2* expression, but also reporter gene expression in *Slitl2-LacZ* knock-in and in *Slitl2-Venus*-BAC transgenic mice. These three approaches produced consistent results and

* externally funded

revealed defined expression from early embryonic stages onwards, suggesting an essential role for Slitl2 in the mouse. And indeed, *Slitl2*-deficient mice, generated *via* classical gene targeting in ES cells, die within 3-4 weeks after birth. We are currently investigating the molecular basis and identifying the pathways leading to the early postnatal death.

Role of Med12 in mouse development

The Mediator complex is commonly seen as a molecular bridge that confers information from transcription factors bound to regulatory regions of genes to the RNA polymerase II machinery assembled on the promoter of these genes. It is a large complex of 30 subunits, some of which seem to play a structural role in the complex and are therefore generally required for transcription. Other subunits, however, are thought to be responsible for specific interactions with defined transcription factors. Med12 is ubiquitously expressed in the embryo (Fig. 1A, B) and in adult tissues (Fig. 1C). The ability of Med12 to bind β-catenin suggests a role in Wnt signaling. Sox9, Gli3, and Sox32 are other factors that have been shown to bind to Med12, and the activation of their target genes is dependent on this interaction. We have targeted the *Med12* gene on the X chromosome in male mouse embryonic stem (ES) cells and generated two *Med12*-mutant alleles, namely $Med12^{flox\text{-}neo}$, a hypomorphic allele, and the conditional $Med12^{flox}$, which behaves as a wild-type allele. Embryos derived from hypomorphic *Med12* mutant ES cells by tetraploid aggregation die around embryonic day 10.5 (E10.5) and have a drastic reduction in Med12 protein levels. We are currently characterizing its function during mouse development and its interaction with other transcription factors like Sox9, and Gli3, that use Med12 as their anchor to the Mediator complex.

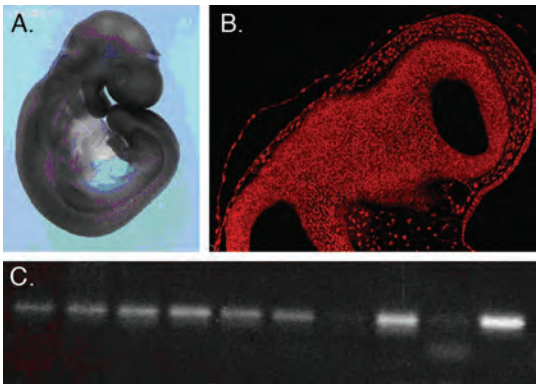

Figure 1: Expression of Med12 in embryonic and adult tissues. ***(A)*** *Mouse E9.5 embryo with Med12 expression detected by whole-mount in situ hybridization (source: MAMEP database).* ***(B)*** *Nuclear Med12 expression in the head region of a E9.5 mouse embryo visualized by fluorescent immunohistochemistry using a α-Med12-specific antibody.* ***(C)*** *RT-PCR shows ubiquitous expression of Med12 in adult mouse tissues (from left: ovary, spleen, small intestine, large intestine, liver, brain, heart, kidney, lung, skin).*

Perspectives

We will continue our studies of *Slitl2* and *Med12* mutants by expression profiling and marker gene expression analyses. Microarray data derived by comparing wild-type and mutant expression profiles will be verified by whole-mount *in situ* hybridization and immunohistochemical techniques. Tissue-specific mutations will be generated by crossing the conditional mutant mice with various Cre recombinase-expressing mouse lines. Resulting tissue-specific mutant embryos and mice will be characterized using the above mentioned techniques.

General information

Selected publications

Birtwistle, J., Hayden, R.E., Khanim, F.L., Green, R.M., Pearce, C., Davies, N.J., Wake, N., Schrewe, H., Ride, J.P., Chipman, J.K. & Bunce, C.M. (2009). The aldo-keto reductase AKR1C3 contributes to 7,12-dimethylbenz(a)anthracene-3,4-dihydrodiol mediated oxidative DNA damage in myeloid cells: implications for leukemogenesis. Mutat. Res. 662, 67-74.

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Awards

P. Rocha: *Mercy Speer Award for best talk at 6th International Conference on Neural Tube Defects*, 2009

External funding

EC, NucSys, Marie Curie Research Training Network, 01/2006 - 12/2009

Medical Research Council, UK, 10/2004 - 09/2007

Work as scientific referee

H. Schrewe serves as referee for the following journals: Journal of Cell Sciences, PLoS ONE, and MoD Gene Expression Patterns.

Organization of scientific events

6. International NucSys Meeting in Berlin (June 20 - 22, 2008) (organizer: H. Schrewe)

General information about the whole Department

Complete list of publications (2006-2009)

2009

Birtwistle, J., Hayden, R.E., Khanim, F.L., Green, R.M., Pearce, C., Davies, N.J., Wake, N., Schrewe, H., Ride, J.P., Chipman, J.K. & Bunce, C.M. (2009). The aldo-keto reductase AKR1C3 contributes to 7,12-dimethyl-benz(a)anthracene-3,4-dihydrodiol mediated oxidative DNA damage in myeloid cells: implications for leukemogenesis. Mutat. Res. 662, 67-74.

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PhD theses

2009

Michaela Mayer: *Functional analysis on the role of Slit-like 2 (Slitl2) in the mouse.* PhD Thesis, Freie Universität Berlin, 10/2009 (supervisor: Heiner Schrewe)

2008

Solveig Müller: *Funktionelle Charakterisierung regulatorischer Gene bei der Bildung der Wirbelsäule der Maus*. PhD Thesis, Freie Universität Berlin, 09/2008 (supervisor: Bernhard Herrmann)

Nathalie Véron: *Untersuchungen zu den molekularen Grundlagen der nichtmendelschen Vererbung in der Maus*. PhD Thesis, Freie Universität Berlin, 12/2008 (supervisor: Bernhard Herrmann and Hermann Bauer)

Student theses

2009

Oliver Sedelmeier: *Systematic analysis of Mettl-gene expression during mouse embryo development*. BSc Thesis, Beuth Hochschule für Technik, Berlin, 09/2009 (supervisor: Philipp Grote)

Judith Proske: *Molekulare Analyse von Genen, die eine mögliche Funktion in der Ausbildung von urorektalen Fehlbildungen während der Säugerembryogenese haben*. Diploma Thesis, Univ. Osnabrück, 2009 (supervisor: Lars Wittler)

2008

Sabrina Schindler: *Etablierung von micro RNA-Konstrukten zur Genexpressionskontrolle von Smok1, Tagap1 und Fgd2*. Diploma Thesis, Technische Fachhochschule Berlin, 2008 (supervisor: Hermann Bauer)

Susanna Sluka: *Analysis of intestinal tumor initiation and progression in APCmin/chromosome substitution strain C3, C12 and C17 mice*. Master Thesis, Humboldt University / Charité - Universitätsmedizin Berlin, 11/2008 (supervisor: Markus Morkel)

2007

Christof Bernemann: *Beta-Catenin/APC kontrolliert die Expression von Kandidatengenen für epithelial-mesenchymale Transition in der intestinalen Karzinogenese.* Diploma Thesis, Freie Universität Berlin, 04/2007 (supervisor: Markus Morkel)

Ruben Schilling: *Elastic registration of 3D volume data*, Diploma Thesis, OPT-3D-registration, University of Freiburg, 2007 (supervisor: Alexander Schliep, Dept. of Computational Molecular Biology, together with Ralf Spörle)

2006

Michael Plötz: *Analyse zur Charakterisierung des Latrophilin-2 Knock-outs*. Diploma Thesis, Freie Universität Berlin, 2006 (supervisor: Heiner Schrewe)

Eun-Ha Shin: *Funktionelle Analyse der Genregulation während der Somitogenese des Wirbeltierembryos*. Diploma Thesis, Institut für Biotechnologie, Technical University Berlin, 09/2006 (supervisor: Bernhard Herrmann)

Teaching activities

Teaching activities at the Charité-Universitätsmedizin Berlin

Preclinical Course: Biology for medical students (each semester term since 2004); Organizer: Schrewe; Tutors: Spörle, Wittler, Bauer, Morkel, Müller, Mayer, Schindler, Shin, Nandy, Proske

Master's Programme: Molecular Medicine (module 3, Developmental Genetics; yearly 3 weeks course since 2006); Organizer: Wittler; Tutors: Bauer, Morkel, Spörle, Neidhardt

Weekend Workshop: Human Genetics (Humangenetik), Charité (since 2006). Tutors: Spörle, Wittler, Grote, Schrewe

Teaching activities at other universities

Wittler: Design of the Module *Developmental Biology of the Vertebrates* as part of the Masters Programme in Molecular Life Sciences, Humboldt University of Berlin; including lecture *Organization of the body axes in the vertebrate embryo*, seminar *animal models in developmental biology* and course *molecular embryology*, summer term 2009

Schrewe: Practical Course: *Developmental Genetics* at the School of Biosciences, University of Birmingham, UK (annually since 2004)

Schrewe: Practical Course: *Embryonic Stem Cell Culture* at the Max-Planck Institute for Molecular Genetics, Berlin (6/2007 and 6/2008)

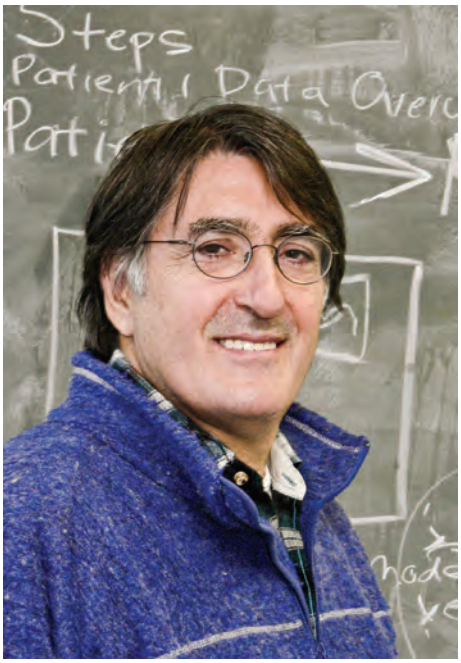
Teaching activities at the MPIMG

PhD Programme, Course 3: *Developmental Genetics* (1 week) Tutors: Morkel, Bauer, Schrewe, Grote, Wittler

Literature Tutorial for Students of the Dept. Develop. Genetics (bi-weekly since 1/2009) Tutors: Grote, Wittler, Morkel

Department of Vertebrate Genomics

(Established: 09/1994)



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Group leaders of the Department
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Dr. Marie-Laure Yaspo (since 96)
Dr. Wilfried Nietfeld (since 10/98)
Dr. Harald Seitz (since 05/00)
Dr. Ralf Herwig (since 02/01)
Dr. Silke Sperling (since 01)
Dr. Margret Hoehe (since 02)
Dr. Zoltán Konthur (since 05/02)
Dr. Bodo Lange (since 05/03)
Dr. James Adjaye (since 03)
Dr. Thorsten Mielke (since 04)
Dr. Georgia Panopoulou (since 03)
Dr. Albert Poustka (since 03)
Dr. Michal Schweiger (since 01/07)
Dr. Alexey Soldatov (since 06/07)
Dr. Andreas Dahl (since 07/07)
Dr. Markus Ralser (since 12/07)
Dr. Jörn Glökler (since 02/08)
Dr. Lars Bertram (since 10/08)
Dr. Christoph Wierling (since 01/09)
Dr. Steffen Hennig (until 10/06)
Dr. Edda Klipp (until 12/06)
Dr. Johan Gobom (until 09/07)
Dr. Claus Hultschig (until 01/07)
Dr. Konrad Büssow (until 02/07)
Dr. Lajos Nyarsik (until 03/07)
Dr. Sascha Sauer (until 07)
Dr. Sylvia Krobitsch (until 08/08)
Dr. Ralf Sudbrak (until 03/08)
Dr. Jürgen Kreutzberger (until 05/08)
Dr. Michal Janitz (until 12/08)
Dr. Eckhard Nordhoff (until 04/09)

Introduction

Structure of the department

The structure of the Department of Vertebrate Genomics, consisting of 19 groups funded to a very large extent (approximately 85%) by outside grants, is owed to different circumstances.

The area of functional genomics and systems biology is dominated by few, very well funded institutes (Sanger Centre, Broad Institute, Washington University, Peking genome centre etc.), able to invest a very large amount of resources either from core funding or from very large grants. Since the core funding of the depart-

ment is, by far, not sufficient to be able to compete on an international level in this research area, we have, from the beginning, been forced to fund most of our work, as well as the infrastructure necessary, from grants. This can be illustrated well by the situation in next generation sequencing (NGS). We have become, as an institute, with currently 14 NGS sequencing systems, the second largest sequencing centre in Europe. In contrast to the situation at most of the other centres, almost all of these machines have had to be financed through grants, or made available through other means (instrument loans etc.). Grants are therefore for us not an 'icing on the cake', but a central requirement to remain competitive in one of the most central and fastest moving research areas of biology, with enormous implications for society. In the current situation, without a large component of grant funded research, we would simply not be able to carry out much of our 'core' research.

Since almost all groups in the department are nearly completely funded through outside grants, this conversely offers a chance to many young scientists, to develop their own, essentially independent group directly after their Ph.D. (e.g. Markus Ralser, Georgia Panopoulou, Albert Poustka, Zoltán Konthur, Sascha Sauer, Ralf Herwig, Christoph Wierling, Andreas Dahl, Konrad Büssow, etc.), after a postdoctoral position (e.g. Marie-Laure Yaspo, Edda Klipp, Bodo Lange, Sylvia Krobitsch, Silke Sperling) or after returning to Germany from a different system (e.g. Lars Bertram). This has created an environment, which is much more similar to American universities (especially medical schools) than usual in Germany, has given many young scientists a chance to become independent much earlier than usual and has helped to develop research, which would not have been happened otherwise.

Research concepts

We can consider life as computational processes, translating the linear information in the genome into the phenotype of the organism in a given environment. To be able to predict the effect of specific changes in these processes, in spite of their daunting complexity, would have immense practical implications, since disturbances in the complex networks involved are likely to be the cause of most or all human diseases, with therapies acting to restore, as much as possible, the original, 'healthy' state. We have therefore attempted to combine tools of genetics, genomics, and systems biology to generate predictive models of the response of complex biological networks, from basic biological processes (e.g. steps in development) to medically important problems (disease processes/therapy response in cancer, neurodegenerative diseases etc.), combining an exhaustive characterisation of the biological system by deep sequencing and other techniques, with pathway information generated by decades of classical research, as well as by new genetic approaches (e.g. the use of chromosome substitution strains). Such predictive models could play an important role in improving our understanding of biology in general, but could also be a key to the development of a new individualised medicine, as well as a generation of new more targeted therapies. This will require improvements in three main areas:

1) Our knowledge of the structure of the networks by a combination of genetics and functional genomics.
2) A detailed analysis of the biology of individuals, e.g. by deep sequencing of genomes and transcriptomes of patient samples.
3) The development of predictive models of biological mechanisms combining known information on relevant pathways with the detailed analysis of the biological networks in the specific individual.

Scientific achievements

Technology development

Predictive models of complex networks require a detailed knowledge of the relevant components specified by the genome, and expressed in a specific state. The rapid improvements in DNA sequencing techniques provided by the NGS systems are a key development in this direction. We continue our efforts in sequencing technology development to optimise the use of the new powerful commercial sequencing systems (Roche 454, Illumina GAII, ABI SOLiD, Danaher Polonator); testing of ligation-based and bead-based sequencing strategies, the development of multiplexing, strand specific RNA sequencing (Soldatov), and work on enrichment protocols (Dahl, Schweiger). The open source sequencing platform Polonator offers us the possibility to test alternative sequencing chemistries as well as novel approaches in single cell analysis (Dahl). Strategic advances in automation and miniaturization (Dahl) can feed into NGS developments, and allow the development of binding reagents with broad applications (Glökler), such as highly affine nucleic acids – Aptamers - able to bind other molecules by the key-lock principle through formation of a sequence-dependent three dimensional structure selected by SELEX (Systematic Evolution of Ligands by EXponential Enrichment). Harald Seitz' group established the combination of rolling circle amplification and enzymatic active nucleic acids as sensitive and simple detection tool.

Beyond sequencing genomes and transcriptomes of multiple organisms, the next steps towards understanding biological processes require the characterization of the protein composition in a given cell, protein function and protein protein-interactions. The department has been very active in this area, e.g. in the development of an automated platform for two-hybrid screens (Wanker), of phagemid selection for antibody libraries (Konthur) and of protein arrays (Büssow, Cahill, Kreutzberger, Seitz). Reverse phase protein microarrays are used for protein quantification (Seitz). Combining phagemid selection with the two-hybrid system has resulted in the development of 'intrabodies', new antibody-derived reagents to selectively interfere with protein-protein interactions in the cell (Krobitsch, Konthur).

Understanding protein function requires knowledge of the structure of a protein in its functional states. Key processes involve large protein complexes, such as the multi-enzyme ribosome and RNA polymerases complexes, whose structure and composition has been analysed systematically, combining mass spectrometry (Gobom, Nordhoff, Lange), and Cryo-Electron microscopy using single-particle approaches (Mielke). Proteomics technology up to now based predominantly on MALDI systems (Gobom, Nordhoff), is now complemented by new ORBITRAP and QTRAP5500 instruments (Sauer, Ralser). The QTRAP5500 will also allow in house metabolite analyses, relevant to several projects (Ralser) and systems biology in general.

In the analysis of gene function within living cells, we have established the cell array technology, providing new ways to test e.g. protein-protein interactions, or promoter function (Janitz, Nietfeld, Yaspo).

Bodo Lange's group has set up an experimental system that combines cell biological, functional genomics and proteomics approaches to study the regulation of cell proliferation pathways in human cells and *Drosophila*. This has identified and functionally characterised the proteome of the *Drosophila* mitotic centrosome, and has contributed to the analysis of protein complexes and signalling pathways relevant for human cancer progression.

Genetics

The department has a long tradition in the application of genetic techniques, back to early work at the roots of modern mouse and human genetics in the early eighties. Genetic analyses have now become even more powerful, uniting the power of genetics, with the enormous capabilities of NGS and other high-throughput techniques. We are currently involved in the mapping and characterization of complex disease loci, e.g. Alzheimer's disease (AD) and schizophrenia (Bertram). A recent genome-wide association study highlighting a number of previously unrecognized novel loci relevant to AD is currently extended by systematic screening of all coding regions based on NGS. The group of Sylvia Krobitsch is involved in the identification of molecular mechanisms contributing to neurodegenerative processes in AD and the polyglutamine disorder Spinocerebellar Ataxia type 2, by combining yeast genetics/models, functional genomics and systems biology approaches, as well as the use of yeast genetics to explore the mechanisms of action of human anticancer drugs. Similarly, the analysis of comprehensive yeast knockout libraries in combination with NGS is used to identify genes modifying the effects of anti-ageing compounds (Ralser). Both groups focused on the dynamic transition in the carbohydrate catabolism, showing that the re-direction of the glycolytic flux from glycolysis to the pentose phosphate pathway is crucial for the adaptation to oxidative stress. *C. elegans* was instrumental to analyse the genetics of ageing (Gerisch, in collaboration with Adam Antebi, Houston/Köln).

To analyse regulatory networks controlling different phenotypic traits in the mouse, we collaborate with the group of Jiri Forejt (Prag) to analyse a set of chromosome substitution strains (consomics) constructed by transferring individual chromosomes from the *Mus musculus musculus* inbred strain PWD into C57BL/6J. Expression phenotypes are identified by analysing the brain transcriptomes by NGS (Yaspo, Soldatov), as well as components of the proteome by 2DE gels (Joachim Klose, Charité). In collaboration with Bernhard Herrmann's department, we have demonstrated the presence of modifiers of the frequency of forming intestinal tumours, as well as epigenetic and transcriptional changes in crosses of C57BL/6J carrying an APC mutation with different B6/PWD consomic strains. Similarly, in collaboration with Kurt Zatloukal (Graz), we are analysing consomics of both the B6/AJ series (Joe Nadeau), and B6/PWD for mapping factors modulating steatohepatitis susceptibility (Yaspo).

Genomics

Large scale sequencing

As one of the centres participating in the sequencing of the human genome, the department has a long track record in large scale sequencing projects, and follow up projects (e.g. chromosome 21, parts of chromosome X in chimpanzee; Yaspo). We also participate, as the only centre in continental Europe, in the 1000 genomes project (Soldatov, Herwig, coop. with Martin Vingron). A proposal to contribute to the analysis of the genomes and transcriptomes of specific childhood tumours as part of the international cancer genome consortium (ICGC) is currently under review (Yaspo, Herwig). In addition, we are carrying out genome and/or transcriptome sequencing of cancer samples as part of NGFN, EU and an IMGuS project (Schweiger) and have started a new project, Treat1000 (www.treat1000.org) to use a combination of deep sequencing and systems biology to improve and individualise the treatment of cancer patients (Yaspo, Schweiger, Dahl, Soldatov, Herwig, Wierling, Regenbrecht, Timmermann, in coop with the Charité Comprehensive Cancer Centre and George Church, Harvard Medical School).

The group headed by Margret Hoehe focuses on genetic variation and its structure. Lines of research have included deep medical re-sequencing and haplotype-based approaches to complex disease. To that aim, a unique haploid reference

resource (100 fosmid libraries) and a next generation sequencing platform have been established, tackling highly variable genomic regions (MHC) and generating haploid sequences on a broader scale.
The group of Heinz Himmelbauer has developed the first program allowing the assembly of sequence contigs *de novo* from Solexa reads with high accuracy. His group was involved in comparative analyses of the mammalian MHC as well as in sequencing the genome of *Beta vulgaris* (coop. between the Centre for Genomic Regulation (Barcelona) and the MPIMG).

Transcriptome analysis/gene regulatory networks

NGS has become the core technology for the analysis of digital transcription profiles, providing quantitative information on expression levels, alternative splicing, allele specific expression pattern and RNA editing (Yaspo). However, cellular resolution of gene expression within organisms requires automated high resolution *in situ* hybridisation techniques. In EURExpress, we have established the pipeline and generated more than 3,000 patterns at E14.5 (EURExpress, Yaspo, coop. with Stefan Mundlos) contributing to the completed ISH atlas of 18,000 mouse genes. To improve our understanding of regulatory networks, the group of Marie-Laure Yaspo has been combining information on transcription factor binding sites (ChIP-Seq), promoter function (cell arrays) and expression profiles for transcription factor RNAi knockouts, complementing the mouse genetics approaches described above.

Informatics

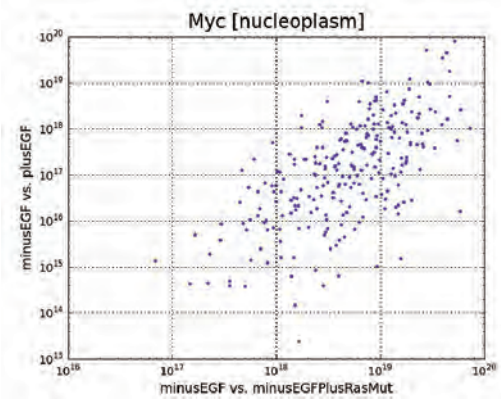
The enormous NGS throughput has prompted the establishment of a significant bioinformatics infrastructure, supported by national and international funding. This includes a network of high-performance computers with > 350 CPUs, a storage capacity of ca. 300 Tbyte, a software environment that allows high standard quality control, mapping, visualisation and follow-up data analyses (Herwig, Soldatov). We have developed a number of new techniques to display and analyse NGS data (Herwig, Soldatov), including a new method, ARH - *alternative splicing robust prediction by entropy*, for the prediction of alternative splicing events from microarray and NGS data. In addition, we developed biological databases, integrating a wide range of data types, such as proteomics, transcriptomics, patient data, genotype data, networks, and computational models (e.g. ConsensusPathDB, DIPSBC) and have applied these methods in the analysis of mutations and identification of disease markers in cancer and other diseases in various collaborative projects (Herwig).

Systems Biology

Over the last ten years, systems biology (SB) approaches have been developed in the department, including novel concepts and tools for the mathematical modeling of biological networks relevant to development and diseases (PyBioS – a modeling and simulation system, GeNGe – an application for the generation and analysis of gene regulatory networks) (Herwig, Wierling).

Systems biology of evolution and development

James Adjaye's group analyses self-renewal in human embryonic stem cells, in particular, iPS cells resembling human ES cells, which can be derived from somatic cells of every individual, in the aim to in-



Investigation of the effects of a Ras mutation via the Monte Carlo approach. The in silico analysis via the Monte Carlo approach shows that a Ras mutation leads to an increased level of the transcription factor c-Myc used as read-out for cell proliferation. The effect of a Ras mutation on c-Myc level is comparable with an activation of the EGF signalling pathway. Each dot represents a single Monte Carlo run.

crease our meagre understanding of the mechanisms underlying cellular reprogramming and to develop techniques to derive patient-specific iPS cells allowing the derivation of tissue-matched differentiated donor cells for therapy, for research on the pathogenesis of complex diseases, for toxicology studies and for drug screening. In collaboration with clinicians iPS cells are generated from Alzheimer's Disease, Type 1 Diabetes, Nijmegen Breakage Syndrome and Steatosis patients.
The group of Georgia Panopoulou and Albert Poustka has been working on the evolution of genome organisation in vertebrate and invertebrate species, e.g. by studying gene and genome duplications, that increase the genetic toolkit of an organism and possibly promote speciation. In particular, gene regulatory networks active in early development in different deuterostomes (e.g. sea-urchin) are analysed and modelled using SB techniques (Poustka/Panopoulou, Wierling).

Systems biology of cardiovascular diseases

The group of Silke Sperling addresses cardiac development by analysing the interplay between transcription factors, co-regulatory elements and epigenetic factors. The group has performed molecular studies and computational developments to gain insights into the transcription networks underlying myogenesis and congenital heart disease. Transcription networks were predicted based on the integration of clinical, phenotypic and molecular data.

Systems biology of autoimmune diseases

The group of Zoltán Konthur has focussed on the application of *in vitro* selection techniques such as phage display and protein array technology for identifying and characterising antibody – protein interaction pairs, with a focus on autoimmunity, including systemic and organ-specific autoimmune diseases. Screening for putative auto-antigens is performed using cDNA expression libraries, patient-derived immunoglobulin fractions as selection targets and NGS. Autoantigene profiling for multiple sclerosis and AD, or therapy response prediction in systemic autoimmune disorders are being performed.

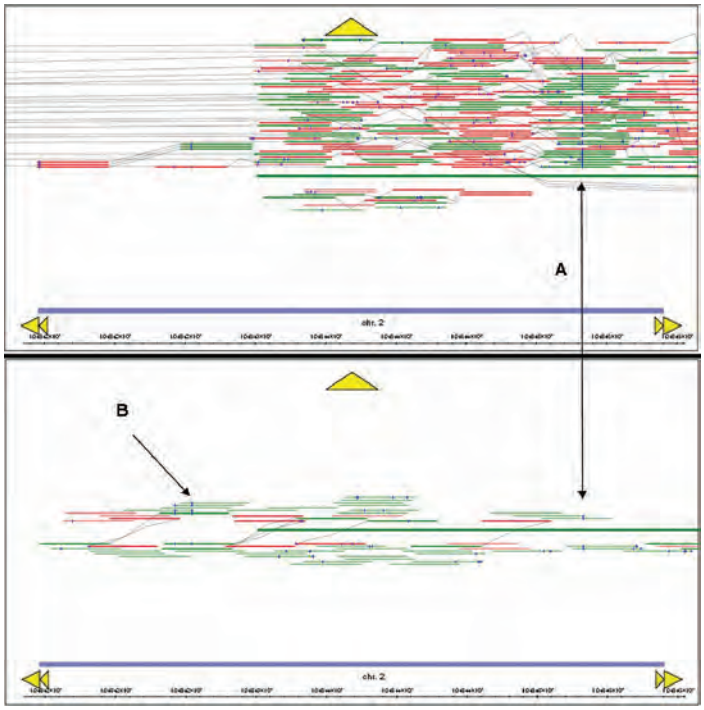
Systems biology of neurodegenerative disorders

AD, Parkinson's disease, or polyglutamine related diseases represent leading causes of disability and death in the human population. The mechanisms are still poorly understood and effective preventive therapies are currently not at hand. Sylvia Krobitsch and Lars Bertram are involved in analyses of molecular mechanisms contributing to neurodegenerative processes (see above). As part of NeuroNet, Bodo Lange's group is analysing the protein interaction networks implicated in the development of neurodegenerative diseases. Wilfried Nietfeld's group has carried out analyses of expression patterns in lymphoblastoid cell lines obtained from AD patients, Parkinson's disease, Amyotrophic Lateral Sclerosis, Spinocerebellar Ataxia and Huntington's disease, and has analysed molecular mechanisms of neuroprotection in cerebral ischemia.

Systems biology of cancer

In spite of massive investments into cancer research, and clear progress in the treatment of some forms of cancer, the survival rates in many of the most common forms of cancer have hardly changed. Many cancer therapies are only effective on a fraction of patients, while causing significant side effects and high costs for all patients. To overcome this problem, we have developed predictive models of effects and side effects of different treatments ('virtual patients'), which can be used to explore treatment options. The models have been based initially on a set of cancer-relevant pathways ('Molecular Biology of Cancer' by R. Weinberg), containing ~830 components and ~1600 individual reactions (Wierling). They incorporate the information on mutational alterations as well as copy number changes identified by NGS in both tumour and germ line genomes/exomes, and tumour,

tumour derived cell line, and tumour stem cell transcriptomes of individual patients. To be able to introduce the molecular effects of mutations, for which the effect on the molecular function cannot be predicted, we have started a project (www.mutanom.org) to identify the functional consequences of the tumour-specific mutations in oncogenes (Lange, coop. with Michael Lappe). Supported by different grants, analyses are currently being carried out on different tumour types (colon cancer, prostate cancer, breast cancer) (Schweiger, Lange, Soldatov, Dahl). In the NGFN network 'Systems Biology of the Cell', the group of Bodo Lange is also studying estrogen receptor (ER) signalling in breast cancer cells upon modulating ER pathway activity.



SNP validation from tumour mRNA (ssRNA-Seq, upper panel) and exome (exon-enriched DNA-Seq, lower panel) sequencing data from Illumina GA2. ***A****: Cross-validated homozygous SNP.* ***B:*** *Heterozygous exome SNP (in splicing region) looks as homozygous in transcriptome, i.e. potential allelic imbalance. Blue dots denote alignment mismatches (potential SNPs).*

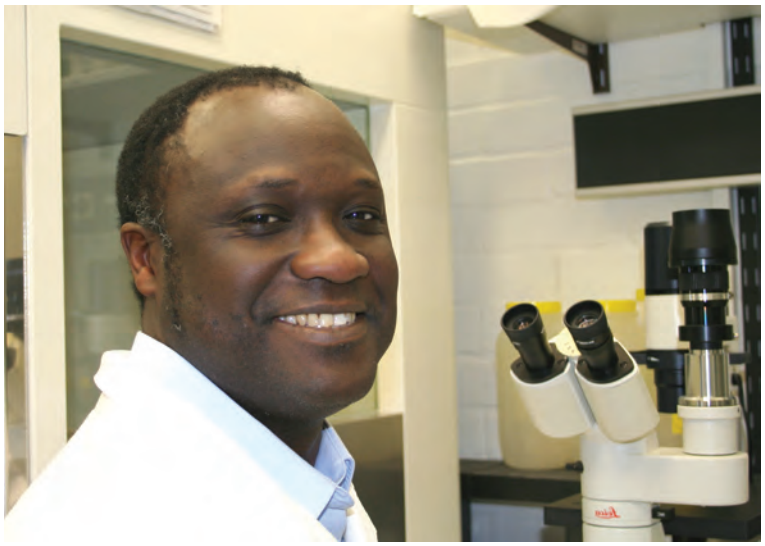
To accelerate the application of this combination of deep sequencing of individual patient samples and predictive modelling, we have started a program (www.treat1000.org) to analyse the genomes of 1000 oncology patients, in a collaboration with the Charité Comprehensive Cancer Centre (Peter Schlag, Reinhold Schäfer, Manfred Dietel), Harvard Medical School (George Church), Alacris Pharmaceutical, a spin-off of the MPIMG, and CollabRx, a US company, which has pioneered the concept of patient funded research in oncology. The first patient with metastatic melanoma has been characterised by deep sequencing of the genome, exome and transcriptome. The establishment of detailed models of tumour of the patient are currently in progress to predict drug effects, which can then be tested on tumour-derived cells and stem cells (Yaspo, Schweiger, Regenbrecht, Soldatov, Dahl, Herwig, Wierling). Early attempts to develop more specific drugs with few side effects for specific genetically defined subgroups of cancer patients by different approaches have been started (Zehetner, Glökler, Lange).

Planned developments

We see the development of systems biology of specific diseases to increase in importance in the work of the department for the next few years. The main problem we address, the development of an individualised medicine of cancer systems, up to the development of new therapeutic principles, can rely on contributions of many groups, both within and outside of the department, as well as many outside collaborations. Work on neurodegenerative diseases will however continue in parallel, able to rely on much of the infrastructure and tools established in our work on cancer.
This will be complemented by a continuing, heavy emphasis on technology development, to solve many of the practical problems we continue to face in the global analyses, as well as a continuing interest in the analysis of regulatory networks/ developmental processes by genetics, genomics and systems biology, complementing work in other departments (Herrmann, Vingron, independent research groups etc.).

Molecular Embryology and Aging

(Established: 2003)



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Katharina Wolfrum* (since 10/08)
Yvonne Welte (since 08)
Björn Lichtner (since 09/09)
Justyna Jozefczuk* (09/05-12/09)
Marc Jung* (03/05-11/09)
Mei-Chih Liao (02/09-11/09)
Thore Brink (12/04 - 10/07)

Scientific overview

The research of our group is divided into six inter-related areas:

1) Transcriptional and signal transduction mechanisms regulating self renewal and pluripotency in human embryonic stem cells, carcinoma cells and induced pluripotent stem (iPS) cells.
2) Reprogramming of somatic cells (healthy and diseased individuals- Alzheimer's Diabetic, Nijmegen breakage syndrome and Steatosis patients) into an ES-like state (iPS cells) and studying the underlying disease mechanisms.
3) Comparative characterisation of functional hepatocytes, cardiomyocytes, pancreatic, and neuronal cells derived from human ES cells and patient-specific iPS cells with the aim of establishing a platform for toxicology studies and drug screens.
4) Systems biology of stem cell fate and cellular reprogramming.
5) Age-associated gene expression patterns and signal transduction mechanisms employing mouse tissues, rat bone marrow-derived MSCs and fibroblast-derived iPS cells from young and aged individuals as model systems.
6) Gene expression and signal transduction mechanisms pertinent to preimplantation development.

*externally funded

Background

Terminal differentiation of embryonic progenitor cells to form distinct adult tissues is the hallmark of organogenesis in complex organisms. Stem cells, in particular embryonic stem cells, which can be expanded indefinitely and are pluripotent (Thomson et al. 1998), have attracted considerable attention as a therapeutic approach for treating for example, diabetes, cardiovascular-, neurological-, and liver-based diseases. However, the use of inner cell mass-derived (ICM) embryonic stem cells in cell replacement therapy remains problematic for a number of reasons, including ethical as well as host rejection of allogeneic cells. As a means of overcoming the problem of host rejection, it has now been demonstrated by numerous laboratories that the combined expression of four transcription factors, OCT4, SOX2, NANOG and LIN28 or OCT4, SOX2, KLF4 and MYC is sufficient to reprogramme human or mouse somatic cells into iPS cells. These cells have normal karyotypes, express telomerase activity, express cell surface makers and genes that characterize ES cells, and maintain the developmental potential to differentiate into advanced derivates of all three primary germ layers. Patient-specific iPS cells can be used to derive tissue-matched differentiated donor cells for therapy, and a source of cells for research into the pathogenesis of complex diseases and also toxicology studies and drug screening.

Current state of research and scientific findings

In earlier studies (see Research Report 2006) we uncovered gene expression patterns which suggest that the delineation of the ICM and TE from the morula may be controlled by signalling pathways which are also crucial for determining cell fates later in embryonic development, in the ontogenesis of some cancers and, the maintenance of human ES and trophoblast stem cells in vitro (Adjaye et al. 2005; Babaei et al. 2007; Greber et al, 2007a,b; Greber et al. 2008). In keeping with the new era of personalized medicine, we now derive (employing viral, episomal plasmids, nuclear protein extracts from pluripotent cells and drug-induced) and characterise iPS cells from various cell types (fibroblasts, keratinocytes, amniotic fluid, hair follicle stem cells and mesenchymal stem cells) of distinct ages and disease states (see figure 1).

We have successfully generated and characterised iPS cells from human skin-derived fibroblast and amniotic fluid cells and comparatively differentiated these into hepatocyte-like cells (see figure 2). These studies are intended to increase our meagre understanding of the mechanisms underlying cellular reprogramming. iPS cells generated from individuals afflicted by diseases such as Alzheimer's, Type 2 Diabetes, Nijmegen Breakage Syndrome and Steatosis provide *in vitro* models for studying the mechanisms underlying these diseases. These studies are carried out in collaboration with relevant clinicians.

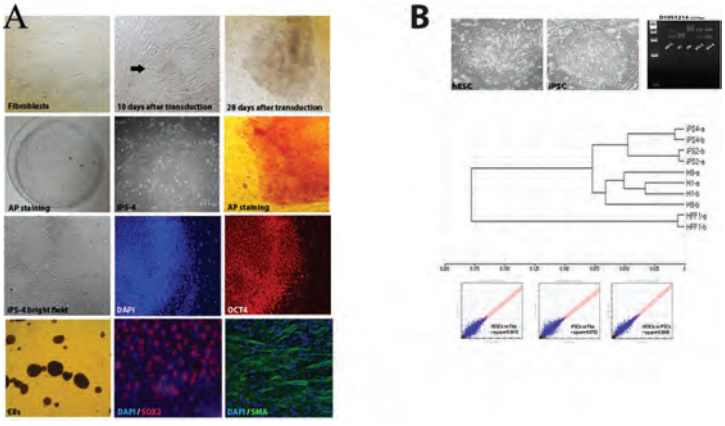


*Figure 1: Derivation and characteristion of fibroblast induced iPS cells. **(A)** A viral-derived (OCT4, SOX2, KLF4 and c-MYC) iPS cell line, stained positive for the pluripotency markers OCT4 and alkaline phosphatase. Positive expression of NANOG, SOX2, TRA-1-60 and TRA-1-81 (not shown). Embryoid body (EB) formation is shown together with the expression of the endoderm marker SOX17 and the mesoderm marker smooth muscle actin (SMA). A full characterisation has been carried but not shown here. **(B)** Human iPS and ES cells share similar morphologies and transcriptomes, furthermore DNA fingerprinting confirms the fibroblast origin of the iPS cells.*

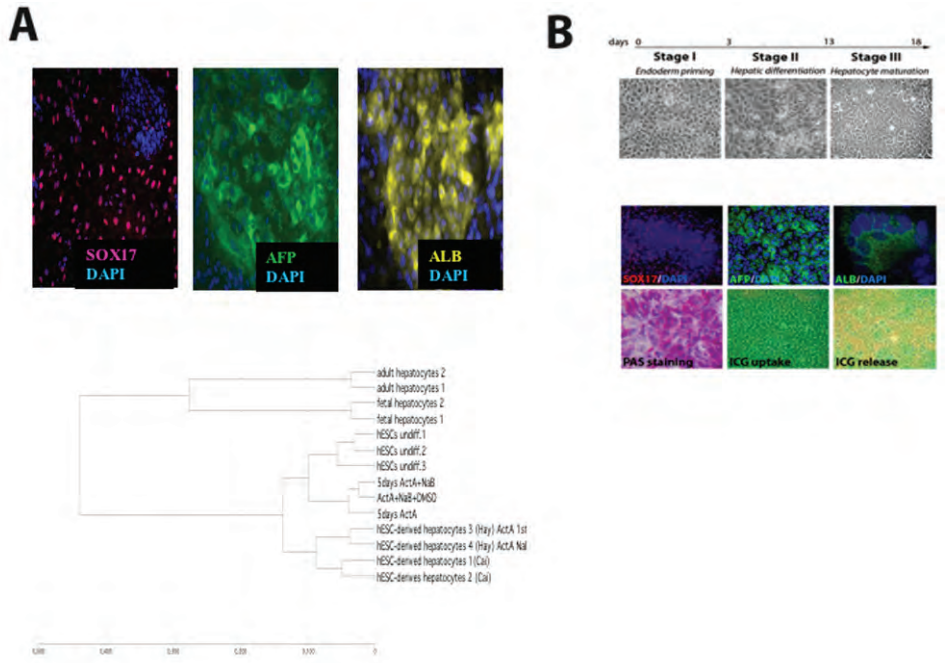


Figure 2: Differentiation of human embryonic stem cells (hESC) and induced pluripotent stem cells into hepatocyte-like cells. The entire protocol for differentiating hESCs into hepatocytes lasts 18 days and involves the induction of the definitive endoderm lineage, followed by hepatic initiation and then maturation. ***(A)*** *Embryonic stem cell derived hepatocyte-like cells. Protein expression of the endoderm marker SOX17 (pink) was detected in approximately 80% of the Activin A-induced endoderm cells whilst expression of the hepatocyte markers AFP (alpha-fetoprotein- green) and ALB (albumin-yellow) were detected in 60% of the hepatocyte-like cells. Nuclei (blue) were stained using DAPI and overlayed with the protein expression. Microarray-based comparison of the transcriptomes of hESC-derived hepatocyte-like employing two independent protocols (Hay et al. and Cai et al.) confirms the robustness of the protocols. The transcriptome correlation co-efficients between hESC-derived hepatocyte-like cells and fetal tissue derived RNA range from 0.63 to 0.69.* ***(B)*** *Fibroblast derived iPS cells can be differentiated into hepatocyte-like cells. The top panel shows the morphologies of the differentiated cells based on phase contrast microscopy. These cells also express SOX17, AFP and ALB. Glycogen storage (PAS staining) and uptake and release of compounds (ICG uptake) which are functional characteristics of hepatocytes are shared by ES- (not shown) and iPS-derived hepatocyte-like cells.*

Systems biology of stem cell fate and cellular reprogramming

Network Reconstruction: In silico modelling of human ES and iPS self-renewal network

The maintenance of pluripotency and self-renewal of human ES and iPS cells are intrinsically complex processes driven by the co-ordinated dynamic expression of a plethora of genes, their encoded proteins and associated signalling pathways in response to external signalling cues such as FGF2. Our systems biology approach combines high throughput approaches (OCT4 ChIP-chip, ChIP-seq, RNAi, metabolomics and cytokine stimulations of hESCs) and advanced computational techniques to dissect the molecular mechanisms of stem cell fate and cellular reprogramming. An OCT4 gene regulatory network is illustrated in figure 3.

Finally we have created a user-friendly database (http://biit.cs.ut.ee/escd/), integrating all OCT4 and stem cell related datasets in both human and mouse ES and EC cells. We have unveiled previously undescribed modules of OCT4 gene regulatory networks and highlighted their importance in the self-renewal transcriptional network in EC cells and ES cells (Jung et al., 2009 (in revision)). In the

current era of systems biology driven research, we envisage that our integrated embryonic stem cell database will prove beneficial to the booming field of ES, iPS and cancer research.
Our research on aging is based firmly on the concept that the same signalling mechanisms that regulate the plasticity of stem cells are altered during aging and in age-related diseases. We also attempt to reverse the aging phenotype of somatic cells by cellular reprogramming as a means to study genome stability, mitochondria biogenesis and stem cell fate. Accordingly, an understanding of molecular and signalling mechanisms underlying the aging process is likely to lead to novel approaches to preventing and treating age-related diseases.

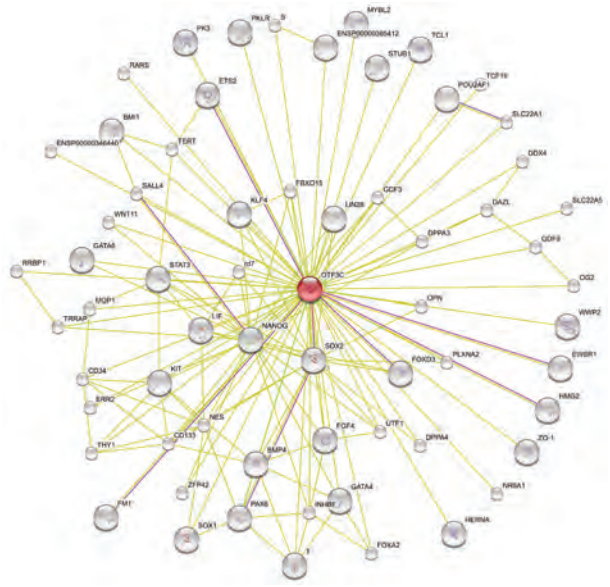


Figure 3: STRING network (v.8.0) based on only OCT4 / OTF3C (red) using high confidence, and network depth 2. Green line indicates links based on textmining, violet based on experiments (http://string.embl.de/).

Cooperations within the institute

- Ralf Herwig (Bioinformatics Group)
- Jörn Glökler (Nucleic acid based technologies Group).
- Christoph Wierling (Systems Biology Group)
- Rudi Lurz (Electron Microscopy Group)
- Lloyd Demetrius (Dept. of Computational Molecular Biology)
- Thomas Manke (Dept. of Computational Molecular Biology)

Planned developments

Integral to our work on individualized iPS cells, we plan to set up a platform for patient-specific drug and toxicity screening. To achieve this goal, patient-specific iPS cells will be differentiated to cardiomyocyte-like and hepatocyte-like cells and then treated with various compounds followed by the analysis of metabolic and transcriptional changes.

Selected information

Selected publications

Brink TC, Demetrius L, Lehrach H & Adjaye J (2009). *Age-related transcriptional changes in gene expression in different organs of mice support the metabolic stability theory of aging*. Biogerontology 10(5):549-564

W.A. Kues, S. Sudheer, D. Herrmann, J.W. Carnwath, V. Havlicek, U. Besenfelder, H. Lehrach, J. Adjaye, & H. Niemann (2008). *Genome wide expression profiling reveals novel clusters of transcriptional regulation during bovine preimplantation in vivo*. PNAS, 105(50):19768-73

Greber B, Lehrach H & Adjaye J (2008). *Control of early fate decisions in human ES cells by distinct states of TGFß pathway activity*. Stem Cells and Development 17(6): 1065-77

Greber B, Lehrach H & Adjaye J (2007). *FGF2 Modulates TGFβ Signaling in MEFs and Human ES cells to Support hESC Self-renewal*. Stem Cells. 25(2):455-464

Babaie Y, Herwig R, Greber B, Brink TC, Wruck W, Groth D, Lehrach H, Burdon T & Adjaye J. (2007). *Analysis of OCT4 dependent transcriptional networks regulating self renewal and pluripotency in human embryonic stem cells*. Stem Cells 25(2): 500-510

Selected invited talks

7Th Annual meeting of the International Society for Stem Cell Research (ISSCR), Industry Symposia, Barcelona, Spain, 08.07.2009

UK-German Regenerative Medicine workshop, British Embassy Berlin, 02.-03. 03.2009

The Berlin-Brandenburgische Akademie der Wissenschaften Minisyposium Stammzellen und Reprogrammierung, Berlin, 30.04.2009

Pluripotency and differentiation in embryos and stem cells, Mini symposium in memory of Prof. Anne McLaren. Pavia, Italy, 17.-18.01.2008

98Th Internationl Titisee Conference- Differentiation, Reprogramming and Regeneration, Titisee, Germany, 05.-09.11.2008

Awards

Thore Brink: PhD prize (2009) from the Berliner Wissenschaftliche Gesellschaft e.V. und Technologiestiftung Berlin

James Adjaye: Ist place winner (2006) of the 7th Royan International Research Award for reproductive biomedicine.

Selected memberships

International Society for Stem Cell Research (ISSCR)

Stem Cell Network - North Rhine Westphalia.

Work as scientific referee

James Adjaye serves as scientific referee for BMC Genomics, BMC Developmental Biology, Basic Research in Cardiology, Bio Techniques, Biochemical Journal, Cancer Research, Cells Tissues and Organs, Cell and Tissue Research, Cell Death and Differentiation, Cell Proliferation, Differentiation, Developmental Dynamics, FEBS Letters, Experimental Dermatology, Human Molecular Genetics, Human Molecular Reproduction, International Journal of Developmental Biology, Journal of Molecular Medicine, Nucleic Acids Research, Physiological Genomics, Reproduction, Stem Cells, Stem Cells and Development, Tissue Engineering

Membership in editorial advisory boards

- Clinical Rehabilitative Tissue Engineering Research
- Dermato-Endocrinology
- Stem Cell Review Letters
- Tissue Engineering and Regenetive Medicine
- World Journal of Stem Cells

Grant reviewing

Jamas Adjaye serves as referee for the following institutions: BBSRC, UK, Medical Research Council, UK, Wellcome Trust, UK, Council for Earth and Life sciences, Holland, Netherlands Organisation for Scientific Research, Holland, Association

Francaise Contre les Myopathies, France, Israel Science Foundation, Israel, National Taiwan University Frontier and Innovative Research Projects, Taiwan.

Appointments of former members of the group

Boris Greber: now as postdoctoral fellow at the Max Planck Institute for Molecular Medicine, Muenster, in the department of Hans Schöler (since 01/08)

Thore Brink: now as postdoctoral fellow at the Medical School at the University of Essen (since 01/09)

External funding

2008 - 2012

ERASysBio, EU: *The systems biology of network stress based on data generated from in vitro differentiated hepatocytes derived from individual-specific human iPS cells*. Co-ordinator: James Adjaye, 03/10-02/12

ENFIN/FP6- LSHG-CT-2005-518254, EU: *Systems biology of self-renewal of human embryonic stem cells.* Co-ordinator: James Adjaye, 02/08-11/10

01GN08073, BMBF: *iPS cells from healthy and Alzheimer's disease somatic cells.* Co-ordinator: James Adjaye, 11/08-10/11

0315398G, BMBF, Medizinische Systembiologie: *Drug-based induction of pluripotent human stem cells (iPS cells) derived from human somatic cells.* 01/09-12/11

DFG/SFB 760: *Transcriptional and signaling mechanism(s) impacting the self-renewal and differentiation capacity of young and old mesenchymal stem cells*. 10/08-09/09

Berliner Krebsgesellschaft e.V.: *Identification and evaluation of stem cell like properties and putative new markers in cancer stem cells (CSCs)*. 08-09

Wilhelm Sander-Stiftung: *Molecular characterisation of thrombin related signalling pathways in glioblastoma stem cells*. (03/09-02/11

2005 - 2007

BMBF, NGFN II, EP: *Genetic etiology of human longevity.*

BMBF, Cell-based regenerative medicine: *Stem-cell based liver regeneration.*

DFG / FOR478, AD184/5-1: *Transcriptomics of bovine preimplantation development - mechanisms of embryo-maternal communication.* until 12/09

DFG- AD 184/4-1 / SPP1109: *Functional characterisation of putative pluripotency controlling genes and their role in establishing transcriptional networks and signalling pathways crucial to stem cell biology.*

Public relations

Articles about our work have been covered on National television networks: ARD, ZDF and SWR. Newspapers and magazines: Frankfurter Allgemeine Zeitung, Die Zeit, Süddeutsche Zeitung, Spiegel, Berliner Tagesspiegel, Cicero, Trillium report, Bio-Spektrum and the French newspaper Le Figaro.

Neuropsychiatric Genetics

(Established: 10/2008)

Head
Dr. Lars Bertram
Phone: +49 (0)30 8413-1542
Fax: +49 (0)30 8413-1380
Email: bertram@molgen.mpg.de

Scientist
Brit-Maren Schjeide*

Database manager
Ute Zauft*

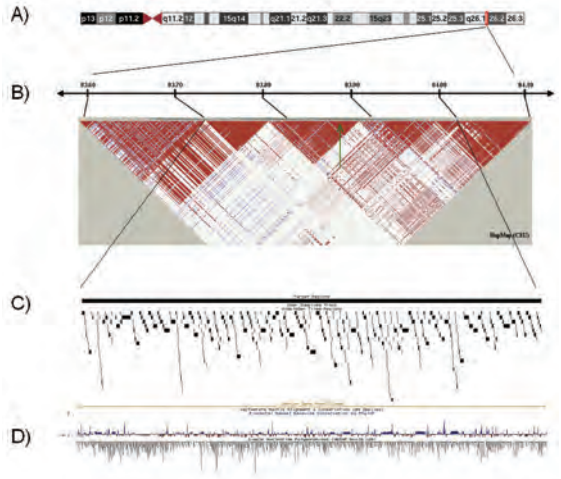
Scientific overview

Dr. Bertram joined the Dept. of Vertebrate Genomics at the MPIMG in October 2008, after heading the Genomics group in the Genetics and Aging Research Unit (led by Prof. Rudolph E. Tanzi) at Massachusetts General Hospital, Boston. In Boston, Dr. Bertram held appointments as Assistant in Genetics (at MGH) and Assistant Professor of Neurology (at Harvard Medical School). His main scientific focus was and continues to be the mapping and characterization of complex disease loci, predominantly in the field of Alzheimer's disease (AD) and schizophrenia. Dr. Bertram's main scientific achievements include the first study to suggest genetic linkage of AD to chromosome 10q (Bertram et al, Science 2000), spearheading the analyses of a high-resolution whole genome linkage scan (Blacker, Bertram et al, Hum Mol Genet 2003), and the identification and functional characterization of UBQLN1 (Bertram, New Engl J Med 2005; Hiltunen, JBC 2006) and IDE (Kim, JBC 2007) as putative novel AD genes. Recently, he spearheaded the design and execution of the first family-based genome-wide association study (GWAS) in AD, which highlighted a number of previously unrecognized novel AD loci independent of APOE (Bertram, Am J Hum Genet 2008). This latter study is currently substantially extended at the MPIMG *via* systematic fine-mapping of the most compelling GWAS regions using "next generation" technologies (see figure).

Apart from the laboratory work, Dr. Bertram's group has pioneered the development of bioinformatic approaches that systematically and quantitatively integrate genetic data for a number of phenotypes including Alzheimer's disease, Parkinson's disease, multiple sclerosis and schizophrenia, two of which have recently been published in Nature Genetics (Bertram et al., 2007; Allen et al, 2008). Due to the systematic and quantitative nature to complex disease genetics of the underlying approach, these projects have already facilitated the design and execution of both genetic epidemiological as well as molecular genetic studies for a large number of research laboratories around the world, including our own (e.g. Schjeide et al, Neurogenetics 2008 and Arch Neurol 2008).

* externally funded

In addition to the studies of mainly late-onset AD genetics listed above, Dr. Bertram is the Principal Investigator in a project aimed at studying the genetic causes of early-onset familial forms of AD. The first phase of this project (funded by the NIA/NIH) was completed at MGH and entailed the fine-mapping and the subsequent mutational screening of linkage regions identified in families of early/mixed onset age in our earlier whole-genome linkage analysis (Blacker, Bertram et al, Hum Mol Genet 2003). To date, several families linked to chromosomes 14 and 21 were identified as carriers of previously described mutations in PSEN1 and APP. For one family, this project also identified a mutation in a predicted microRNA (miRNA) binding region in the 3'UTR of APP. Using a variety of *in vitro* experiments, we showed that the affected miRNA is expressed in brain and can regulate the expression of endogenous APP in HEK-293 cells. For the first time, these data suggest that genetic variants within a potentially functional miRNA target site in the 3' UTR of APP can modify predisposition for AD (manuscript in preparation). At the MPIMG, this project has been extended (funded by the BMBF as part of the NGFNplus initiative) to a systematic screening of all coding regions in probands of early-onset multiplex AD families excluded to carry any of the known disease-causing AD mutations. This cutting edge technology allows to efficiently enrich all known coding regions, i.e. approx. 180,000 exons across 20,000 genes, plus all known ~550 miRNAs, in genomic DNA samples from AD patients. The "exome" enrichment is followed by massively parallel, ultra-high throughput re-sequencing using one of the MPIMG's next generation sequencing instruments.



Fine-mapping of the chromosome 14q31 GWAS signal using next generation technologies.
***A)** Chromosome context; **B)** Linkage disequilibrium structure and location of the GWAS SNP (green arraow) in a 500 kb interval; **C)** Location and tiling of oligonucleotide probes in 400 kb interval targeted for sequence capture and follow-up sequencing; **D)** Cross-species conservation and SNP map of targeted region. [Figure adapted from Fig 3. in Bertram et al. Am J Hum Genet 2008; 83(5):623-32.]*

Selected information

Selected publications

Schjeide BM, McQueen MB, Mullin K, DiVito J, Hogan MF, Parkinson M, Hooli B, Lange C, Blacker D, Tanzi RE, Bertram L. *Assessment of Alzheimer's disease case-control associations using family-based methods.* Neurogenetics. 2009 Feb;10(1) :19-25. Epub 2008 Oct 2.

Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ, Tanzi RE, Bertram L. *Systematic Meta-Analyses and Field Synopsis of Genetic Association Studies in Schizophrenia: The SzGene Database.* Nat Genet. 2008 Jul;40(7):827-34.

Bertram L, Lange C, Mullin K, Parkinson M, Hsiao M, Hogan MF, Schjeide BM, Hooli B, Divito J, Ionita I, Jiang H, Laird N, Moscarillo T, Ohlsen KL, Elliott K, Wang X, Hu-Lince D, Ryder M, Murphy A, Wagner SL, Blacker D, Becker KD, Tanzi RE. *Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE.* Am J Hum Genet. 2008 Nov;83(5):623-32. Epub 2008 Oct 30.

Bertram L & Tanzi RE. *Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses*. Nat Rev Neurosci. 2008 Oct;9(10):768-78. Review.

Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE.. *Systematic Meta-Analyses of Alzheimer's Disease Genetic Association Studies: The AlzGene Database*." Nat Genet. 2007 Jan;39(1):17-23.

Selected invited talks

International Conference on Alzheimer's Disease, Vienna, Austria, 07/2009

Serono Symposia International Foundation Satellite Symposium: Alzheimer, Parkinson, Multiple Sclerosis: role of genes; 19th Meeting of the European Neurological Society, Milan, Italy, 06/2009

Wenner Gren Foundations International Symposium: Alzheimer therapy: still a challenge; Stockholm, Sweden, 05/2009

Rudbeck Seminar Series at Uppsala University; Uppsala, Sweden, 03/2009

HuGENet Workshop on Networks, Genome-Wide Association Studies, and the Knowledge Base on Genetic Variation and Human Health; Atlanta, GA, 01/2008

Awards

Recipient of the National Alliance for Research on Schizophrenia and Depression "Independent Investigator Award", 2009

Work as scientific referee (selection)

Lars Bertram serves as scientific referee for Am J Hum Genet, Ann Neurol, Arch Neurol, Brain Research, Eur J Hum Genet, Hum Mol Genet, Mol Psych, Nature Genet, Nature Neuroscience, Neurobiol Aging, Neurogenetics, New Engl J Med, PNAS, Trends in Genetics

Grant reviewing (selection)

Alzheimer's Association, Bethesda, MA, USA; Health Research Board, Dublin, Ireland; Michael J. Fox Foundation for Parkinson's Research, New York, NY, USA; National Institutes of Health, Bethesda, MD, USA; Stichting voor Alzheimer Onderzoek - Fondation pour la Recherche sur la Maladie d'Alzheimer, Belgium; Wellcome Trust, London, UK

Membership in editorial boards

Neurogenetics, Eur J Clin Invest, Int J Mol Epidemiol Genet

External funding

BMBF (06/08-05/11): *Identification and Functional Characterization of Novel Early-Onset Alzheimer's Genes.*

Cure Alzheimer Fund (01/07-12/11): *The AlzGene database (Phases II & III).*

Cure Alzheimer Fund (01/09-12/09): *Fine-mapping of Chromosome 14q31 Using Next Generation Technologies*.

Michael J. Fox Found. (10/05-09/10): *Creation of a Publicly Available Parkinson's Disease Genetics Database*.

NARSAD (01/06-21/09): *Creation of a Publicly Available Genetics Database for Schizophrenia*.

NARSAD (10/09-11/11): *Deep resequencing of SZ loci using next generation technologies.*

Automation

(Established: 1998)

Senior scientists

Andreas Dahl* (since 07/07)
Phone: +49 (0)30 8413-1517
Fax: +49 (0)30 8413-1380
Email: dahl@molgen.mpg.de

Wilfried Nietfeld* (since 10/98)
Phone: +49 (0)30 8413-1405
Fax: +49 (0)30 8413-1380
Email: nietfeld@molgen.mpg.de

Harald Seitz* (since 05/00)
Phone: +49 (0)30 8413-1614
Fax: +49 (0)30 8413-1380
Email: seitz@molgen.mpg.de

Claus Hultschig* (10/00-01/07)
Lajos Nyarsik* (07/98-03/07)
Ralf Sudbrak* (until 03/08)
Michal Janitz* (until 12/08)

Scientists

Florian Mertes (since 09/08)
Wolfram Brenner* (10/02-12/05)
Yuhui Hu (08/06-01/09)

PhD students

Gina Ziegler* (since 04/06)
Daniela Köster* (since 03/07)
Robert Wild* (since 05/09)
Katja Köhler* (since 09/09)
Young-Sook Baek (until 05/09)
Marina Tagliaro Jahns (04/08-03/09)
Anika Andersson* (until 05/07)

Engineers

Thomas Przewieslik* (04/96-06/08)
Axel Fischer (09/07-06/08)
Matthias Lange* (03/02-02/08)
Thomas Nitsche* (01/02-10/06)
Tillmann Wegwerth* (11/05–08/06)

Technicians

Aydah Sabah* (since 11/08)
Uta Marchfelder (since 03/09)
Carolin Stockmeyer* (since 03/09, 50% part time)
Marion Schmidt* (since 05/09, 50% part time)
Ulrike Borgmeier (11/01-12/08)
Pamela Kepper* (07/08-10/08)
Kerstin Zurth (until 04/08)
Regine Schwartz* (01/03-06/07)
Nicole Greiner* (08/01-10/06)
Ninette von der Dellen* (11/02-04/04)
Corinna Kober-Eisermann* (09/02-03/04)

* externally funded

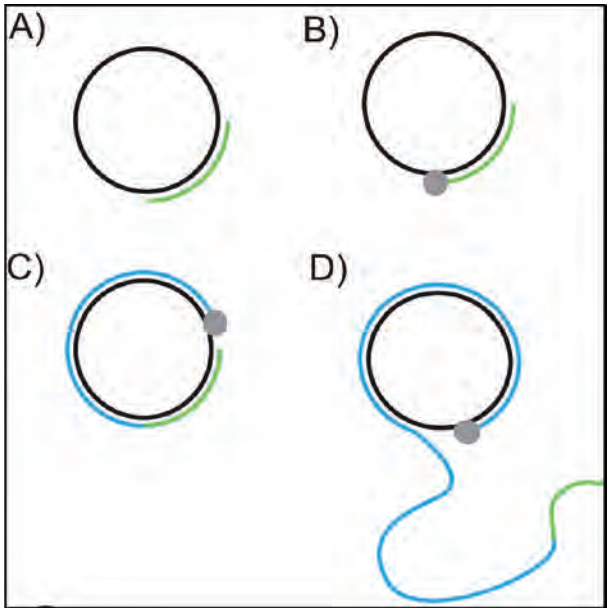
Scientific overview

Functional genome analysis

The research of *Wilfried Nietfeld's* team is focused on the development of resources for functional genomics, e.g. generation of gene specific tags in *Arabidopsis* (CATMA) and human promoter-reporter constructs as well as providing this resources for scientific collaborations. The group was a member of a consortium of European *Arabidopsis* genome centres and bioinformatic partners. More than 2000 *Arabidopsis* samples were produced and analyzed under standardized conditions and profiled on CATMA arrays for building a compendium of gene expression. Within the NGFN II SMP DNA project "Promoter Resources" a unique set of human promoter-reporter constructs was generated in collaboration with S. Schreiber (U. Kiel), M. Nöthen (U. Bonn), S. Wiemann (DKFZ) S. Haas (MPIMG) and ML. Yaspo (MPIMG). In cooperation with other research groups the infrastructure for gene expression analysis is provided. In collaboration with E. Wanker (MDC) and J. Priller (Charité) gene expression profiling studies with lymphoblastoid cell lines obtained from Alzheimer's Disease (AD), Parkinson's Disease (PD), Amyotrophic Lateral Sclerosis (ALS), Spinocerebellar Ataxia (SCA) and Huntington's Disease (HD) patients and healthy individuals as well as disease-gene expressing cell lines are performed to identify disease pathways, diagnostic markers and potential therapeutic interventions. In collaboration with U. Dirnagl and G. Trendelenburg (Charité) molecular mechanisms of neuroprotection are analyzed with respect to their functional role in cerebral ischemia, based on expression profiling data in combination with animal models aiming to discover novel therapeutic interventions in stroke. In collaboration with A. Frischauf, F. Aberger (University of Salzburg) and C. Wierling, S. Krobitsch (MPIMG) the role of Hedgehog (HH)/GLI, their signalling pathways and regulatory networks in cancer is analyzed. Gene expression profiles using microarrays and in selected cases Solexa "Deep sequencing" or "ChIP sequencing" are used to validate computer models and predictions of HH/GLI signalling pathways and regulatory networks in human cancer. In collaboration with Y. Shiloh (U. Tel Aviv), A. Venkitaraman (University of Cambridge, UK) and ML. Yaspo (MPIMG) microarrays and "Deep sequencing" are applied to analyze alteration in transcriptional networks induced by ionizing radiation (IR), to identify the major regulators that induce it, and to dissect this transcriptional response into sub-networks according to the upstream regulators that control them. Furthermore resources are provided for genome-wide genotyping (U. Lindenberger (MPIB) and L. Bertram (MPIMG)). Currently 2,200 participants of different ages attending the Berlin Aging Study II are genotyped.

Functional protein analysis

Over the past years the group of *Harald Seitz* was involved in several projects focusing on establishing protein microarray based techniques. Currently the group is involved in three externally funded projects. The first project is aiming to adopt alternative detection systems for DNA and protein microarrays. Recently, rolling circle amplification of a circular template and enzymatic active DNA sequences so called DNAzymes were successfully combined. This combination creates a powerful detection technique for nucleic acids (DNA and RNA) and proteins. This method will be adapted to nano-wells. The second project is part of the BMBF consortium MoPS (Modelling pain switches) by T. Hucho (Dept. Ropers) and C. Stein (Charité). The aim of the subproject is the relative quantification of proteins involved in signal transduction pathways in neuronal cells with a focus on post-translation modifications mainly phosphorylation of those proteins. The usage of reverse protein microarrays allows the parallel analysis of several time points and



Schema of Rolling Circle Amplification. ***A)*** *A primer is hybridised to a circularized DNA strand.* ***B)*** *Phi29 DNA Polymerase synthesize a complementary DNA strand,* ***C)*** *displacing the previously synthesized strand. The resulting rolling circle product contains linked copies of the original strands (D).*

stimuli with tiny amounts of cell lysates. The data obtained will be combined with data generated in the other part project to refine a petri-net based model of signal transduction in neuronal cells. The transcription factor CREB is very well described and one key player in several diseases. Upon stimulation of neuronal cells with EGF or NGF CREB is phosphorylated and able to activate several genes. Mutations of residues phosphorylated by different kinases shed some lights about the fine tuning of CREB activation. CREB is one target of transglutaminase TG2 (D. Walter, Dept. Ropers). This adds another putative regulation option. The third project aims to identify potential biomarkers in patients after kidney transplantation. During kidney transplantation the immune system is suppressed. This could lead to a rejection of the newly transplanted kidney caused by a BKV infection. A peptide microarray based screening approached tries to identify putative peptides that can serve to predict the likelihood of a rejection. Therefore peptide microarrays on conventional glass slides as well as Flexchip will be used. Flexchips is a SPR based techniques that allows the real time detection of up to 400 interactions in parallel. Beside a yes/no answer (binding – no binding) this technique allows the calculation of association and dissociation rates, adding an additional layer of information. All protein and DNA microarrays necessary for the projects will be generated using a piezo spotter.

Miniaturization and its combination with NextGen Sequencing

Since March 2007 *Andreas Dahl* is leading the miniaturization group continuing the work of Dr. L. Nyarsik. Focus of research is the development of miniaturized systems for high-throughput genome and transcriptome analysis with internal and external collaboration partners. A µPCR Chip platform has been developed consisting of advanced nanoliter dispensing system, a thermo cycling and fluorescence detection for volumes down to 18 nl. A novel highly sensitive and robust fluorescence reader has been developed for clinical diagnostics. Additionally several low-volume assays and assay formats were developed for SNP genotyping (cooperation University of Kiel), methylation analysis and salmonella subspecies serotyping. Within the last year, next generation sequencing technologies were included in the analysis. A high-density nanowell technology is going to be applied in single cell analysis, since it provides high number of individually addressable reactors. This is going to be used for preparation of sequencing libraries of multiple single cells. Transcriptome analysis is done using the SOLiD sequencing platform that is constantly run by the group and provided as a resource to the department. Additionally, the open source sequencing platform Polonator is used for development of alternative sequencing chemistries as well as for novel approaches of single cell analysis in a collaborative effort together with the University of Uppsala within the READNA project.

Cooperations within the institute

- Lars Bertram; Dept. Vertebrate Genomics
- Bernd Timmermann; MPIMG service group
- Michal Schweiger; Dept. Vertebrate Genomics
- Aleksey Soldatov; Dept. Vertebrate Genomics
- Sylvia Krobitsch; Neurodegenerative Disorders Group, Otto Warburg Laboratory
- Richard Reinhardt; MPIMG service group
- Marie-Laure Yaspo; Dept. Vertebrate Genomics
- H.-Hilger Ropers, Tim Hucho, Diego Walter; Dept. Human Molecular Genetics
- Martin Vingron, Stefan Haas, Thomas Manke; Dept. Computational Biology
- Bernhard Herrmann, Lars Wittler; Dept. Developmental Genetics

Selected information

Selected publications

Solomun T, Seitz H, Sturm H. (2009). *DNA Damage by Low-Energy Electron Impact: Dependence on Guanine Content*. J Phys Chem B. 2009 Aug 3. [Epub ahead of print]

Baek YS, Haas S, Hackstein H, Bein G, Hernandez-Santana M, Lehrach H, Sauer S, Seitz H. (2009). *Identification of novel transcriptional regulators involved in macrophage differentiation and activation in U937 cells*. BMC Immunol 10:18 ("highly accessed" article end of July)

Parsons MJ, Grimm CH, Paya-Cano JL, Sugden K, Nietfeld W, Lehrach H, Schalkwyk LC. *Using hippocampal microRNA expression differences between mouse inbred strains to characterise miRNA function*. Mamm Genome 2008 Aug;19(7-8):552-60.

Sclep G, Allemeersch J, Liechti R, De Meyer B, Beynon J, Bhalerao R, Moreau Y, Nietfeld W, Renou JP, Reymond P, Kuiper MT, Hilson P. *CATMA, a comprehensive genome-scale resource for silencing and transcript profiling of Arabidopsis genes*. BMC Bioinformatics. 2007 Oct 18;8:400.

Dahl A, Sultan M, Jung A, Schwartz R, Lange M, Steinwand M, Livak KJ, Lehrach H, Nyarsik L. *Quantitative PCR based expression analysis on a nanoliter scale using polymer nano-well chips*. Biomed Microdevices. 2007 Jun;9(3):307-14.

Selected invited talks

Daniela Köster: Presentation during the 11th Status Seminar Chip Technologies, 05.-06.03.2009, Frankfurt, Germany

Harald Seitz: Presentation during the "Application-oriented Flexchip Meeting", 14.06.2007, IBCP (Institut de Biologie et Chimie des Protéines) Université Lyon, France

Harald Seitz: Presentation during the "Protein Science Biacore Meeting", 11.04.2007, Cologne, Germany

Harald Seitz: Presentation during the "Scienion Practical Workshop", 25.-26.09.2006; Dortmund, Germany

Harald Seitz: 5th annual HUPO World Congress, 28.10.–01.11.2006; Long Beach, California, USA

Harald Seitz: CIGB 3rd International Workshop on Genomics 04.-10.11.2006; Varadero, Cuba

Awards

Harald Seitz: Member spotlight "The Science Advisory Board" September 2008

Work as scientific referee

The senior scientists of the Automation Group serve as referees for the following journals: Nucleic Acids Research, BMC Genomics, Pharmacogenomics, Biotechniques, Expert Review of Proteomics, Nucleic Acids, Journal of proteome research, Molecular and cellular proteomics, Molecular Genetics and Genomics

Membership in professional societies

Human Brain Protein Project within the Human Proteome Organization

German Society for Proteome Research (DGPF) (Seitz)

Society of Biochemistry and Molecular Biology (GBM), Arbeitskreis Nachwuchswissenschaftler in der GBM (Seitz)

Bridge Programm (Österreichische Forschungsförderungsgesellschaft (FFG) and Fonds zur Förderung der wissenschaftlichen Forschung (FWF)) (W. Nietfeld)

Patents

M. Jacobsen, W. Nietfeld, D. Repsilber, H. Mollenkopf, and S. Kaufmann. *Multiple normalization of heterogeneous sample analyses by constant molecular markers with relevance for clinical applications*. (EP-05 01 0351.4)

External funding

IBB project "*Virus-immunesystem interaction*", since 03/09

BMBF project "*MoPS*", since 02/09

IBB project "*RCA amplification on BioChips*", 10/06 – 09/08

BMBF project "*NGFN2 – SMP protein*", 11/04 – 05/08

EU grant MolTools, 01/01 – 06/07

EU grant QLK3-CT-2002-02035: *CAGE: Compendium of Arabidopsis Gene Expression,* 11/02 – 04/06

EU grant 028594: *APES - Comparative analysis of primate genomes, transcriptomes and proteomes with an emphasis on cognitive capabilities,* 10/06 - 03/10

EU grant QLRT-2001-01049: *Whipple's disease - Clinical features associated with Tropheryma whipplei infection in an European setting - Pathogenesis, diagnosis and treatment of Whipple's disease,* 11/02 - 10/06

BMBF 01GR0472: Nationales Genomforschungsnetz 2: Systematisch-methodische Plattform „*protein*", since 11/04

BMBF 01GS0426: NGFN2, KG *Diseases due to environmental factors pathway mapping: Systematische Beschreibung intrazellulärer Signalwege krankheitsassoziierter Gene,* TP: NUW-S23T16, 11/04 – 10/07

BMBF 01GR0414: NGFN2 SMP-DNA *Resequenzierung und Mutation,* TP: PDN-S02T17, 07/05 – 06/08

BMBF 31P3674: NGFN2, EP *Untersuchungen in der Welt der nicht-kodierenden RNAs: Rnomics trifft auf Proteomics,* TP: EP-S32T01, 07/05 – 06/08

BMBF 01GR0414: NGFN2, SMP-*DNA Promotor Ressources,* TP: PDN-S02T14, 07/05 – 06/08

EU project in the 6. Research And Technological Development Framework Programme (LSHG-CT-2004-503155). *Advanced molecular tools for array-based analyses of genomes, transcriptomes, proteomes, and cells (MolTools)*, 01/04-06/06

BMBF 0313349 Bioprofile: *Technologie zur Hochdurchsatzanalyse von Genexpressionsprofilen mit Applikation für Herz/Kreislauferkrankungen*, 10/04 – 12/08

EU FP7 HEALTH-2007-1.1-3: *REvolutionary Approaches and Devices for Nucleic Acid analysis - READNA*, 06/08 – 05/12

BMBF 0315394A: *Systems level analysis and modeling of Hedgehog/GLI signaling and regulatory networks in cancer – MoGli,* 01/09 – 12/11

EU FP7 Health-F4-2009-223575: *Systems-Level, Multi-layer Understanding of Cellular Responses to Ionizing Radiation – TRIREME*, 01/09 – 12/11

BMBF 01GS08171 NGFNplus: *Neurodegenerative Disease Networks: Connecting small molecules, proteins, and disease phenotypes – NeuroNet*, 06/08 – 05/11

BMBF 01UW0808 UA: *Die Berliner Altersstudie (BASE): Fortführung und Erweiterung - BASE II*, 10/08 – 06/09, with possible extension

Teaching activities

Lecture: *Development of Array Technologies for Genome Research,* German Institute of Human Nutrition, Potsdam-Rehbrücke, WS2005/2006 (W. Nietfeld)

Lecture: *Development of Array Technologies for Genome Research,* Technische Universität Berlin, SS2006 (W. Nietfeld)

Lecture at the University Kassel, Department of Biochemistry since WS2005/2006 undergraduate and advanced students for biology, biochemistry and nanotechnology (H.Seitz)

Since WS 2005/2006 lecture at the Free University of Berlin (21 678 V) (H. Seitz)

WS 2007/2008 practical course and seminar "*Protein-Protein Interactions*" Free University of Berlin (21 631a/ & 21 631b) (H.Seitz)

WS 2008/2009 practical course and seminar about "*Modern Detection methods in molecularbiology*" University of Kassel (FB18-036) (H.Seitz)

Organization of scientific events

Harald Seitz is initiator and organiser of the annual international meeting *Molecular interactions*, first meeting held in 2005

Public relations

17.05.2006: *Chromosom 3 des Menschen gibt seine Geheimnisse preis*

Koester D., Mayer-Enthart E., Sialelli J., Rurack K., Resch-Genger U., Seitz H. (2008): *Hula Hoop für DNA – Eine hoch sensitive Detektionsmethode für DNA Microarrays*. GenomXpress; 1/08

Nucleic Acid-based Technologies

(Established: 02/2008)

Head
Dr. Jörn Glökler
Phone: +49 (0)30 8413-1122
Fax: +49 (0)30 8413-1380
Email: gloekler@molgen.mpg.de

Scientist
Hannsjörg Braun*

PhD student
Tatjana Schütze*

Technician
Nicole Greiner*

Scientific overview

Our goals are

- Selection of nucleic acid libraries for specific ligand interactions
- Development of procedures that avoid PCR bias and binding artefacts
- Automation and high throughput screening technology
- Identification of transcription factor binding sequences
- Selection of nucleic acids (aptamers) for highly specific and affine binders
- Aptamer generation for affinity chromatography and detection
- Finding new applications for nucleic acid-based *in vitro* evolution

Nucleic acids as specific ligand binders

Aptamers are highly affine nucleic acids that are able to bind other molecules by the key-lock principle through formation of a sequence-dependent three dimensional structure (see figure 1). Aptamers were first isolated by Gold and Tuerk in 1989 using the *in vitro* selection procedure SELEX (**S**ystematic **E**volution of **L**igands by **EX**ponential Enrichment). SELEX is employed for the identification of RNA or DNA molecules that bind to their target molecule with high affinity. Starting with combinatorial libraries with up to 10^{15} different molecules, the specific binders are isolated by an iterative process of ligand binding, washing, recov-

* externally funded

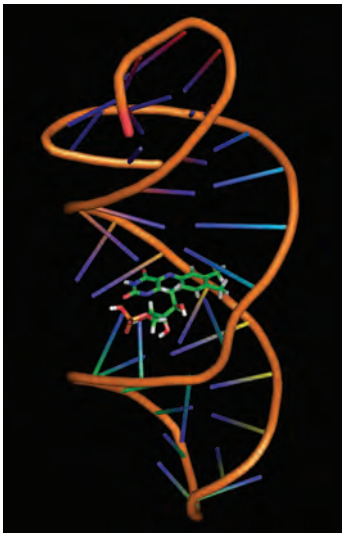


Figure 1: FMN-RNA aptamer complex

ery (elution), and amplification (see figure 2). This has yielded aptamers with affinities ranging from sub-picomolar to nanomolar affinities thus comparable to other well established biomolecules like monoclonal antibodies. Several hundreds of aptamers have already been identified to various kinds of targets as small organic molecules, proteins, virus particles up to entire cells and tissues. (see *Ellington Lab Aptamer Database:* http://aptamer.icmb.utexas.edu/) In comparison to other binding molecules, aptamers have the advantage to not elicit unwanted immune responses and are able to easily penetrate biological tissues *in vivo*. In addition, as oligonucleotides they can be easily chemically synthesised thus highly reproducible and allow the facile introduction of further modifications. As such the use of steric isomers of the biogenic D-ribose, so called spiegelmers are resistant to the ubiquitous nucleases and therefore to degradation in various environments including the human body. This spiegelmer technology has been developed and patented by Fürste, Bald, and Erdmann at the FU Berlin. Aptamer applications are ranging from therapeutics and diagnostics to biosensors, nanotechnology, and affinity chromatography. Despite this enormous potential only few aptamers are commercialised like Macugen (Eyetech and Pfizer), a treatment for neovascular age-related macular degeneration. Depending on the application, different properties and selection methods may be required for optimal solutions.

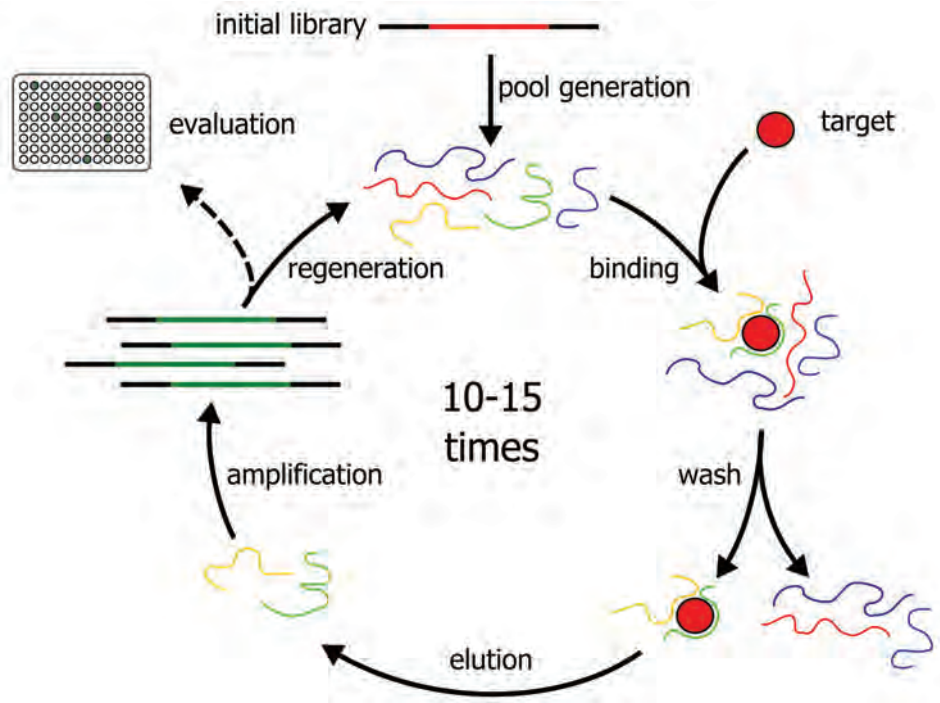


Figure 2: General SELEX procedure

Caveats

Despite the advantages of aptamers as being easily chemically synthesised, modified for various applications, and cheaply available some drawbacks remain. Most importantly, negatively charged molecules are poor targets because of electrostatic repulsion by the likewise negatively charged nucleic acids. Many binders have also shown to be poorly active upon immobilisation and proper controls have to be implemented in order to ensure that true binders have been obtained. These shortcomings are partially reflected by the fact that most publications demonstrating proof-of principle applications of this technology are conducted with robust ssDNA aptamers binding to thrombin or ATP.

Our task

In order to cope with these problems, a high throughput selection could be employed to quickly yield and evaluate binders to a wide range of targets. Thus it is the scope of this group to establish this technology in high throughput and optimise the selection procedures to obtain the most useful nucleic acid binders for any given application.

Current state of research and scientific findings

Selection automation including evaluation by next generation has been established. New protocols for monitoring sequence diversities are submitted for publication. The use of emulsions in selection experiments greatly reduces artefacts and bias. A new sequencing technology is currently under development. Novel mechanosensors based on nucleic acids are being evaluated. New applications for nucleic acids in the fabrication of smart biomaterials are developed together with the Max Planck Institute of Colloids and Interfaces. Some of these developments are submitted to the patent department of the Max Planck Society. Extensive co-operations with other research groups in and outside of the institute are necessary to optimise our scientific results.

Co-operation within the institute

Due to the similar nature of *in vitro* selection technologies, a strong co-operation in many different areas ranging from library design and synthesis to target protein expression is ongoing with the group of Zoltán Konthur.
SELEX technology can be applied to numerous scientific problems. The most obvious being the selection of transcription factor binding sites. Several strategic co-operations inside the Lehrach dept. with groups of Marie-Laure Yaspo, Michal Schweiger, and the depts. of Vingron and Herrmann concerning transcription factor selections are ongoing.
Selections for DNA aptamers are being conducted for the groups of James Adjaye, and Zoltán Konthur, and with the independent research groups of Sylvia Krobitsch, Uli Stelzl, and most notably RNA aptamers to ribosomal targets for Knud Nierhaus. Additional co-operation dealing with novel diversity assays takes place with the group of Markus Ralser. Novel sequencing approaches are developed together with the group of Andreas Dahl. As we are constantly trying to optimise our technology, new enzymes for improved protocols for molecular biology are produced and tested in cooperation with Jochen Hecht from the research group of Stefan Mundlos.

External academic cooperations

- Prof. Erdmann, FU Berlin – ProFIT project partner, thus involved in all research co-operations
- Prof. Schmülling, FU Berlin – aptamer selection to phytohormones
- Dept. Lipowsky, MPI of Colloids and Interfaces, Potsdam - selection of novel motor protein variants for nanotechnology
- Dept. Antonietti (AG Cölfen), MPI of Colloids and Interfaces, Potsdam - selection of nucleic acids that modulate crystal growth
- Dept. Jäckle, MPI for Biophysical Chemistry – selection of sequences binding to the HMG domain of the transcriptional repressor Capicua (Cic) from *Drosophila.*
- Prof. Wollenberger, University of Potsdam - aptamers in redox biosensors
- Prof. Mario Mörl, University of Leipzig – selection of aptamers to microbial surface antigens
- Jürgen Kreutzberger, Robert Koch Institute, Berlin – selection of aptamers to an HIV epitope
- Karl Skriner, Charité - Universitätsmedizin Berlin – selection of aptamers to modified peptides; selection of DNA modification by transglutaminases
- Zida Wu, Charité - Universitätsmedizin Berlin– selection of aptamers to HGH and its receptor
- Prof. Szewzyk, TU Berlin – monitoring of microbial communities by a novel diversity assay
- Prof. Lisdat and Prof. Frohme, FH Wildaus – direct sensing of DNA folding by surface plasmon resonance
- Prof. Kurreck, University of Stuttgart – selection of aptamers to coxsackie virus surface receptor

Industrial cooperations

- Roboklon GmbH, Berlin
- LGC AGOWA Genomics, Berlin
- TransTissue Technologies GmbH, Berlin
- RiNA GmbH, Berlin
- Chemicell GmbH, Berlin

General information

Selected publications

Schütze, T., Arndt, P.F., Menger, M., Wochner, A., Vingron, M., Erdmann, V.A., Lehrach, H., Kaps, C., Glökler, J. (2009). *A calibrated diversity assay for nucleic acid libraries using DiStRO-a Diversity Standard of Random Oligonucleotides*. Nucleic Acids Res (accepted)

Wochner A., Menger M., Orgel D., Cech B., Rimmele M., Erdmann V.A., Glökler J. *A DNA aptamer with high affinity and specificity for therapeutic anthracyclines*. Anal Biochem. 2008 Feb;373(1):34-42.

Wochner, A., B. Cech, M. Menger, V.A. Erdmann, Glökler J. *Semi-automated selection of DNA aptamers. Biotechniques*. 2007 Sep;43(3):344, 346, 348.

Angenendt P, Kreutzberger J, Glökler J, Hoheisel JD. *Generation of high density protein microarrays by cell-free in situ expression of unpurified PCR products*. Mol Cell Proteomics. 2006 Sep;5(9):1658-66. Epub 2006 Jul 5.

Selected book articles

Konthur Z, Glökler J, Skriner K. *Protein Interaction Analysis: Phage Display*. In: Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine, eds: D. Ganten and K. Ruckpaul. Springer; 2006.

Menger M, Glökler J, Rimmele M. *Application of aptamers in therapeutics and for small-molecule detection*. Handb Exp Pharmacol. 2006;(173):359-73

Book cover

RNAi, Martin Latterich, editor. Taylor & Francis. Nov 2007. Pb: 978-0-415-45950-6

Work as scientific referee

Journal of Biotechnology,

Membership in professional societies

- RNA Society
- RiNA e.V.

External funding

EU, *EFRE:* ProFIT grant *SELEX Technologieentwicklung im Hochdurchsatz*, contract no. 10139409

Teaching activities

Part of the lecture series at FU Berlin *Von der Funktionellen Genomforschung zur Systembiologie*

Guest lecture at the Institute of Biochemistry, University of Leipzig

Public relations

Presentation and supervision of "*Unser Erbgut, die DNA*" at the Lange Nacht der Wissenschaften 2008 and 2009

Bioinformatics

(Established: 02/2001)

Head
Ralf Herwig, PhD
Phone: +49 (0)30 8413-1741
Fax: +49 (0)30 8413-1769
Email: herwig@molgen.mpg.de

Scientists
Wasco Wruck*, since 03/01
Reha Yildirimman*, since 07/06
Felix Dreher*, since 08/2006
Marcus Albrecht*, since 09/07
Christopher Hardt*, since 11/08
Mireia Villardel*, since 09/09

PhD students
Axel Rasche*, since 11/04
Mario Drungowski*, since 11/04
Lukas Chavez Wurm*, since 04/06
Atanas Kamburov*, since 10/07

Scientific overview

Current state of research and scientific findings

The bioinformatics group develops methods and tools for the analysis and interpretation of biological data, predominantly in the domain of human diseases. Furthermore, the group is internationally active, for example within the European Network of Excellence EMBRACE, in the development of biological databases and data integration systems as well as systems biology methodology. The work of the group is structured in methods development, resources development and application to human diseases.

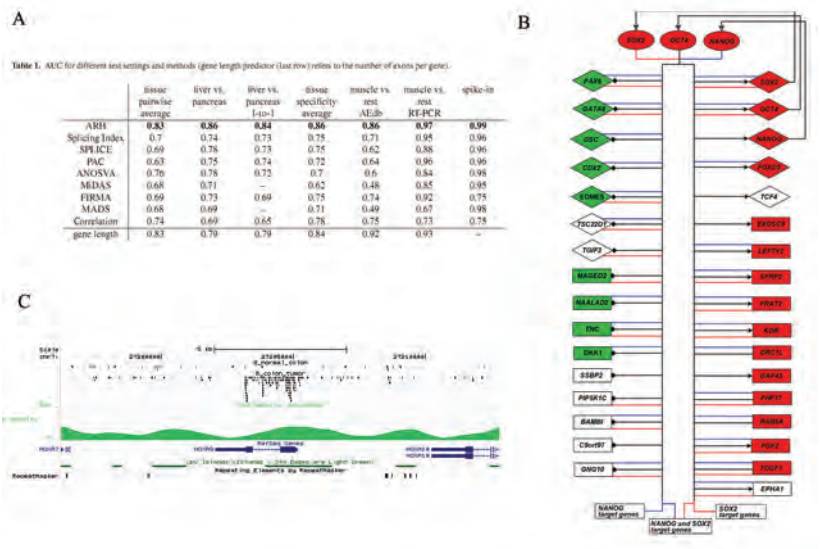


*Figure 1: **A)** Method comparison on benchmark data comparing the ARH method to existing splicing prediction methods. Best values of area under curve (AUC) are shown in bold. **B)** Core regulatory network of OCT4 as derived from overlaying different high-throughput data. **C)** Example of a MEDIP-Seq enrichment of a human colon cancer in comparison to normal tissue. Data have been incorporated in a local installation of the UCSC browser.*

Methods developments

Among others we developed a new method, ARH – *alternative splicing robust prediction by entropy*, for the prediction of alternative splicing events from microarray data and next generation sequencing (Rasche and Herwig, 2009) based on the information theoretic concept of entropy. Using published benchmark data we were able to show that the method outperforms existing methods (fig. 1A). Additionally, we focus on integrative meta-analysis of data

* externally funded

in many different projects since high-throughput data typically has a large bias (Rasche et al., 2008). As an example, we have correlated information from RNAi, ChIP-on-Chip data and promoter sequence information in order to annotate a gene regulatory network in the context of human embryonic stem cells (Chavez et al., 2009; fig. 1B). Furthermore, as part of the German National Genome Research Network and the 1000 Genomes Project we are developing methods for the analysis of next generation sequencing applications such as RNA-Seq, MEDIP-Seq, genome and exome sequencing (fig. 1C).

Resources developments

Recent achievements include, for example, the integration of a critical mass of fourteen human interaction databases into a unified system, ConsensusPathDB. In contrast to previous work where integration of interactions of homogeneous types, for example protein-protein interaction data sets, had been the focus, the network integration performed with the ConsensusPathDB includes diverse heterogeneous interaction types, such as protein-protein, signalling, metabolic and gene regulatory interactions (Kamburov et al., 2009; figure 2). Thus, functional modules derived from the ConsensusPathDB reflect more accurately biological processes. Several disease networks have been annotated with ConsensusPathDB, for example a cancer network, a type-2 diabetes network and a network for early human development. These networks build the basis for computational analyses in many international research projects, for example the EU FP7 project APO-SYS on systems biology of apoptosis or the EU FP6 project carcinoGENOMICS on the development of alternative testing systems for chemicals.

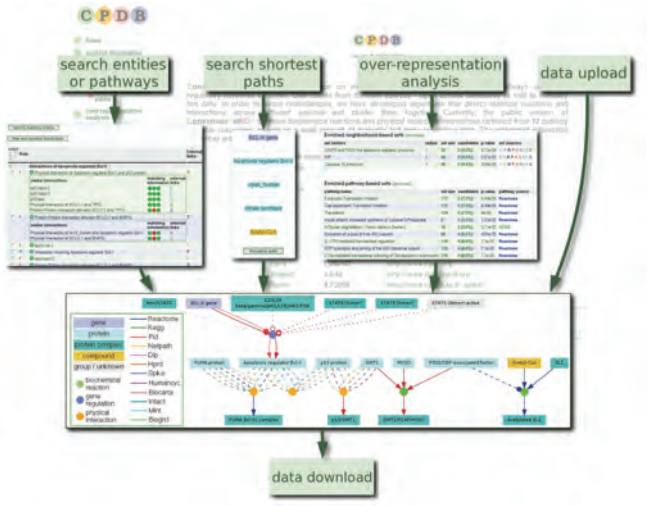


Figure 2: Main features of the ConsensusPathDB web interface allowing over-representation analysis and gene enrichment as well as topological investigations of the interaction graph.

Furthermore, we developed a data integration system, DIPSBC – *data integration platform for systems biology collaborations*, that is based on XML representation of different data types followed by indexing and querying data through a collaboration system. This system is currently used in five large collaborative projects integrating a wide range of data types, such as proteomics, transcriptomics, patient data, genotype data, networks, and computational models. Until December 2008 the group was also developing resources for systems biology, such as the modelling system PyBioS (Wierling et al., 2007) and the GeNGe (Hache et al., 2009) system for analysis of regulatory networks. Since January 2009 these systems are further developed in the newly founded systems biology group (C. Wierling).

Application to human diseases

The group has a long-year collaboration with the DKFZ (German Cancer Research Centre, Heidelberg) on the identification of disease markers and associated networks in several cancer entities (lung, prostate, breast). Several clinics are involved in these projects, for example the Charité Berlin, the Medical Universities Graz and Innsbruck and the Karolinska Institute. The group is integrated in several projects on the national (National Genome Research Network) and international level (EU project APO-SYS) that target computational modelling of cancer processes, for example apoptosis, carcinogenesis induced by chemicals as well as

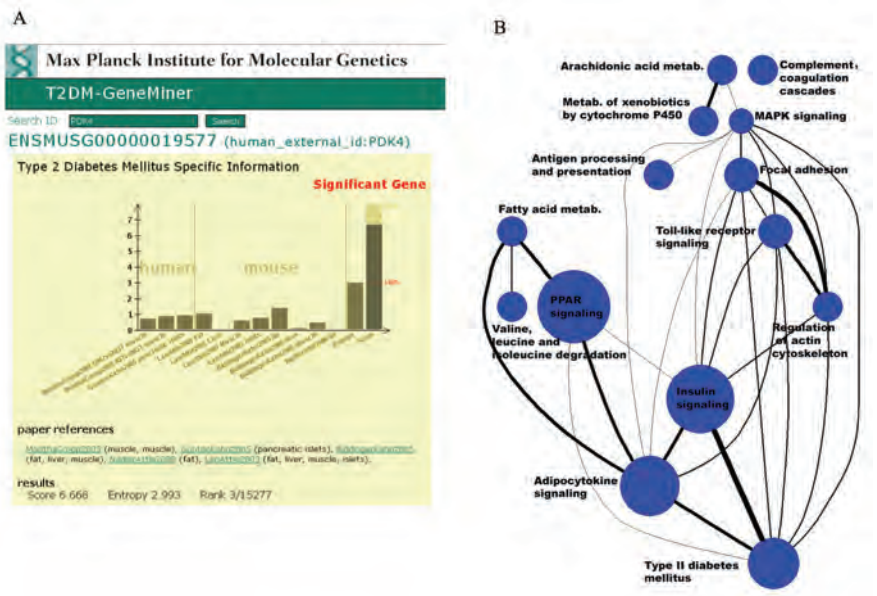


*Figure 3: **A)** Web browser that shows results for type-2 diabetes mellitus candidate genes across multiple integrated data sets. **B)** Pathway cross-talk involving 213 genes that were identified as potential disease markers in the meta-analysis. Pathways are visualised according to relevance.*

drug action. Furthermore, using national and EU-funding (SysProt) the group is developing disease networks for type-2 diabetes mellitus (Rasche et al., 2008; Dreja et al., 2009; figure 3) together with the DIfE (German Institute for Human Nutrition) and commercial partners. Together with the Charité Berlin, the University Clinic Essen and Bayer Healthcare we aim to identify new biomarkers for uremic toxins important in chronic renal failure and cardiovascular disease.

Cooperation within the Institute

- Bernhard Herrmann: MEDIP-Seq data analysis,
- Martin Vingron: promoter sequence analysis,
- Bodo Lange: protein function and interaction networks in human cancer,
- Ralf Sudbrak: 1000 Genomes Project, Cancer Genome Project data analysis,
- Marie-Laure Yaspo: Trisomy 21 data analysis,
- Michal Schweiger, Andreas Dahl: next generation sequencing data analysis of cancer samples,
- Christoph Wierling: computational modelling,
- James Adjaye: identification of markers and networks in human ES and iPS cells,
- Sylvia Krobitsch: yeast microarray data analysis,
- International Max Planck Research School: Ralf Herwig is staff member of the research school.

Special Facilities / equipment

The bioinformatics group hosts two systems, the ConsensusPathDB for human (and recently mouse) interactions and the XML-based data integration system that is in use in several collaboration projects. Furthermore, it provides web service access to several computational tools that are integrated in the EMBRACE registry hosted by the EBI/University of Manchester.

Planned developments

The planned developments of the group focus on two directions: further exploration and development of tools and methods for analysis and interpretation of next generation sequencing and the application of these methodologies with respect to human diseases, in particular cancer systems biology. Towards this perspective,

the group has been successful recently in maintaining its national and international funding. Ralf Herwig is member of the 1000 Genome Project and, very recently, of the Cancer Genome Project where large cohorts of individuals are analyzed with new sequencing technology. Furthermore, he is coordinating the PREDICT project within the Medical Systems Biology program of the German Ministry of Education and Research (BMBF). The purpose of the PREDICT project, that started February 2009, is the analysis of somatic mutations and the characterization of individual tumor entities by these mutation profiles.

General information

Selected publications

Chavez, L., Bais, A. S., Vingron, M., Lehrach, H., Adjaye, J., Herwig, R. (2009). *In silico identification of a core regulatory network of OCT4 in human embryonic stem cells using an integrated approach*. BMC Genomics 10:314, *highly accessed*.

Klipp, E., Liebermeister, W., Wierling, C., Kowald, A., Lehrach, H., Herwig, R. (2009). *Systems Biology: A Textbook*. Wiley Blackwell

Kamburov, A., Wierling, C., Lehrach, H., Herwig, R. (2009). *ConsensusPathDB - a database for integrating human functional interaction networks*. Nucleic Acids Research 37: D623-D628.

Rasche, A., Al-Hasani, H., Herwig, R. (2008). *Meta-Analysis Approach identifies Candidate Genes and associated Molecular Networks for Type-2 Diabetes Mellitus*. BMC Genomics 9:310, *highly accessed*.

Makrantonaki, E.(*), Adjaye, J.(*), Herwig, R.(*), Brink, T.C., Groth, D., Hultschig, C., Lehrach, H., Zouboulis, C.Z. (2006) (*equal contribution). *Age-specific hormonal decline is accompanied by transcriptional changes in human sebocytes in vitro*. Aging Cell, 5(4):331-344.

Selected invited talks

Ralf Herwig: *Systems modelling as an iterative approach towards understanding mechanisms of toxicity and carcinogenesis*. VII. World Congress on Alternatives & Animal Use in Life Sciences, 30.08.-03.09.2009, Rome, Italy

Ralf Herwig: *Interpreting genomic data with functional modules*. ICCA-LRI Workshop on Connecting Innovations in Biological, Exposure, and Risk Sciences: Better Information for Better Decisions, 16.-17.06.2009, Charleston, US

Ralf Herwig: *Integration of human molecular interactions (keynote lecture)*. 1st Annual Meeting of the National Genome Research Network in the Program of Medical Genome Research, 11.-13.12.2009, Munich, Germany

Ralf Herwig: *Predictive biological models - an EGEE Grid application (keynote lecture)*. EGEE 2008 Conference, 22.-26.09.2008, Istanbul, Turkey

Ralf Herwig: *Computational modelling of human diseases*. ICT for Bio-Medical Sciences Conference, 29.-30.06.2006, Brussels, Belgium

Awards

Christoph Wierling: Heinz-Billing Award for Scientific Computing 2005, 3rd prize.

Work as scientific referee

Ralf Herwig serves as referee for the following journals (selection): Nucleic Acids Research, Bioinformatics, BMC Bioinformatics, Nature Publishing Group

Ralf Herwig serves as referee for the following institutions: Wellcome Trust, German Science Foundation, European Commission Frameworks 6 and 7, Landesstiftung Baden-Württemberg.

Appointments of former members of the group

Steffen Hennig, PhD, now Imagenes GmbH, Berlin-Buch,

Matthias Steinfath, PhD, now University Potsdam,

Claudia Schepers, PhD, now Merck Darmstadt,

Detlef Groth, PhD, now University Potsdam,

Raffaello Galli, Computer Scientist, now Mount Sinai Hospital New York,

Christoph Wierling, PhD, now Systems Biology group, MPIMG

Elisabeth Maschke-Dutz, Mathematician, now Systems Biology group, MPIMG

Andriani Daskalaki, Bioinformatician, now Systems Biology group, MPIMG

Hendrik Hache, PhD, now Systems Biology group, MPIMG

Patents

Application: EP 1832 "*Computer implemented model of biological networks*".

External funding

(selected ongoing projects)

German Ministry of Education and Research:

- NGFN-Plus Modifiers
- NGFN-Transfer NT-CVD
- MEDSYS PREDICT
- Fugato-Plus REPORI
- 1000 Genomes Project

European Commission:

- FP6 IP carcinoGENOMICS
- FP6 SME-STREP SysProt
- FP6 Network of Excellence EMBRACE
- FP7 Collaborative Project APO-SYS

German Science Foundation (DFG):

- Research Group FOR-753

Organization of scientific events

Workshop *Biostatistics* within the EU-FP7 Integrated Project APO-SYS, 19.-21.01.2009, Berlin, Germany

Workshop *Bioinformatics* within the EU-FP6 Integrated Project carcino GENOMICS, 12.11.2008, Dublin, Ireland

Workshop *Web services in systems biology* within the 9th International Conference on Systems Biology (ICSB), 28.08.2008, Gothenburg, Sweden

Workshop *Bioinformatics* within the EU-FP6 Integrated Project AnEUploidy, 05.-08.05.2008, Berlin, Germany

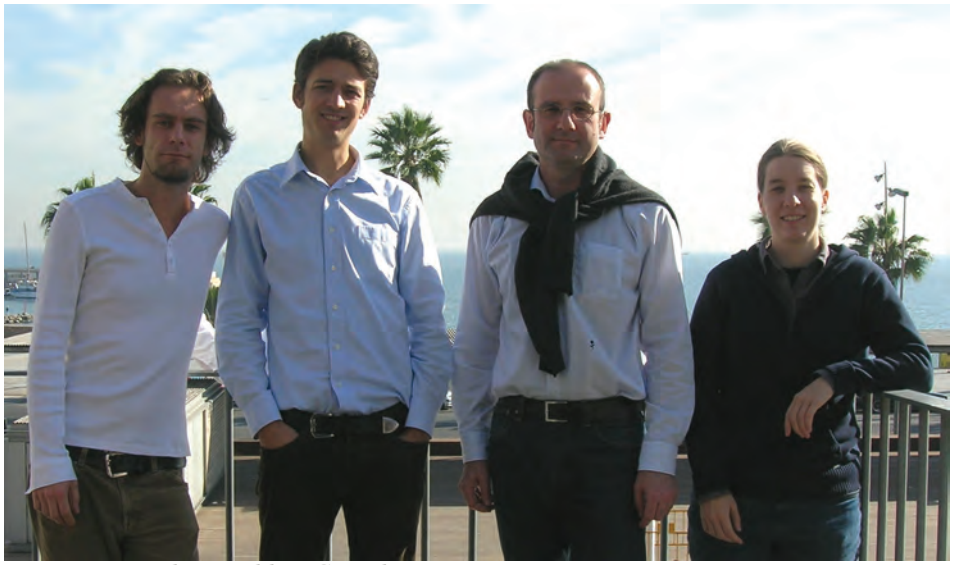
Public relations

Interview about Next generation sequencing, 25.08.2009, *In Sequence* Journal

Wierling, C., Herwig, R. (2006) *Screening - Trends in drug discovery (article)*

Comparative and Functional Genomics

(07/1995 - 05/2008)



Not present: Tobias Nolden, Cornelia Lange

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Scientists
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Dr. Andreas Ludewig* (until 09/06)

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Stefanie Palczewski* (until 05/08)
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Steve Hermann* (until 12/06)

PhD students
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Tobias Nolden* (since 05/04)
Juliane Dohm* (until 09/08)
Ruben Rosenkranz* (until 02/08)
Florian Mertes* (until 02/07)

The group moved to the Centre for Genomic Regulation (CRG), Barcelona, Spain, on 01.06.2008.

Scientific overview

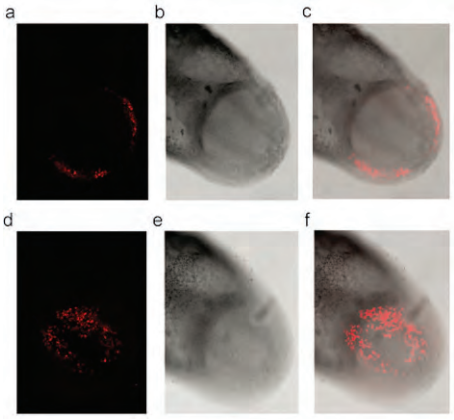
Current state of research and scientific findings

With the sequence of the human genome and many model organisms completed, the focus shifts to comparative genome analysis and to the analysis of gene functions. Our model system since 1995 has been the mouse. Complementing work on medaka (rice fish) was initiated in 2001. For a deeper understanding of major histocompatibility complex (MHC) structure and evolution we sequenced the MHC of rat and platypus, a monotreme. The mapping and sequencing of a plant genome (sugar beet) is under way, utilising next-generation sequencing (NGS) technology. A major focus of the group in the last three years has been the development of tools for the analysis of sequence data sets produced on NGS platforms. This work lead, for instance, to the first program that allowed the assembly of sequence contigs *de novo* from Solexa reads with high accuracy.

Characterization of short read sequence datasets and development of tools for the analysis of short read data

Next generation sequencing (NGS) has profoundly altered the way biological questions can be addressed. In particular, a wealth of data can be generated in short time, at affordable costs. While data generation is fast, tools for the analysis and interpretation of data were not available. Our effort was focused onto the characterization of NGS data produced on the Illumina platform. For once, we characterized Illumina sequence data regarding biases and error occurrences in such data sets. We observed, for instance, that the sequence context surrounding wrong basecalls and the frequency of substitution errors was not random. Considering the properties of Illumina reads (short reads; high error rates), *de novo* genome assemblies based on such data are very demanding. We solved this problem by implementing SHARCGS, a *de novo* assembler for BAC clones and bacterial genomes. With single-read 36mer data, we generated an error-free assembly with an N50 length of 3.7 kb that covered 98% of the genome of *Helicobacter acinonychis* (1.55 Mb genome size). We also developed strategies to analyze transcriptome datasets, and used mouse ES cell Illumina profiles for comprehensive data characterization (GC bias; 3' bias; comparison to bead array data).

Mouse functional genomics



Expression of an SV40 largeT NLS tagged DsRed transgene under the control of the endoderm specific Sox17 promoter. DsRed is expressed in the definitive and visceral endoderm, but is absent from the primitive streak region and the early head fold of the day 8.0 p.c. old Sox17+/- mouse embryo. Shown are two different optical sections (a-c) and (d-f); optical sections were taken with a Zeiss Apoptome *Axiovert microscope at x10 magnification. DsRed (a, d); brightfield (b,e); merge (c,f).*

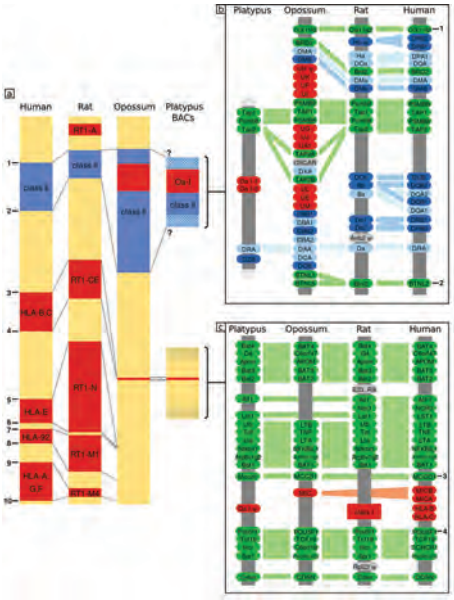
The chemical mutagenesis of mouse ES cells was complemented by other approaches for the generation of mouse mutants. Sulfotransferase gene knockouts (*Sult1a1*, *Sult1d1*) were accomplished by BAC recombineering, and three adipositas candidate gene mouse knockout lines were generated using the Genetrap approach (cooperation with W. Meinl, A. Schürmann, and H. Al-Hasani, DIfE, Potsdam). In the context of the FunGenEs (Functional genomics in embryonic stem cells) consortium, we generated and characterized engineered ES cells harboring fluorescent transgenes, under the control of lineage-specific promoters, using BAC recombineering and knock-in strategies. A Sox17-DsRed reporter cell line was characterized by differentiation assays, FACS sorting, and expression profiling, leading to the discovery of numerous novel candidate genes for early endoderm specification.

Evolution of the mammalian major histocompatibility complex (MHC)

Building on experience gained with sequencing the rat MHC, we embarked on sequencing segments of the platypus MHC, and characterizing the equivalent regions in the short-beaked echidna, another monotreme species. The analysis revealed an MHC core with class I and II genes. Using FISH hybridization (collaboration with F. Grützner, University of Adelaide, Australia), we discovered that the monotreme MHC is not contiguous, and locates within the pseudoautosomal region of two pairs of their sex chromosomes. We located the core MHC on platypus and echidna X3/Y3. Echidna X4/Y4 and platypus Y4/X5 showed synteny to the human distal class III region and beyond. We discovered an intron containing class I pseudogene on platypus Y4/X5 at a genomic location equivalent to human HLA-B,C, suggesting ancestral synteny of the monotreme MHC. In addition, the data showed that the complex sex chromosome system of monotremes is dynamic and still evolving.

Mapping and sequencing the genome of medaka (*Oryzias latipes*)

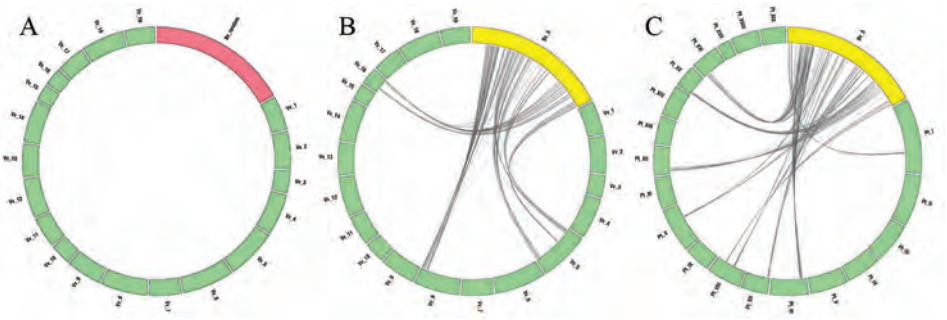
Upon completion of a physical map of the medaka genome, the map was used for map-based cloning of ethylnitrosourea-induced mutations. To date, the mutated genes in two mutants have been cloned and published, *hokecha*, a thymus development defect (collaboration with Makoto Furutani-Seiki, University of Bath, U.K.), and *ojoplano*, causing eye malformation (collaboration with J. Wittbrodt, EMBL, Heidelberg, and with J.-R. Martinez-Morales, UPO/CSIC, Sevillla, Spain). In addition, the map was used to sequence medaka linkage group 22 (collaboration with N. Shimizu, Keio University Medical School, Tokyo, Japan).



Comparative map of the mammalian MHC. ***(a)*** *Aligned MHC regions of human, rat, opossum and the sequenced portions of the platypus MHC: class I regions (red); class II regions (blue); framework regions including class III (yellow). Numbers denote framework genes that typically flank these intervals. Dotted lines link orthologous positions defined by genes 1-10 in the four species. ''?'' indicates that borders of the class I/II region in platypus have not been cloned.* ***(b)*** *Comparison of eutherian class II regions to class I/II regions of opossum and platypus. Red: class I genes; light blue: class II á chain genes; dark blue: class II â chain genes; grey: genes/pseudogenes without conserved position.* ***(c)*** *Map of* Bat4 - Cdsn *interval including a class I gene block.*

Mapping and sequencing the genome of sugar beet (*Beta vulgaris*)

Sugar beet (*Beta vulgaris*; order Caryophyllales) is a diploid crop plant with a genome size of 758 Mb. Phylogenetically, sugar beet is only a distant relative of other eudicot plant species with genomes already sequenced (e.g. Arabidopsis, poplar), or in progress (e.g. tomato). We have generated a physical map of the sugar beet genome in bacterial artificial chromosome (BAC) clones, using a hybridization-based strategy, utilizing >10,000 gene-derived probes. Chromosomal assignments of >2600 probes and clones allowed integration of BAC contigs and genetic map. The data were furthermore utilized to develop a synteny map of sugar beet in comparison to poplar, grapevine, and Arabidopsis. Towards sequencing the sugar beet genome and transcriptome, we have generated a wealth of sequence resources, including whole genome shotgun (WGS) and cDNA data (454), 2.5 kb and 4.5 kb paired end data (Illumina) and miRNA sequences (Illumina). These resources, together with further data including 454 WGS reads, and end sequences form BACs and fosmids (collaboration with B. Weisshaar, University of Bielefeld), are presently used to assemble and annotate a draft of the sugar beet genome.



Syntenic regions between Beta vulgaris and Vitis vinifera ***(B)*** *or Populus trichocarpa* ***(C)****, respectively, and the empty scenario for a random B. vulgaris chromosome* ***(A)****. The 683 source sequences of probes in the physical map of B. vulgaris chromosome 3 were used for a blast search against the gene sets of Vitis and Populus (tblastx -e 1e-5). Matches with at least 60% identity and a length of at least 30 amino acids were kept only if 10 or less Vitis genes or Populus genes were found for one B. vulgaris sequence. Of those, only the top three best matches were kept. Lines were drawn between B. vulgaris chr. 3 and chromosomes of Vitis or Populus, if at least 10 genes matched within an interval of 1 Mbp in the genomic sequences. The random chromosome was generated by randomly choosing 655 probes (average size of all nine B. vulgaris chromosomes) from the physical map.*

Cooperation within the institute

- Richard Reinhardt: genomic sequencing
- Ralf Spörle, Department Developmental Genetics: *in situ* hybridization
- Tatiana Borodina, Department Vertebrate Genomics: Illumina sequencing
- Claudio Lottaz, Department Computational Biology: NGS data analysis and software development

Planned developments

The group moved to the Centre for Genomic Regulation (CRG) in June 2008, following Heinz Himmelbauer's appointment as head of the CRG Ultrasequencing Unit (http://www.crg.es/ultrasequencing_unit). The sugar beet genome project is carried out in cooperation between CRG and MPIMG in the context of the BeetSeq project, funded by the Ministry of Education and Science (BMBF).

General Information

Selected publications

Dohm JC, Lange C, Reinhardt R, Himmelbauer H. *Haplotype divergence in Beta vulgaris and microsynteny with sequenced plant genomes*. Plant J. 2009 Jan;57(1):14-26

Dohm JC, Lottaz C, Borodina T, Himmelbauer H. *Substantial biases in ultra-short read data sets from high-throughput DNA sequencing*. Nucleic Acids Res. 2008; 36, e105.

Rosenkranz R, Borodina T, Lehrach H, Himmelbauer H. *Characterizing the mouse ES cell transcriptome with Illumina sequencing*. Genomics 2008; 92, 187-194.

Dohm JC, Lottaz C, Borodina T, Himmelbauer H. *SHARCGS, a fast and highly accurate short-read assembly algorithm for de novo genomic sequencing*. Genome Res. 2007 Nov;17(11):1697-706.

Dohm JC, Tsend-Ayush E, Reinhardt R, Grützner F, Himmelbauer H. *Disruption and pseudoautosomal localization of the major histocompatibility complex in monotremes*. Genome Biol. 2007;8(8):R175.

Selected invited talks

Deep Sequencing: Challenges and Pitfalls, Centro Nacional de Investigaciones Oncológicas, Madrid, Spain, March 25, 2009.

The BeetSeq project: a genome sequence for sugar beet (Beta vulgaris). Tagung der Gesellschaft für Pflanzenzüchtung AG Genomanalyse, Göttingen/Einbeck, Nov. 10, 2008.

Massively parallel sequencing. Promises, prospects, pitfalls. BCI 2008, Trieste, Italy, Sept. 9, 2008.

Assembly and annotation of short-read datasets. Symposium Future of Genome Research in the Light of Ultrafast Sequencing Technologies. Bielefeld University, July 4-7, 2007

External funding

GABI-FUTURE: *BeetSeq - a reference genome sequence for sugar beet (Beta vulgaris),* 2008-2010 (coordinator)

EC, FP6 Integrated Project: *Functional genomics in embryonic stem cells (FunGeneEs)*, 2004-2007.

BioProfile-Nutrigenomics: *Gene-nutrition interactions in the pathogenesis of the metabolic syndrome and its complications*, 04-07

GABI2: *BeetPhysMap, Construction of a physical map of sugar beet (Beta vulgaris) and its connection to the genetic map*, 04-07

BioProfile-Nutrigenomics: *Humanised mouse models for xenobiotics-metabolizing enzymes,* 03-07

Teaching activities

Computermodellierung in den Biowissenschaften, COMOBIS II, 1 SWS per term, Universität Salzburg, Austria, 2003-2009

Genetic Variation, Haplotypes & Genetics of Complex Disease

(Established: 2002)

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Dr. Anja Bauerfeind* (until 12/08, guest scientist* since 1/09)
PD Dr. Thomas Sander (guest scientist since 07/09)
Dr. Johannes Dapprich* (until 03/09)
Dr. Cornelia Platzer* (until 01/09)
PD Dr. Werner Terhalle* (until 12/07)
Dr. Birgit Mentrup* (until 10/07)
Dr. Dmitri Parkhomchuk* (until 06/07)
Dr. Thomas Kroslak* (until 10/06)
Karolina Janitz* (until 09/06)
Bernd Timmermann* (until 04/04)

Technicians/Engineers
Stefanie Palczewski*
Sabrina Schulz*
Sandy Thottakara (until 11/08)
Annett Neubert* (until 05/08)
Mario Sontag* (until 6/2004)
Pamela Kepper* (until 2/2006)

Scientific overview

Major focus is the systematic analysis of human genetic variation and its structure, in genes, genomic regions of interest, and genomes. Longstanding lines of research (Hoehe MR, 1990, www.molgen.mpg.de/~genetic_variation_program/), development and production have included 1) establishment of deep medical resequencing technologies ('Multiplex PCR Sequencing', high throughput capillary sequencing, 'Next Generation Sequencing'); 2) implementation of bioinformatic approaches to analyze genetic variation, predict haplotypes, establish genotype/haplotype-phenotype relationships, and assemble and analyze molecular haplotype sequences directly. Major motivation has been to assess essentially complete information on DNA sequence variation and its 'true' underlying molecular haplotype structures in human, mandatory to establish valid relationships between gene/genome variation, function and phenotype.

Deep medical re-sequencing of candidate gene loci

Substantial data sets have been generated by re-sequencing on average 333 (up to 1050) individuals in more than 30 candidate gene loci for common disease. Essentially complete DNA sequence information within such defined segments of the genome demonstrated tremendous gene sequence and haplotype diversity.

* externally funded

The systematic comparative evaluation of our high resolution (HR) data in particular against corresponding HapMap data (up to version 22, Phase II, March 2007, including ~ 2.6 Mio SNPs polymorphic in CEU) showed, that HapMap SNPs represent at most 80% of all common variation (MAF > 5 %), one fourth of the total existing variation, two third of the common haplotype structures only, and at best two third of information content. The richness (> one third) of rare variants identified at HR and, as a consequence, an increasing fraction of rare haplotypes (information content up to 50%) may ultimately challenge the concept of common haplotypes over extended regions.
These results shed first light on the major challenges arising with targeted and whole genome re-sequencing efforts currently underway, such as the '1000 Genomes' project. We have explored first approaches to filter signal from noise against a background of high genome sequence diversity.

Genetic variation and haplotypes in candidate genes related to obesity

The dataset described has been expanded by re-sequencing and large-scale genotyping of an additional 83 candidate genes in 1440 individuals affected with severe obesity and controls. To facilitate comprehensive analyses, we have developed a software tool for efficient data processing and semi-automated modular analysis of variation data. Integrating a maximum of information from multiple sources, about 5000 candidate genes had been sequentially filtered, with a focus on major obesity-related pathways. A remarkable number of SNPs and both risk and protective haplotypes were found highly significantly related to the obesity phenotype, in what may be considered one of the most comprehensive case-control studies in obesity at present.

Taken together, our deep re-sequencing data in conjunction with an increasing evidence for abundant structural variation demonstrate a much higher complexity, diversity and richness of genetic variation and its underlying haplotype structures, indicating the limitations of *in silico* haplotype prediction. The concept of a reference sequence may seem no longer applicable. Therefore, it is increasingly important to be able to directly determine individual molecular haplotypes.

A unique 'Haploid Reference Resource' for determination of molecular haplotypes

We have now established a unique 'Haploid Reference Resource' (HRR) of 100 human fosmid libraries (200 haploid genomes) from a representative German population cohort. Individual samples are genetically and phenotypically well characterized. Haploid genomes of an individual are physically covered by redundant fosmids, formatted in pools of fosmids containing one of both possible haploid fosmids in the same pool (detailed description see figure 1).
The advent of next generation sequencing (NGS) technologies in combination with the HRR allows direct determination of molecular haplotypes & haploid sequences for any region of interest and even whole exomes by application of targeted enrichment technologies. Haploid fosmid sequences are of particular importance for complex and structurally variable regions, where different molecular haplotypes may compose the diploid human genome.

Establishment of an independent next generation sequencing (NGS) data production & analysis pipeline

We have established an independent SOLiD NGS platform. Since its installation October 31st, 2008, we have produced ~ 300 GB of mappable sequence data using > 40 different super-pools (15.000 fosmids), about 20 pools of 5000 fosmids and sets of enriched fosmids. More than 220 sequencing libraries were prepared, in-

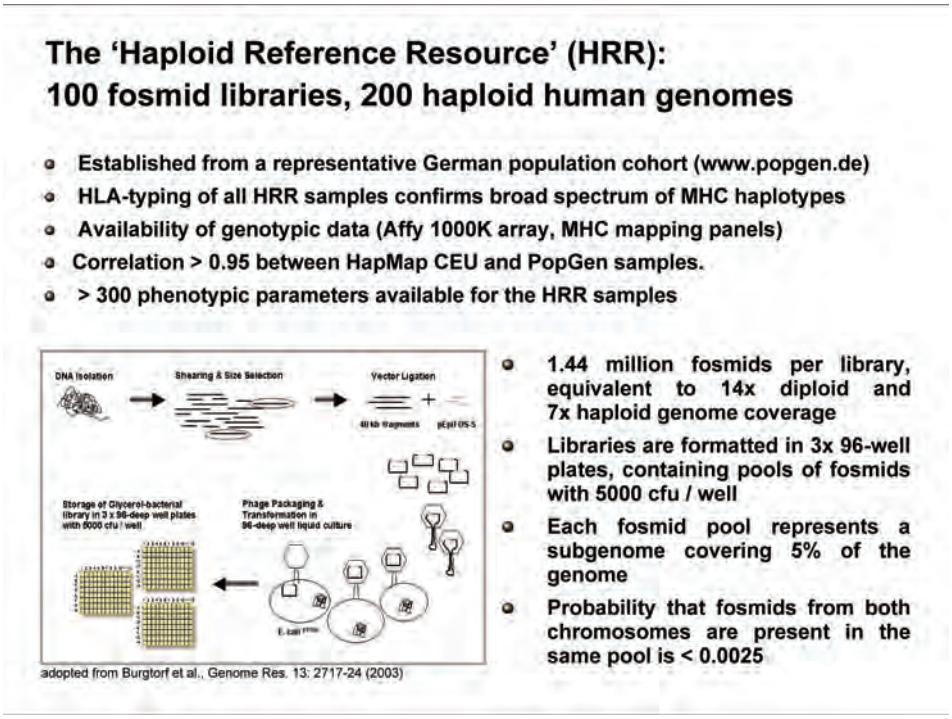

Figure 1

cluding 30 mate-pair (MP) libraries (inserts 600 bp up to 12 kb) and 48 barcode libraries. As to data analysis, project-specific developments such as fosmid detection modules, sub-genome matching and components to identify phase-informative contigs have been integrated into the standard SOliD pipeline, providing the necessary basis for fosmid and MHC specific analyses. NGS of 10 BAC clones from the PGF reference haplotype resulted in 99,996% sequence identity for the MHC, demonstrating high quality performance of our SOLiD sequencing & data analysis pipeline.

MHC haplotype sequencing: An integrated approach to common disease

Our key resources and technologies are presently being applied to generate individual molecular MHC haplotype sequences (~ 4 MB), as a research project central to NGFN-Plus. The following approaches have been taken in parallel: 1) SNP-based mapping and isolation of MHC haplotype-informative fosmids from the clone pools, separate assembly of two MHC fosmid tiling paths, subsequent NGS; 2) hybridization-/PCR-based enrichment of MHC sequences from the fosmid clone pools; 3) direct NGS of entire haploid clone pools. We have determined presence and position of MHC haplotype-informative fosmids in our HRR by HT SNP typing of our fosmid libraries. On average, ~ 94% of the mapped MHC region was found physically covered. Enrichment of MHC sequences is being comparatively tested. First results using microarray-based enrichment protocols are encouraging. With direct NGS of entire fosmid pools sufficient sequence coverage has to date been accumulated to allow first assembly of MHC haplotype sequences. Obviously, NGS of fosmid pools also does accumulate genome-wide sequence coverage (principle illustrated in figure 2), thus preparing the ground for whole haploid genome re-sequencing.

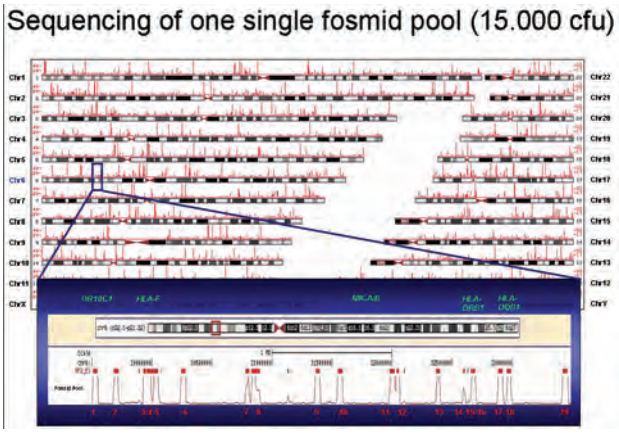

Figure 2

With the projected increase of sequencing throughput (100 GB in 2010), direct NGS of entire fosmid pools becomes viable in the future to generate MHC haplotype sequences - at the added value of whole haploid genomes. The basic principle to generate contiguous haploid sequences relies on the tiling/phasing of identified fosmid sequences by identical alleles in the overlapping regions (see figure 3), a working segment presently addressed. Taken together, our recent lines of research and production provide a valuable platform to tackle highly variable genomic regions and generate haploid sequences on a broader scale.

Perspectives

Continued analysis of DNA sequence variation and its underlying structures in relation to gene structure, function, regulation and (disease) phenotype, ultimately preparing the ground for an individualized medicine. Generation and analysis of haploid sequences of genes, regions of interest and genomes as the basis for a 'haploid biology'.

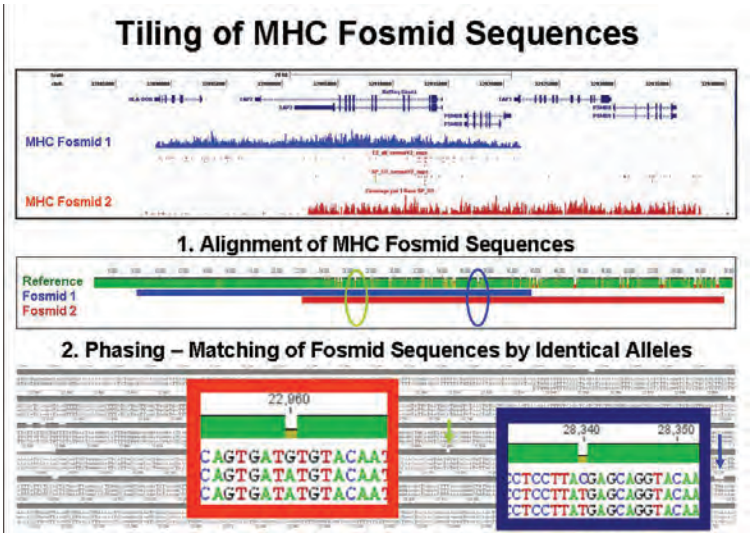

Figure 3

Internal cooperations

- M. Vingron, MPIMG
- H. Lehrach, R. Herwig, MPIMG

External cooperations

- G. M. Church, Harvard Medical School, Boston
- R. Shamir, Tel Aviv University, Tel Aviv
- E. Ziv, D. Hui, UC-Berkeley, Berkeley
- J. Ott, Rockefeller University, New York
- E. Eichler, University of Washington, Seattle
- J. Korbel, EMBL, Heidelberg
- G. McVean, University of Oxford, Oxford
- K. Kidd, H. Zhao, Yale University, New Haven
- J. Zhang, University of York, York
- J. Reich, K. Rohde, Max-Delbrück-Center, Berlin
- S. Schreiber, Christian-Albrechts-University, Kiel
- A. F. H. Pfeiffer, DIfE & Charité, Berlin
- F. F. Horber, Clinic Hirslanden, Zürich/ Clinic Winterthur
- H.E. Wichmann/I. Heid, Helmholtz Zentrum Munich/University Regensburg
- B. Korn, DKFZ, Heidelberg
- R. Wambutt/S. Krüger, AGOWA, Berlin
- N. Schracke, R. Guimil, febit biomed GmbH, Heidelberg
- A. Ullrich, MPI Biochemistry, Martinsried
- H. Ehrenreich, MPI Experimental Medicine, Göttingen

General Information

Selected publications

Hoehe MR (2007). *Individual variation in response to mu opiate receptor challenge - past, present, and future: a "personal" history of investigation*. Dialogues Clin Neurosci. 9: 471-75

Zhang J, Vingron M, Hoehe MR (2005). *Haplotype Reconstruction for Diploid Populations*. Hum Hered 59: 144-56

Hoehe MR (2003). *Haplotypes and the systematic analysis of genetic variation in genes and genomes*. Pharmacogenomics 4: 547-70 (Invited Review, Paper of the Month)

Branson R, Potoczna N, Kral JG, Lentes K-U, Hoehe MR, Horber FF (2003). *Binge eating is a major phenotypic characteristic of melanocortin-4 receptor gene mutations*. New Engl J Med. 348: 1096-1103

Burgtorf C, Kepper P, Hoehe M, Schmitt C, Reinhardt R, Lehrach H, Sauer S (2003). *Clone-based Systematic Haplotyping (CSH) – a procedure for physical haplotyping of whole genomes*. Genome Res. 13: 2717-24

Open-access publication

Heid IM et al. (> 10 authors) (2009). *Meta-analysis of the INSIG2 association with obesity including 74,345 individuals: does heterogeneity of estimates relate to study design?* PLoS Genet. Oct;5(10):e1000694. Epub Oct 23

Selected invited talks

ESHG Meeting, Vienna 2009

2nd PGP-Meeting, Harvard Medical School, Boston, 2008

University of Kent at Canterbury, 2008

Appointments, honors

Who's Who in Science and Engineering, 10th ed. Marquis Who's Who, 2008-2009

Who's Who in the World. 25th ed. New Providence: Marquis Who's Who, 2008

Appointment as Adjunct Professor at The Rockefeller University, NYC, effective 01.06.2005

Work as scientific referee

Referee for the European Science Foundation (ESF), BMBF BioFuture, GIF, and other national and international funding agencies

Reviewer for international journals, such as Hum Mol Genet, Pharmacogenetics BMC Med Genetics, Genomics, Anal Biochem, J Biotechnol, Pain, J Mol Med, Dialogues Clin Neurosci, and others.

Editorial Board member 'Dialogues in Clinical Neuroscience'; coordinator of issues on 'Addictive Substances' 2008, 'Epilepsy and Psychiatry' 2008, 'Genetics and Genomics' 2009/2010

Chairperson and organizer of the Weimar Conference of the German Society of Genetics on 'Genetic Variation in Man'

Member, Scientific Advisory Board, PATH (Patients Tumorbank of Hope)

External funding

BMBF/NGFN-Plus: IG *MHC Haplotype Sequencing: An Integrated Approach to Common Disease*

BMBF/NGFN2: *Haplotype approaches to disease gene discovery: A systematic investigation and establishment of reference resources*

German-Israeli Foundation (GIF): *Haplotyping and Association Algorithms and their Applications to Model Disease Genes*

BMBF BioProfile Potsdam Berlin: '*Genetics and Pharmacogenomics of Obesity*'

BMBF BioProfile Potsdam Berlin: Verbundvorhaben *Innovation des Therapiekonzeptes für das Metabolische Syndrom - Teilprojekt: 'Haplotypenanalyse'*

BMBF/NGFN: *Comparative Candidate Gene Sequencing, Haplotype Analysis and Genetic Risk Profile Identification*

GlaxoSmithKline Award: *Analysis of high-resolution genetic variation data with particular emphasis on haplotype structures and LD patterns*

Public relations

Interview Q&A, In Sequence / Sequencing / GenomeWeb, 2009

Interview 'Technology Review', 'Using HapMap data to unravel diabetes', 2006

In vitro Ligand Screening

(Established: 05/2002)

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Dr. Josmar Langner* (02/05 – 08/07)
Dr. Jürgen Kreutberger* (until 01/07)
Dr. Jörn Glökler (01/04 – 06/04)
Dr. Axel Wettich (10/03 – 12/03)

Engineer
Jeannine Wilde*, Dipl. Ing. (FH) (05/02 – 05/08)

Technicians
Carola Stoschek (since 05/02)
Annette Poch-Hasnek* (07/05-04/08)

PhD students
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Stephan Klatt* (since 10/08)
Florian Rubelt* (since 10/08)
Theam Soon Lim* (09/06 – 08/09)
Chenna Reddy Galiveti* (11/04-08/09)

Scientific Overview

The focus of the group centres on the application of *in vitro* selection techniques such as phage display and protein array technology for the identification and characterisation of antibody – protein interaction pairs. Primary goals of our work are the development of research reagents in form of human recombinant antibodies, and the elucidation of antibody-antigen interaction patterns in human diseases, with a special focus on autoimmunity.

Generation of research antibodies

Antibodies, or more commonly binder molecules, are inevitably important tools for the functional characterisation of human gene products. Next to immunisation of various laboratory animals, a number of *in vitro* selection techniques exist to obtain highly specific binders, of which phage display is the most common. Within the last few years, we have worked on a conveyer-belt type production pipeline for the generation of human recombinant antibody molecules applying the phage display technology.

Selection of phage display-derived antibodies

Within the NGFN 2 funding period we successfully participated in the SMP "Antibody Factory", which set out to evaluate all necessary processes for establishing phage display of human antibody fragments as a routine method to generate binders against human gene products on large scale. Our subproject was concerned

with the semi-automation of panning and evaluation procedures. We considered all necessary aspects ranging from the expression of *in vivo* biotinylated antigens in *E. coli* or mammalian cells and more recently *Leishmania tarentolae* as hosts. Further, we developed a selection process based on robotic manipulation of streptavidin-coated magnetic bead, as well as protein microarray-based screening methods for fast determination of target-specificity by applying the Multiple Spotting Technique. Additionally, we developed a laboratory information management system (LIMS) to store all generated clones and relevant experimental data. Finally, we succeeded with the assembly of streamlined selection pipeline which now could go into production if funding were available.

Selection of binder molecules with inhibitory properties

This project is dedicated to the selection of binder molecules, which allow specific blocking of protein-protein interactions and was developed in close collaboration with the group of Dr. Sylvia Krobitsch in our department (now Otto Warburg Laboratory). We successfully established a combined *in vitro* / *in vivo* selection scheme to obtain inhibitory antibodies, called intrabodies. First, phage display libraries are enriched *in vitro* on immobilised target proteins and once the diversity of the libraries are reduced to a complexity amenable to reverse yeast-2-hybrid screens, the selection is continued *in vivo*, where the selection is carried out with interaction pairs of the target proteins. Resulting binders not only bind their respective target proteins, but also inhibit defined protein-protein interaction of the targets *in vivo*. The method is applied using protein-protein interaction pairs involved in Spinocerebellar Ataxia type 2 (Ataxia UK).

Exploring autoimmune antibody libraries

On the quest to obtain the best antibody scaffold for phage display, we have generated several new semi-synthetic antibody libraries in different formats and are currently assembling a large naïve antibody library exclusively from donors with autoimmune disorders. This group of individuals possess large number of antibodies directed against self-proteins and should therefore be a great resource for developing binders against human gene products in future. Furthermore, we are interested to find out if there is a correlation between individual V-gene usage and autoimmune disorders. For this purpose, we intend to select specific binders towards known autoantigens in different autoimmune disorders and combine the outcome of these selections with the knowledge of general antibody repertoire of autoimmune patients we obtain applying next generation sequencing technologies.

Discovery of antibody–antigen interaction pairs in human disease

Many human diseases are characterised by the presence of antibodies directed towards self-proteins. In most cases, the existence of self-reactive antibodies is not fully understood, as the pathogenic role of such antibodies is – with some exceptions – not known. This is also clearly demonstrated by the fact, that every individual has autoantibodies even without being affected by disease and that autoantigenicity patterns overlap (Figure 1). Furthermore, our knowledge about the role of certain autoantibodies in disease progression, whether being of significance or simply a bystander effect, is still vague.

In this respect, we focused our work in recent years in close collaboration with the Charité on the characterisation of autoantigenicity patterns in healthy and diseased individuals. We concentrated primarily on autoimmune disorders and the diseases under investigation ranged from systemic to organ-specific autoimmune diseases (BMBF-Nutrigenomics project), including systemic lupus erythematosus, rheumatoid arthritis, celiac disease and thyroiditis (Graves' and Hashimoto).

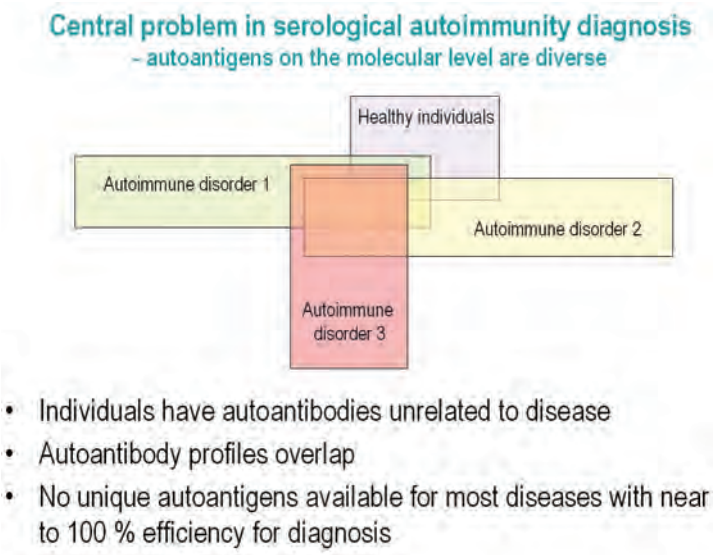

Figure 1

Further, screening with sera of dilated cardyomyopathy patients were performed (SFB-TR19 project) and currently autoantigenicity profiling for multiple sclerosis and Alzheimer's disease are on the way (BMWi-ZIM project). Additionally, we expanded our efforts to identify biomarker sets for therapy response prediction in systemic autoimmune disorders on the example of second line treatment of rheumatoid arthritis with tumour necrosis factor alpha (TNFα) blocking agents.

Next to protein arrays, screening for autoantigens is additionally performed using cDNA expression libraries cloned in M13 or T7 display vectors presenting the recombinant proteins on the bacteriophage surface. Selection is carried out in an iterative process – essentially based on affinity enrichment – using patient-derived immunoglobulin fractions as selection targets and finally, mass sequencing the cDNA inserts applying next generation sequencing technology of individual bacteriophage molecules identifies the putative autoantigens (Figure 2).

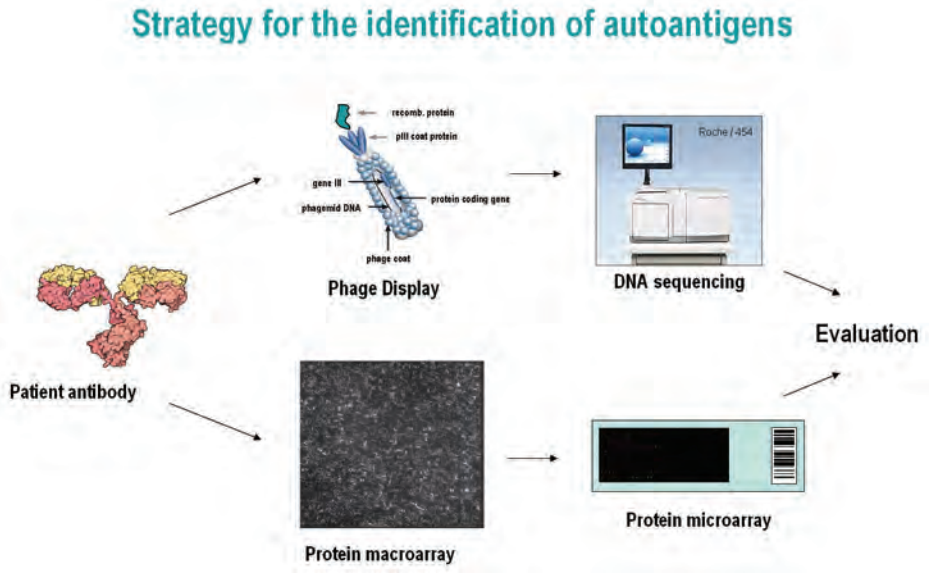

Figure 2

Generating autoantigenicity patterns applying protein arrays

For basic screening we apply high-density protein macroarrays carrying >38.000 clones expressing human recombinant proteins, which are comprised of >10.000 different genes and splice variants thereof. Using this technology, which was developed in our department in the late 90's, led to the identification of ~1.600 clones expressing potential autoantigens against which healthy and diseased individuals reacted. Notably, the number of autoantigens detected in different disorders decrease with organ specificity. For instance, in rheumatoid arthritis and celiac disease, more than 500 different antigens were scored positive, while in thyroiditis, only ~ 200 clones reacted.

To elucidate the autoreactivity pattern of celiac disease patients in more detail, we have generated protein microarrays with a selection of 160 purified recombinant human proteins and controls, such as commonly used diagnostic markers for the

disease (tissue transglutaminase and wheat gliadin) and compared the screening results of 142 patients with that of 50 healthy individuals. Finally, we could identify a number of autoantigens which might serve for diagnostic purposes in patients with IgA-deficiency in future. This is particularly useful, since coeliac disease patients with IgA-deficiency remain frequently undetected in routine diagnosis.

Identification of diagnostic markers for therapy response prediction

In systemic autoimmune disorders, treatment comprises the administration of corticosteroids or immunosuppressive medication. In rheumatoid arthritis, first line treatment is carried out with disease-modifying antirheumatic drugs (DMARDs), such as Methotrexate (MTX). Second line treatment of MTX-resistant patients is carried out with biologicals, mainly tumour necrosis factor alpha (TNFα) blocking agents, such as monoclonal antibodies (infliximab, adalimumab) or soluble TNFα-receptor (etanercept). The major drawback of treating rheumatoid arthritis with TNFα inhibitory biologicals is its high cost as well as the fact that 30-40% of treated individuals do not respond or only poorly respond to treatment. In a pilot study, we have identified a set of biomarkers according which we can potentially discriminate between therapy responders and non-responders and are currently evaluated (BMBF-KMU Innovativ). Furthermore, a clinical study is on its way to recruit 150 patients treated with etanercept (sponsored by Wyeth BioPharma GmbH). Screening autoantigenicity patterns with these specific patient sera shall allow the identification of therapy prediction biomarkers specific for etanercept.

Cooperations within the institute

- Lars Wittler - *in vivo* application of intrabodies (Dept. of Developmental Genomics)
- Jörn Glökler - technology development & crystallography (Dept. of Vertebrate Genomics)
- Michal Schweiger - generation of research antibodies & intrabodies (Dept. of Vertebrate Genomics)
- Sylvia Krobitsch - generation of research antibodies & intrabodies (OWL)

External academic cooperations

- Stefan Dübel, Institute for Biochemistry and Biotechnology, Technical University Braunschweig
- Ronald Frank, Department of Chemical Genomics, GBF, Braunschweig
- Jürgen Brosius, Institute for Experimental Pathology, University Münster
- Steffen Hennig, German Resource Center for Genome Research - RZPD, Berlin
- Karl Skriner, Gerd-Rüdiger Burmester, Falk Hiepe, Dept. Rheumatology & Clin. Immunology, Charité – University Medicine Berlin
- Stephan B. Felix, Policlinic for Internal Medicine, University Greifswald
- Uwe Völker, Faculty of Medicine, University Greifswald
- Reto Crameri, Swiss Institute of Asthma and Allergy Research, Davos, Switzerland
- Bernd Müller-Röber, Inst. for Biochemistry & Biology, University Potsdam
- Wolfram Saenger, Institute for Chemistry and Biochemistry, Dept. of Crystallography, Free University Berlin
- Martin Steup, Institute for Biochemistry and Biology, University Potsdam
- Reinhard Lipowsky, MPI for Colloids and Interfaces, Potsdam
- Mario Mörl, Institute for Biochemistry, University Leipzig

Industrial cooperations

- Imagenes GmbH, Berlin
- IMTEC Immundiagnostika GmbH, Berlin
- in.vent Diagnostica GmbH, Hennigsdorf
- RiNA GmbH, Berlin
- Wyeth BioPharma GmbH Münster

General information

Selected publications

Gloriam DE, Orchard S, Bertinetti D, Björling E, Bongcam-Rudloff E, Bourbeillon J, Bradbury AR, de Daruvar A, Dübel S, Frank R, Gibson TJ, Haslam N, Herberg FW, Hitke T, Hoheisel JD, Kerrien S, Koegel M, Konthur Z, Korn B, Landegren U, van der Maarel S, Montecchi-Palazzi L, Palcy S, Rodriguez H, Schweinsberg S, Sievert V, Stoevesandt O, Taussig MJ, Uhlén M, Wingren C, Woollard P, Sherman DJ, Hermjakob H (2009). *A community standard format for the representation of protein affinity reagents*. Mol Cell Proteomics 2009 Aug 11 [Epub ahead of print].

Menzel C, Schirrmann T, Konthur Z, Jostock T, Dübel S (2008). *Human antibody RNase fusion protein targeting CD30+ lymphomas*. Blood 111, 3830-3837

Konthur, Z. (2007). *Automation of Selection and Engineering*. In *Handbook of Therapeutic Antibodies*. Wiley-VCH (*S. Dübel, Ed.*), ISBN: 978-3-527-31453-9, 413-431.

Taussig MJ, Stoevesandt O, Borrebaeck CA, Bradbury AR, Cahill D, Cambillau C, de Daruvar A, Dübel S, Eichler J, Frank R, Gibson TJ, Gloriam D, Gold L, Herberg FW, Hermjakob H, Hoheisel JD, Joos TO, Kallioniemi O, Koegl M, Konthur Z, Korn B, Kremmer E, Krobitsch S, Landegren U, van der Maarel S, McCafferty J, Muyldermans S, Nygren PA, Palcy S, Pluckthun A, Polic B, Przybylski M, Saviranta P, Sawyer A, Sherman DJ, Skerra A, Templin M, Ueffing M, Uhlen M (2007). *Proteome Binders: planning a European resource of affinity reagents for analysis of the human proteome*. Nat Methods 4, 13-7.

Hultschig C, Kreutzberger J, Seitz H, Konthur Z, Büssow K, Lehrach H (2006). *Recent advances in protein microarrays*. Curr Opin Chem Biol 10(1):4-10

Selected invited talks

Konthur, Z. (2009). *From gene to antibody – A phage display selection pipeline for antibody generation.* 8th International Congress on Recombinant Antibodies, Pre-Conference Symposium W, Cologne, Germany

Konthur, Z. (2008). *In vitro Selektion & Evolution molekularer Bindungspartner*. Kolloquium „Molekulare Biotechnologie“, University Potsdam, Germany

Konthur, Z. (2006). *The use of high-density protein arrays and phage display for the identification of novel autoantigens*. DECHEMA - Technologieforum Diagnostik, Frankfurt a.M., Germany

Galiveti, C.R. (2006). *RNomics – Prediction and analysis of in silico-derived non-protein coding RNAs in the human genome*. HUGO's 11th Human Genome Meeting, Helsinki, Finland

Sievert, V. (2006). *A short overview of abdb – a LIMS for managing antibody screening data in the Antibody Factory*. Proteome Binders Workshop on Standards and Ontologies for Binder Data Representation, St. Emilion, France

Membership in professional societies

- German Society for Proteome Research (DGPF)
- German Society for Biochemistry and Molecular Biology (GBM)
- International Society of Molecular Recognition
- Human Proteome Organisation (HUPO)

Work as scientific referee

Zoltán Konthur serves as referee for the following journals: Nucleic Acids Research, Cell Stress & Chaperones, Journal of Biotechnology, European Journal of Cancer, Mini-Reviews in Medicinal Chemistry

Patents

Konthur, Z., Skriner, K., Lehrach, H. (2009) Diagnostic Prediction of Rheumatoid Arthritis and Systemic Lupus Erythematosus (filed)

Konthur, Z., Skriner, K., Lehrach, H. (2008) Biomarker for the prediction of responsiveness to an anti-Tumour Necrosis Factor alpha (TNFalpha) Treatment (WO/2009/056633)

External funding

BMBF – KMU-Innovativ: *pre.markTNF - Anti-TNFalpha Therapy Response Prediction*, 01/2009 – 12/2011

Robert Bosch Stiftung: *Investigations into patients immune response to the parasite Schistosoma japonicum*., 09/2009 – 09/2010

BMWi – ZIM: *Detection of Biomarkers by Differential Screening applying Phage-Display, Next Generation Sequencing and Protein-Array Technology*, 05/2009 – 04/2011

Wyeth BioPharma: *PREDICT - Evaluation of diagnostic markers for prediction of response to Etanercept*, 01/2009 – 12/2010

EU-FP6: *Proteome Binders - A European Infrastructure of Ligand Binding Molecules against the Human Proteome*, 03/2006 – 02/2010

BMWi – ProInno II: *Application of in-vitro protein biosynthesis for microarray formats*, 03/2007 – 02/2009

Ataxia UK: *Investigations into the cellular function of Ataxin-2 by the use of ssRNA aptamers*, 09/2005 – 08/2008

DFG – SFB TR19: *Inflammatory Cardiomyopathy – Molecular Pathogenesis and Treatment*, 07/2004 – 06/2008

BMBF – NGFN-2: *SMP Antibody Factory*, 01/2005 – 05/2008

BMBF – BioProfile Nutrigenomics: *Identification of novel diagnostic markers for Coeliac Disease & Investigation of immunoreactive compounds in nutritional sources*, 05/2005 – 04/2008

BMBF – NGFN-2: EP *Exploring the world of non-messenger RNA: RNomics meets Proteomics*, 11/2004 – 10/2007

Teaching activities

Single Lecture as part of the lecture series *From functional genome research to systems biology*, Free University Berlin, winter term 06/07, 07/08, 08/09, 09/10

Lecture series *High-throughput methods in biomedical research*, Technical University Braunschweig, winter term 06/07, 08/09, 09/10

Laboratory course *Biochemical laboratory methods*, Technical University Braunschweig, winter term 06/07

Laboratory course *Molecular Biotechnology I*, Technical University Braunschweig, summer term 08, 09

Public Relations

Konthur, Z. Interview for: A Planck Walk. The Scientist, 2008; 22(7), 46-53.

Schirrmann T, Hust M, Konthur Z, Frank R, Toleikis L, Dübel S (2007). *Schlüsselreagenzien für die Proteomforschung im Hochdurchsatz*. Laborwelt 2/07, 16-20.

Protein Complexes and Cell Organelle Assembly

(Established: 05/2003)

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Dr. Thomas Kessler* (since 07/07)
Marie-Laure Fogeron* (04/05-09/09)
Dr. Christian Regenbrecht* (05-07)

Engineer
Tanja Kurtz* (since 07/06)

Technicians
Karl-Heinz Rak (since 01/72)
Verena Lehmann* (since 07/03)
Anne Oelmann* (since 11/07)
Melanie Hessler* (since 08/09)
Daniela Sauvageot* (06/08-04/09)
Stephanie Siebert (01/07–10/07)

PhD students
Karin Habermann* (since 12/05)
Anne-Kathrin Scholz* (since 05/06)
Armin Haupt* (since 10/07)
Nicole Hallung* (since 06/08)
Seon-Hi Julia Jang* (since 09/08)

Scientific overview

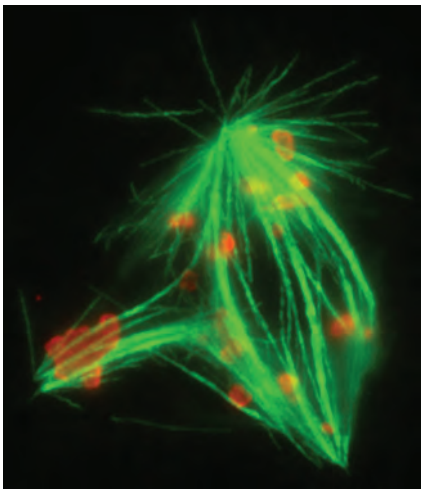
Background

New opportunities are presently available for the direct investigation of the molecular basis of diseases caused by mutation of genes. The technology in the area of functional genomics has matured and can now be applied to basic questions in the fields of developmental biology, cell biology and molecular medicine. This, in turn, facilitates the study of many parameters in parallel (mRNA and protein profiles, morphological cellular or subcellular analysis) and their dynamic changes upon modulating cues (*e.g.* cellular or environmental stress). Such complex data sets require now system models to understand and predict diseases development. In this context, the study of the protein complexes and protein-protein interactions of the components of cellular proliferation and signalling pathways is now an opportunity and highly relevant for our understanding of the molecular basis of development and diseases.

Current state of research and scientific findings

The main research focus of our group is the regulation of cell proliferation pathways in human cells and *Drosophila*. Critical for cellular division and tissue homeostasis is the cytoskeleton and the centrosome (the microtubule organizing centre in the eukaryotic cell).

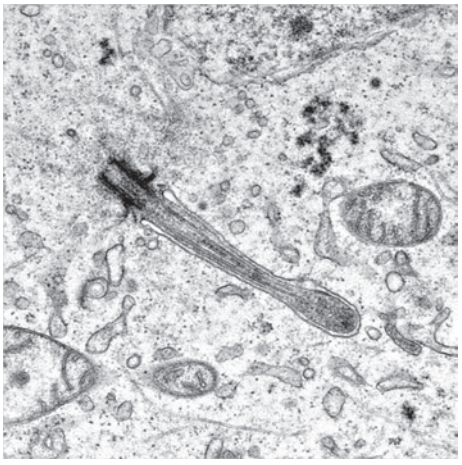
* externally funded



Immunofluorescence microscopy staining of a Drosophila SL2 cell, uncovering the function of an identified centrosomal component from our RNA interferences functional screen. Chromosomes (red) are displaced from the metaphase plate, while microtubuli filaments (green) have formed an extra half spindle in vicinity to the main spindle.

We have set up an experimental system that combines cell biological, functional genomics and proteomics approaches. Using these approaches, we identified the proteome of the *Drosophila* mitotic centrosome and we functionally characterised all the components, *via* RNAi. From the 260 identified proteins we could assign new functions to 27 proteins. We could describe a core set of 11 proteins that is required for maintaining centrosome integrity. We focused subsequently our work on these 11 proteins as centrosome stability is frequently compromised in human cancer cells. Characterisation of the human orthologues of these 11 proteins identified 4 functionally highly conserved components that are now being analysed in detail. The first results of this study have been published (Müller *et al.* 2006) with a detailed follow-up analysis in revision (Müller *et al.*).

From the identified centrosomal proteins, those with novel centrosome-associated functions are analysed *via* two basic approaches: First they are characterised on the developmental, cellular and biochemical level in the fly. To this end, we employ tissue-specific overexpression of target proteins and conditional depletion of proteins through RNAi, transgenic or mutant fly stocks. Second, we identify the protein complexes, *via* which these molecules excert their function, using protein complex isolation from *Drosophila* embryo extracts and tandem affinity purification (TAP) from human cells. Both approaches have defined overlapping and complementary lists of proteins that we exploit for our subsequent work. Our TAP experiments have identified an interaction network of 2700 proteins. From this set, we selected 20 proteins that were annotated in databases to be deregulated in cancer. These proteins have been characterised by RNAi and over-expression approaches in human tissue culture revealing striking effects on cell cycle regulation and for centrosome function (Fogeron & Lange *unpublished*).



Transmission electron microscopy of a basal body and primary cilium in a human U2OS osteosarcoma cell. In mammalian cells primary cilia are an essential part of the cellular signaling in development. Our experiments identified novel human centrosome components depletion of which leads to the abrogation of the basal body (dark tubular structure on the left) that subtends the cilium (Fogeron & Lange).

In addition, the group is participating in three medically related and complementary network projects. As part of *Mutanom,* (www.mutanom.org), which I coordinate, we study the consequence of cancer-related mutations on the cellular and organism level, aiming to build predictive models for disease development and for the identification of new molecular targets for site-specific therapies. Second, as part of *NeuroNet*, we are investigating the protein-interaction networks implicated in the development of neurodegenerative diseases employing our TAP approach. Third, as part of the network *Systems Biology of the Cell,* we are studying estrogen receptor (ER) signalling upon modulating the ER pathway activity. In all three projects we contribute with the methods established in the group (human isogenic cell line system, expertise in protein complex isolation, phenotypical assays) to analyse the consequence of disease-related genes on a biochemical and cellular functional level.

Cooperation within the Institute

- Ralf Herwig, Christoph Wierling, Dept. of Vertebrate Genomics: Modelling of pathways (Mutanom project)
- Marie-Laure Yaspo, Dept. of Vertebrate Genomics: Characterisation of BACH1 and AIRE1 transcription factors, ChIP, TAP (Mutanom project)
- Michal Schweiger, Dept. of Vertebrate Genomics: Second-generation sequencing of cancer cells & tissues (Mutanom project), TAP of viral DNA-binding proteins (viral signalling).
- Thorsten Mielke, Rudi Lurz, Electron Microscopy Group: Cryo-electron microscopy of protein complexes; gamma-TuSC (USN & AWZ projects)

General information

Selected publications

Varmark, H.; Llamazares, S.; Rebollo, E.; Lange, B.M.H.; Reina, J., Schwarz, H., Gonzalez, C., (2007). *Asterless Is a Centriolar Protein Required for Centrosome Function and Embryo Development in Drosophila.* Curr Biol 17(20): 1735-1745

Müller, H., Fogeron, M.-L., Lehmann, V., Lehrach, H. and B.M.H. Lange (2006). *A Centrosome-Independent Role for γ-TuRC Proteins in the Spindle Assembly Checkpoint.* Science 314: 654-656

Lehmann, V.; Müller, H. and Lange, B.M.H. (2005). *Immunoisolation of centrosomes from Drosophila melanogaster.* Curr Protocols in Cell Biol 29: 3.17.1-3.17.13.

Sauer, S., Lange, B.M.H., Gobom, J., Nyarsik, L., Seitz, Harald and Lehrach, H. (2005). *Miniaturization in functional genomics and proteomics*. Nature Reviews Genetics, 6(6): 465-476

Selected invited talks

Keynote lecture at the Biotechnica 2008, Hannover

Work as scientific referee

Bodo Lange serves as referee for the following journals: Cancer Research, Cell Motility and the Cytoskeleton, Contributing Member of Faculty of 1000, EMBO Journal, Human Molecular Genetics, Journal of Cell Science, Trends in Cell Biology, Oncogene, PLOS Biology

Grant evaluation

Bodo Lange serves as referee for the following institutions: GEN-AU, DFG, Boehringer Ingelheim Fonds, Humboldt Foundation, Czech Science Foundation.

External funding

BMBF: GABI CentroPlanta, 2007 - 2010

BMBF: *Mutanom (coordination),* 2008 - 2011

BMBF: Neuronet, 2008 - 2011

BMBF: Systemsbiology of the Cell , 2008 - 2011

EC: PHGEN II, 2009 - 2012

EFRE: Anwenderzentrum, 05/2006 - 11/2008

EFRE: Ultrastructure Network, 06/2003 - 06/2006

BMBF / NGFN2: SMP Protein, 2004 - 2008

Thyssen Foundation, 2005 - 2007

Teaching activities

Biannual practical course in *Drosophila Genetics and Cell Biology* at the FU Berlin (part of main studies), 2003 - current

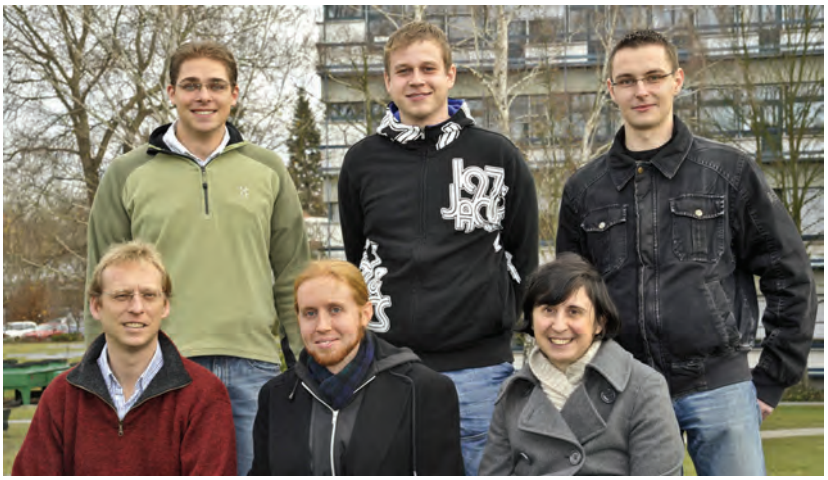
Practical course in *Drosophila Developmental and Cell Biology* as part of the Module 3 of the Molecular Medicine Master Course, Charité, 2006/2007

Public relations

Participation and demonstration of group research in the “Lange Nacht der Wissenschaften” i.e. open night for the public

Evolution & Development

(Established: 2003)



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Dr. Alexander Kühn (since 08/09)
Dr. Detlef Groth (until 03/07)
Maryam Khorasani* (until 11/06)

PhD students
Peggy Matz (since 06/09, guest)
Udo Georgi (since 08/09)
Alexander Kühn (until 07/09)

Technician
Vesna Weisse (until 01/08)

Scientific overview

Our research focuses on the evolution of genome organisation with the purpose of contributing to the question of how novel characters and complexity arise. We investigate how gene and whole genome duplications (WGDs) increase the genetic toolkit available to an organism, modify gene order *via* the rearrangements they trigger and promote speciation. Furthermore, we study the degree of flexibility within a given regulatory module and in extent in a gene network during evolution *via* experimentally analysing and computationally modelling the endo-mesoderm and apical organ networks in sea urchins. Finally, the group within the frame of its research focus has generated genomics resources and databases that have been extremely helpful to the scientific community (see Research Report 2006 and http://www.molgen.mpg.de/~evolutiondevelopment/).

Genome duplications at the origin of vertebrates

Via the analysis of conserved gene order between vertebrate genomes and amphioxus we provided sufficient evidence supporting the *2R hypothesis*. This hypothesis claimed the existence of two rounds of WGDs during early vertebrate evolution. The *2R hypothesis* has been a hotly debated topic for many years - in large part because phylogeny-based approaches and the lack of sequence data from key organisms could not distinguish WGD from other gene duplication models (see Research report 2006; Panopoulou et al. 2003; Panopoulou and Poustka 2005).

* externally funded
** financed by Martin Vingron

The recent release of the amphioxus genome allowed us to reevaluate the *2R hypothesis*. Even with the amphioxus genome present, identifying syntenic segments and estimating genome rearrangement rates in the ancient vertebrate lineages is not trivial as there is no reliable method that could use genomes in scaffold-level assemblies, such as the current amphioxus genome assembly. We developed an own gene pair method to identify syntenic segments which once more illustrated the traces of the vertebrate WGD events. We surprinsingly found that, while amphioxus remains the best representative of the early chordate genome, its genome structure is not particularly well conserved and it cannot be considered a fossilization of the vertebrate pre-duplication genome (Hufton et al. 2008).

Polyploidy and rearrangements

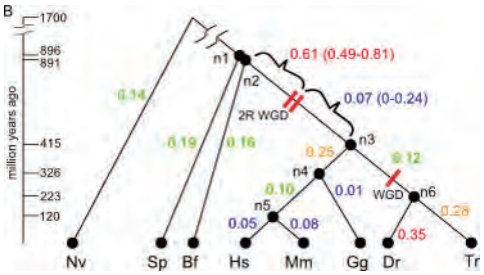

Figure 1: Syntenic distances were apportioned to the known species tree and then divided by the estimated evolutionary time in each branch to obtain rates of synteny loss. Internal nodes are labeled n1–n6. The highest rates of loss are observed in the period after the vertebrate divergence from amphioxus but before the early vertebrate WGD events (n2–2R WGD), and in the terminal zebrafish lineage (n6–Dr). Species abbreviations are human (Hs), mouse (Mm), chicken (Gg), fugu (Tr), zebrafish (Dr), amphioxus (Bf), sea urchin (Sp), sea anemone (Nv).

Evidence from research on plant polyploidy suggests various mechanisms by which WGD stimulates genome rearrangement, including aberrant recombination, transposable element activation etc. Applying an own metric for calculating syntenic distances and estimating synteny loss rates across vertebrate and invertebrate genomes, we found that vertebrate WGD events have not induced genome rearrangement (figure 1), but they may have been a symptom of a pre-existing predisposition toward genomic structural change (Hufton et al. 2008). In our recent review of the latest evidence regarding genome rearrangements after WGD, we find that genomic responses to WGD are also highly varied (Hufton and Panopoulou 2009).

Fates of gene duplicates and regulatory elements

It is proposed that cis-regulation may play a key role in determining which genes are retained after duplication *via* the evolution of new beneficial functions (neofunctionalization) or the division of the function of the ancestral gene between the newly formed duplicates (subfunctionalisation). Examples of expression partitioning *via* the subdivision of regulatory elements exist in vertebrates and in yeast where genes with complex cis-regulation are over-retained after duplication. An alternate model is the Gene Balance Hypothesis (GBH) which proposes that selection against gene dosage imbalances determines which duplicates will be retained.

To understand the role of cis-regulation in duplicate retention, we initially developed an approach that would identify conserved non-coding elements (CNEs) between evolutionary distant genomes which relies on sensitive local similarity searches in the genomic regions surrounding phylogenetically defined gene families (Hufton et al 2009). Using this method we were the first to identify a large number of CNEs that have been conserved between vertebrates and nonvertebrates, including more than a thousand CNEs in the invertebrate amphioxus which diverged prior to the vertebrate duplications. We tested 42 of our CNEs in transgenic zebrafish assays (figure 2) - including examples from vertebrates and amphioxus - and found that the majority are functional enhancers (Hufton et al. 2009).

Via classifying mouse and zebrafish genes according to association with CNEs, synteny conservation and duplication history we made two key observations: i) subfunctionalization of conserved cis-regulation has not been the primary determinate of gene duplicate retention in vertebrates. Instead, the data support the

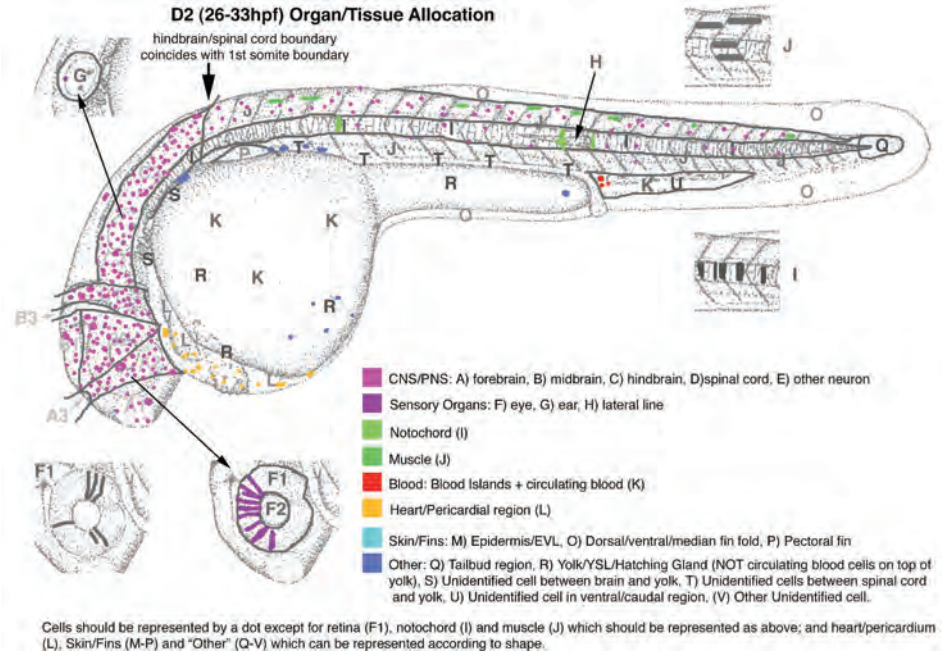

Figure 2: Tissue specific expression pattern driven by the Zf-SoxB1-Element 5 in the zebrafish 24hr embryo.

gene balance hypothesis. ii) CNEs located outside of their predicted target genes (extragenic CNEs) are strongly associated with the conservation of synteny (Hufton et al. 2009).

Gene regulatory networks (GRNs)

Via treatments of seaurchin embryos with LiCl2 that leads to excess endomesoderm and ZnCl2 that blocks endomesoderm formation and the subsequent hybridisation of the respective RNAs on cDNA arrays, we isolated novel tissue specific markers (Poustka et al. 2007). Whole-mount *in situ* hybridization (WISH) analysis of 700 differentially expressed genes showed that the apical organ region is eliminated in lithium-treated embryos. Conversely, apical and neural markers are expressed more broadly in zinc-treated embryos where the number of serotonergic neurons is amplified by at least tenfold (figure 3). To study the extent of conservation of the seaurchin apical organ GRN, we completed a WMISH screen of the orthologs of 50 seaurchin apical organ patterning transcription factors isolated from the sea anemone *Nematostella vectensis*.

While details about many GRNs are emerging, their extent of completeness is unknown. This uncertainty stems from limited experimental data, which is the main bottleneck for creating detailed dynamical models of cellular processes. We

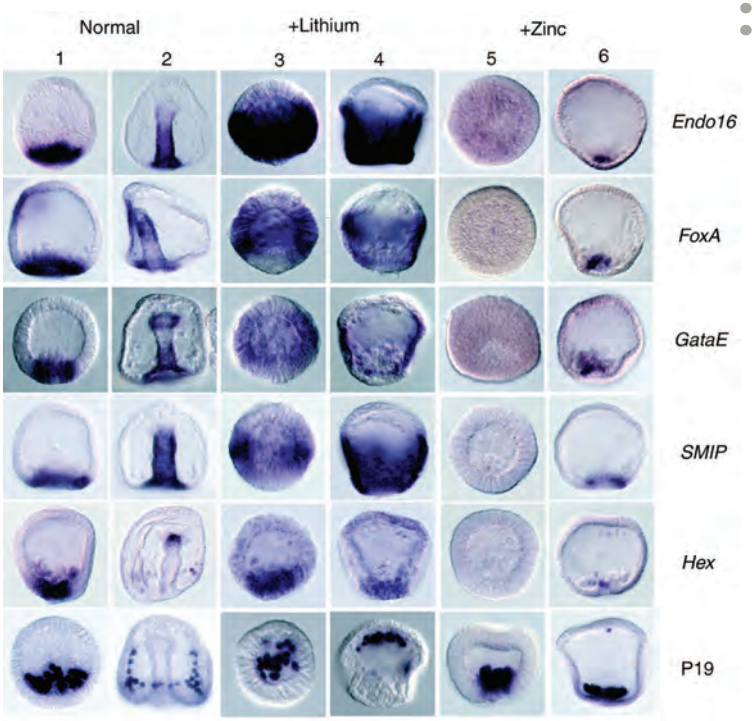

Figure 3: Expression of endomesoderm markers in normal, lithium-treated and zinc-treated embryos. Shown are whole-mount in situ hybridizations (WISHs) of endomesodermal marker genes on blastula stage (columns 1, 3, and 5) and gastrula stage (columns 2, 4, and 6) sea urchin embryos. The gene names are listed on the right hand side.

developed a method, based on random parameter estimations through Monte-Carlo simulations to measure completeness grades of GRNs (Kuhn et al. 2009) which we applied on the Endomesoderm GRN. Our method could be especially useful for GRNs involved in human diseases, where often the amount of connectivity is unknown and/or many genes/interactions are missing.

Perspectives

The evolution of new gene functions is one of the keys to evolutionary innovation. Our goal is to understand the involvement of duplications and rearrangements in this process. The evolution and plasticity of GRNs, the synteny breakpoints and their re-use during chromosome evolution and the evolution of transcription binding sites will remain our focus.

Internal cooperations

- Martin Vingron, Dept. Computational Molecular Biology
- Stefan Mundlos, Development & Disease Group

External cooperations

- Vladimir Benes, Genomics Core Facility, EMBL
- Patrick Lemaire, LGPD, IBDM, Marseille, France
- Eric Davidson, Div. of Biology, California Institute of Technology, USA
- D. McClay, Department of Biology, Duke University, Durham, USA.
- Cynthia A. Bradham, Biology Department, Boston University
- Mike Thorndyke, Kristineberg Marine Research Station, Sweden

General information

Selected publications

Kühn C, Wierling C, Kühn A, Klipp E, Panopoulou G, Lehrach H, Poustka AJ. *Monte-Carlo analysis of an ODE Model of the Sea Urchin Endomesoderm Network*. BMC Syst Biol. 2009 Aug 23;3(1):83

Hufton AL, Panopoulou G. *Polyploidy and genome restructuring: a variety of outcomes.* Curr Opin Genet Dev. 2009; Nov 7.

Hufton AL, Mathia S, Braun H, Georgi U, Lehrach H, Vingron M, Poustka AJ, Panopoulou G. *Deeply conserved chordate noncoding sequences preserve genome synteny but do not drive gene duplicate retention*. Genome Res. 2009;19(11):2036-51.

Hufton AL, Groth D, Vingron M, Lehrach H, Poustka AJ, Panopoulou G. *Early vertebrate whole genome duplications were predated by a period of intense genome rearrangement*. Genome Res. 2008; 18(10): 1582-91.

Poustka AJ, Kühn A, Groth D, Weise V, Yaguchi S, Burke RD, Herwig R, Lehrach H, Panopoulou G. *A global view of gene expression in lithium and zinc treated sea urchin embryos: new components of gene regulatory networks*. Genome Biol. 2007; 8(5):R85.

Teaching

Otto Warburg International Summer School and Workshop on Evolutionary Genomics, August 2006, Berlin

Work as scientific referee

Georgia Panopoulou und Albert Poustka serve as scientific referees for the following journals: Trends in Genetics, Genome Research, Genome Biology, BMC Evolutionary Biology, PLoS Computational Biology

Grant evaluations

National Science Foundation (NSF), USA

External funding

FP6 EU: Network of Excellence *Marine Genomics* (2003-2008)

Molecular Biology of Metabolism

(Established: 12/2007)

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PhD students
Nana-Maria Grüning, since 02/09
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Scientific overview

The metabolic network is of modular architecture and robust. It adapts to biological stimuli, perturbations or environmental pressure, which requires an intensive crosstalk with the transcriptome and proteome. Two categories of *reporter metabolites* bridge between the small-molecule and macromolecular universe of the cell: General metabolic cofactors, responsible for most of the interconnectivity within the metabolome, are monitored to sense the overall state of the network. In contrast, pathway-specific intermediates cooperatively tune idiosyncratic biological processes. Since metabolic transitions can affect multiple reporter metabolites with a different kinetic, metabolic pathways off the possibility to control biological timing. We are interested to identify basic mechanisms, how dynamic transitions in the metabolic flux are induced, and how they interfere with regulation of the transcriptome and proteome of the cell. Furthermore, we develop techniques that allow the identification of biological targets of bio-active small molecules. These techniques are currently applied in studies of the aging process, an unavoidable consequence of metabolic activity.

Current state of research and scientific findings

Starting and central point of our investigations on the cellular metabolome is a dynamic transition in the central carbohydrate catabolism. We found that a redirection of the glycolytic flux from glycolysis to the pentose phosphate pathway is central for eukaryotic cells to cope with oxidative perturbations [Ralser et al., 2007]. This deviation stabilizes the pool of redox co-factors required for the antioxidative machinery, and yet unpublished results demonstrate that this metabolic redirection *per se* is capable to induce the anti-oxidative response. Interestingly, the biochemical induction of this redirection is compartmentalized over time: first, within seconds after a perturbation, a central enzyme if glycolysis is inactivated and acts as redox switch. Later (minutes after the perturbation), transcriptional control takes over this process and allows the cell to return to normal metabolism [Ralser et al, 2009a] (Figure 1).

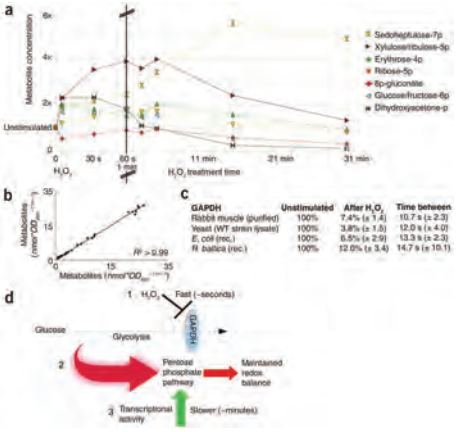

*Figure 1: The metabolic reconfiguration of the antioxidant-response is compartmentalized over time (from Ralser et al, Nature Biotechnology, 2009). **(A)** Concentrations of glycolytic and PPP intermediates of oxidant-treated cells are given as relative change compared to the concentration of an unstimulated culture. The first time point of the H2O2 treatment is highlighted. **(B)** Reproducibility of metabolite quantification by LC-MS/MS illustrated by linear regression; Metabolites quantified from biological replicates are plotted against one another. **(C)** Relative GAPDH activity (substrate conversion per time) after oxidant treatment of rabbit muscle GAPDH or extracts of transgenic yeast expressing different GAPDH paralogues. Bracketed values indicate standard deviation. **(D)** Timeline of the anti-oxidant response: Inactivation of GAPDH (1) enforces a protective re-configuration of the carbohydrate flux (2) within seconds after the perturbation. Later, changes in the transcriptome become prominent (3), contributing to this process and allowing the cell to return to normal metabolic activity.*

Several metabolites of glycolysis have a regulatory role in cellular processes. Therefore, we believe that dynamic oscillations and deviations in this pathway have to be studied in this context. For instance, we provided evidence that the flux through a central enzyme is important for the function of a relevant epigenetic factor, the histone deacetylase *SIR2* [Ralser et al., 2009b]. In addition, we showed that a glycolytic inhibitor which has promising anticancer properties, 2-Deoxy-D-glucose, does not block cell proliferation by solely causing an energy deprivation [Ralser et al., 2008a]. Moreover, we discussed and investigated the influence of alterations in the these pathways on aging processes of model organisms and humans [Ralser et al, 2008b; Ralser & Benjamin, 2008]. Currently, we are working to establish a novel technological pipeline for the identification of targets of biologically active small molecules, termed Chemical-genetic profiling. This technique, first presented by Parsons et al. [Parsons et al., 2006] is based on parallel fitness tests of comprehensive yeast libraries. We extended this system by using Solexa sequencing technology, and use it currently for screening the yeast collection to identify small-molecule targets of biological compounds such as Resveratrol (Figure 2). Lastly, ongoing work aims to invent a method of parallel chronological aging experiments, which will allow the identification of novel metabolic processes that are important factors of the aging process.

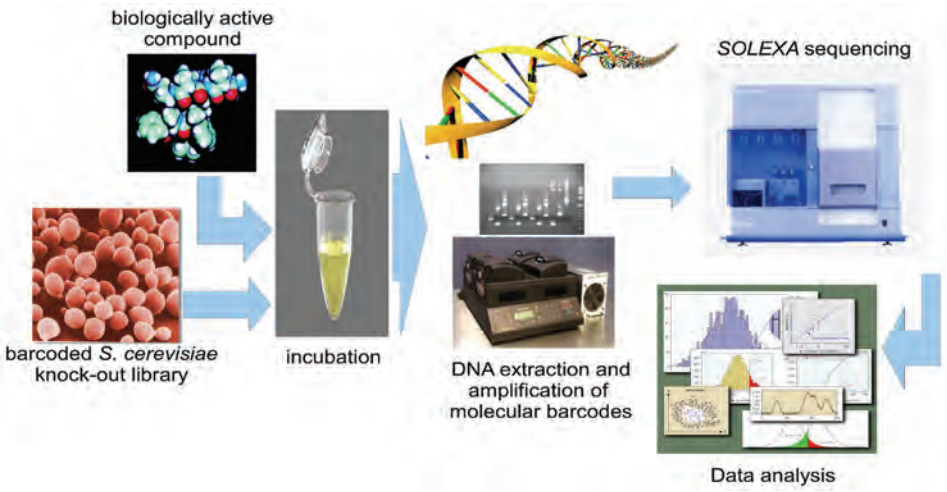

Figure 2: Chemical-genetic profiling via second generation sequencing. A complex mixture of yeast strains labelled with a genome-integrated molecular barcode is exposed to a biological compound. This exposure causes a selection procedure, resistant strains overgrow the pool, and sensitive strains are depleted. The change in the population structure is detected via sequencing of the molecular barcodes by Solexa technology. This approach is suitable to systematically detect chemical-genetic interaction of any kind of soluble compound.

Cooperation within the Institute

- Bernd Timmermann, Whole genome sequencing of *S. cerevisiae* to identify functional relevant alleles
- Andreas Dahl, Development of sequencing strategies for chemical-genetic profiling of dual-barcoded strains
- Heiner Schrewe, Generation and Analysis of Mouse-models with alterations in central Carbohydrate Flux

Planned developments

The directors of the Max Planck Institute for Molecular Genetics decided to extend the biological analytics capacities of the institute. The *Molecular Biology of Metabolism group* will take responsibility of the Mass spectrometry part and quantify peptides and small molecules for own projects, but also in collaboration and as service for other research groups at the MPIMG. For this, we use a MALDI-TOF-MS from Bruker Daltonics (Ultra-Flex II) and a state of the Art Triple-Quadrupole MS currently ordered form Applied Biosystems/MDS/SCIEX. These modern systems will be used to systemically uncover processes of gene regulation caused by metabolic alterations.

General information

Selected publications

Ralser M, Wamelink MM, Latkolik S, Jansen EE, Lehrach H, Jakobs C. *Metabolic reconfiguration precedes transcriptional regulation in the antioxidant response*. Nat Biotechnol. 2009 Jul;27(7):604-5.

Ralser M, Zeidler U, Lehrach H. *Interfering with glycolysis causes Sir2-dependent hyper-recombination of Saccharomyces cerevisiae plasmids*. PloS One. 2009;4(4): e5376

Ralser M, Wamelink MM, Struys EA, Joppich C, Krobitsch S, Jakobs C, Lehrach H. *A catabolic block does not sufficiently explain how 2-deoxy-D-glucose inhibits cell growth*. Proc Natl Acad Sci U S A. 2008 Nov 18;105(46):17807-11.

Ralser M, Nebel A, Kleindorp R, Krobitsch S, Lehrach H, Schreiber S, Reinhardt R, Timmermann B. *Sequencing and genotypic analysis of the triosephosphate isomerase (TPI1) locus in a large sample of long-lived Germans*. BMC Genet. 2008 May 29;9:38

Ralser M, Wamelink MM, Kowald A, Gerisch B, Heeren G, Struys EA, Klipp E, Jakobs C, Breitenbach M, Lehrach H, Krobitsch S. *Dynamic rerouting of the carbohydrate flux is key to counteracting oxidative stress*. J Biol. 2007 Dec 21;6(4):10

Open access activities

Our philosophy is, that open access publishing is very important for publicly funded science. Since 2005, M. Ralser has published 10 articles under an open access licence.

Selected invited talks

Dept. of Systems Biology, Univ. of Cambridge, UK, 09/2009

Max Planck Institute for Biophysical Chemistry, Göttingen, GER, 08/2009

Free University of Amsterdam, Metabolic Unit, NL, 02/2009

University of Salzburg, AUT, 02/2008

Free University of Amsterdam, Dept. of Clinical Chemistry, NL, 11/2007

Awards

Biomed-Central Research Award Biology, London, UK, 2008

Faculty of 1000, Genomics Section. Associate Member of Hans Lehrach, 2009

Scientific referee

Markus Ralser serves as scientific referee for the following journals: Current Aging Science, Free Radicals in Biology and Medicine, Journal of Theoretical Biology, Biochemistry.

Teaching activities

One-week-lecture at University of Salzburg, Austria (2 ECTS credits for students of *Molecular Biology*): *Dynamics in Metabolic Pathways: Implications for signalling, ageing and disease*, Summer 2009

Contribution to the lecture of Prof. Hans Lehrach at the Free University of Berlin, *Von der Funktionellen Genom-forschung zur Systembiologie*, lecture *Transitions in Metabolic Pathways (2 hrs)*

Organization of scientific events

MPG/CNRS Miniworkshop *Robustness of Metabolic Pathways,* 20.-22. July 2009, Berlin (Organizers: Ovidiu Radulescu, Univ. of Rennes, Markus Ralser, MPIMG, Berlin)

Public relations

Ralser M, Lehrach H, and Timmermann *B. Chemical-genetic profiling mittels Next Generation Sequencing. Laborwelt* 2009(3); 16-17 (German language laboratory magazine)

Cancer Genomics

(Established: 01/2007)

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Stefan Börno* (since 07/08)
Melanie Isau* (since 04/09)

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Anna Kosiura* (since 05/08)
Nada Kumer* (since 04/08)

Scientific overview

According to the world health organization (WHO) malignant neoplasms will be the most common cause of death worldwide in 2010. Despite intensive research on carcinogenesis this frightening scenario will persist mainly due to the overall increase of lifetime expectancy. Furthermore, most cancers are only diagnosed in an advanced stage, which prohibits curative treatment and a large proportion of patients do not respond to their chemotherapy. In a concerted action, based on the recent improvements of methodological techniques, we develop strategies for the identification of patients at risk and tumors at an early stage. In addition, we intend to identify prognostic and predictive biomarkers as guides for patient's successful treatment at different stages of the disease. These clinical orientated projects are the basis for several mechanistic research projects: Using Next Generation Sequencing (NGS) technologies we are identifying genome wide mutational alterations, changes in DNA methylation patterns and we are also performing mRNA and small RNA profiling experiments on human tumor material. In collaborations with structural biologists (Michael Lappe) and computer modelling experts (Christoph Wierling) we identify hubs relevant for tumor progression and metastasis. These central points are further investigated in protein-protein interaction assays (tandem affinity purification and yeast two hybrid systems) as well as several functional assays for the identification of functional consequences of the tumor-specific alteration.

* externally funded

Studies on sequence analyses of healthy human material such as the 1000 genomes project are in progress. Much less has been done so far in the field of oncology. Until now only the complete genome of one patient suffering from a cytogenetically normal acute myeloid leukaemia (AML) has been published. Further studies within the international cancer genome consortium (ICGC), where the department is a partner of, concentrate on the basic characterization of genetic alterations within tumors, whereas tumor stages and chemotherapies are primarily disregarded. Thus, to put more weight on patient - related interests our main goal is to identify mechanisms responsible for therapy resistance and the identification of genetic alterations underlying tumor progression.
To realize these ambitious research projects we are primarily facing three major challenges: 1. Acquisition of clinically well characterized tissue material, 2. Conservation of the tissue material and 3. Adaptation of NGS technologies for studies on human tissue material in statistical significant numbers.

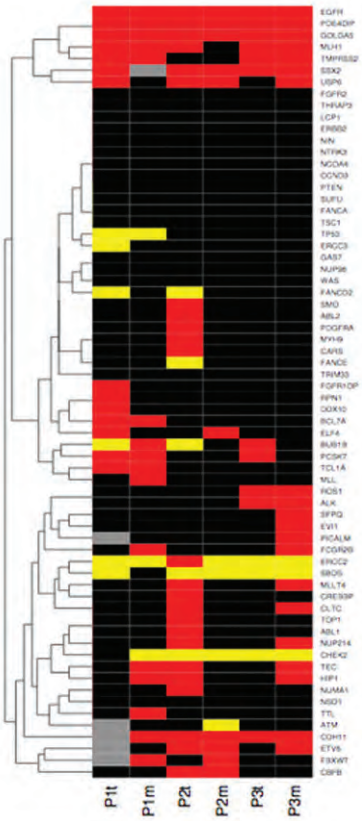
1. Acquisition of clinically well characterized tissue material:
For the recruitment, clinical characterization of patients and the preparation of human tissue samples, we rely on numerous clinical collaborations with the Universitätsklinikum Charité, Berlin (Department of Gastroenterology, Department of Gynaecology), the University Hospital Eppendorf, Hamburg (Department of Urology and Department of Abdominal Surgery), the University Hospital in Innsbruck, Austria (Department of Urology) and the University Hospital of Graz, Austria (Department of Pathology).

2. Conservation of the tissue material
Concerning the patient's material we have found that the Illumina Genome Analyzer (Solexa) technology can be used for the sequencing of formalin-fixed and paraffin-embedded (FFPE) tissue samples. This technological application is important because tissue samples are routinely stored as FFPE samples in pathology departments. However, FFPE preparation is incompatible with many down-stream molecular biology techniques such as PCR based amplification methods and gene expression studies. Their usability for NGS technologies now opens up access to a variety of clinical questions with patients' acquisitions over the last 100 years.

3. Adaptation of NGS technologies for studies on human tissue material in statistical significant numbers
We make use of the NGS techniques to analyse all steps in the information flow of biological systems - from genomic DNA to mRNA and miRNA and we are about to build up a network between different levels of information, e.g. the influence of DNA methylation patterns on mRNA expression data or the interconnection between mutations and miRNA - function. For the generation of sequencing data we are using the Roche/454 FLX, the Illumina/ Solexa Genome Analyzer and the Applied Biosystems SOLiD system. The sequencing is supported by Andreas Dahl and Bernd Timmermann. In view of the identification of common tumor progression mechanisms and factors responsible for therapy resistance we established five different targeted se-

Image taken during a SOLiD sequencing run. Each dot represents one DNA fragment.

quencing strategies. Using array-based, in solution hybridization techniques and rolling circle mechanisms we are able to specifically enrich DNA fragments from 1Mb up to 34 Mb, corresponding to the whole exome, and use them for sequencing. These approaches are crucial for our projects in regard to costs and sequencing capacities. Finally, we have also set up primary sequence data analyses for heterogeneous tissues - as it is frequently found in tumors and – are now performing correlation analyses to identify connections between genetic and epigenetic alterations and clinical parameters. Bioinformatics modelling are done in collaboration with Ralf Herwig and Christoph Wierling. Studies on epigenetic alteration in colon cancer are done in collaboration with Christina Grimm and Marie-Laure Yaspo. We have already identified a set of new tumor relevant mutations and collaborate on functional analyzes with Bodo Lange's group. For some identified protein-protein interactions we are already in the stage to develop 'intrabodies' and 'aptamers'. These experiments are done together with Jörn Glökler, Zoltán Konthur and Sylvia Krobitsch. In the future, these molecules will hopefully be useful as potent tumor inhibitors.



Heat map generated from NGS data reflecting relevance of nucleotide exchanges (NE) in cancer genes in six different tumor tissues (red: highly relevant NE, yellow: likely irrelevant NE, black: wt sequence.

Specific Projects

Identification of genetic and epigenetic alterations underlying colon cancer progression

Colon cancer is the third most common cancer type worldwide and in 2004 over one million new cancer cases have been diagnosed. Thus, one focus of the group is to identify colon cancer progression markers e.g. mutations, methylation, gene and miRNA expression changes. So far we have already collected extensive data on miRNA and mRNA expressions in different colon cancer stages and we are now performing functional characterizations. For the genome-wide methylation analyzes we are using MeDIP-seq techniques. For this, methylated DNA is enriched by a chromatin immunoprecipitation with an antibody targeting methylcytosine followed by NGS. The project is part of the 'Modifiers of Intestinal Tumor Formation and Progression' network. Using consomic mouse models on one side and human cell cultures as well as primary tissue materials on the other side the network aims at the indentification of intestinal modifiers of epigenetic alterations in cancers.

Development of diagnostic and therapeutic markers for prostate cancer treatment

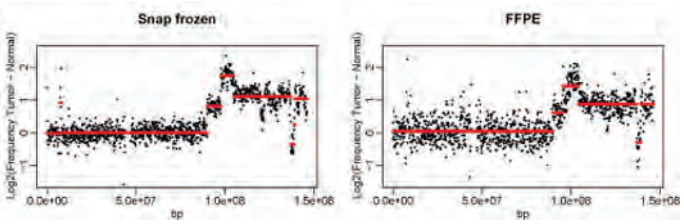
In Germany every year approximately 60,000 men are diagnosed with prostate cancer. Prostate cancers are classified according to the Gleason score and are selected according to this score for radical prostatectomy. However, the diagnosis is difficult and the false positive rate is high. Our goal together with the DKFZ

(Deutsches Krebsforschungszentrum), the University Hospital in Innsbruck and the University Hospital Eppendorf is to develop new diagnostic algorithms. Our project part focuses on the classification and characterization of prostate cancers based on genomic analyses such as targeted genomic sequencing with mutation analyses and genome-wide methylation analyses.

Functional analyses of cervical cancer pathogenesis and development of antiviral substances

Cervical cancer is the second most common cancer among women with a worldwide prevalence of 2,27 millions and with an estimated 490,000 new cases and 270,000 deaths per year. 'High risk' human papillomaviruses are detected in over 98% of cervical carcinoma cases. Together with Prof. Dr. Peter Howley (Harvard Medical School, Boston) we have found that the interaction between the viral E2 protein and the cellular bromodomain containing protein Brd4 is required for the genome maintenance and the viral transcriptional regulation functions and thereby regulate cervical cancer pathogenesis. We are now working on specific inhibitors of this interaction and we are using NGS technologies for an investigation of functional consequences for cervical cancer pathogenesis. Our experiments show that a disruption of the interaction between E2 and Brd4 leads to a curing of cells from papillomavirus infections and thereby might prevent cervical cancer.

Taken together, the goals of the Cancer Genomics Group are to integrate different fields of medicine, biology and natural science in order to better understand how tumor cells work and how carcinogenic processes are regulated. Knowledge of disease- relevant alterations in gene sequences and molecules of the metabolic network will reveal targets for effective diagnostic and therapeutic applications. With the availability of these techniques we are on a turning point of cancer diagnosis and treatment.



Copy number variations of chromosome 8 from snap frozen and FFPE breast cancer tissues in relation to normal tissues The x-axis represents the genomic position, the y-axis the log2 ratio of fragments per bin of tumor versus normal tissue.

External cooperations

- Prof. Dr. George Church, Harvard Medical School, Boston
- PD. Dr. Severin Daum, Department of Gastroenterology, Charité, Berlin
- Prof. Dr. Peter Howley, Harvard Medical School, Boston
- Prof. Dr. Helmut Klocker, Department of Urology, University of Innsbruck
- PD Dr. Christine Sers, Department of Pathology, Charité, Berlin
- PD. Dr. Holger Sültmann, DKFZ, Heidelberg
- Prof. Dr. Kurt Zatloukal, Department of Urology, University of Graz

General information

Selected publications

Boerno ST, Grimm C, Lehrach H, Schweiger MR. *Next Generation Sequencing Technologies for DNA Methylation Analyses in Cancer Genomics*. Epigenomics 2009 (in press)

Gang Z, Schweiger MR, Martinez-Noel G, Zheng L, Smith JA, Harper JW, and PM Howley: *Brd4 Regulation of Papillomavirus E2 Protein Stability*. J Virol. 2009 Sep; 83(17):8683-92.

Schweiger MR, Kerick M, Timmermann B, Albrecht MW, Borodina T, Parkhomchuk D, Kurt Zatloukal K and H Lehrach: *Genome - wide Massively Parallel Sequencing of Formaldehyde Fixed - Paraffin Embedded (FFPE) Tumor Tissues for Copy-Number- and Mutation-Analysis*. PLoS One. 2009 May ;4(5):e5548.

Schweiger M, Ottinger M, You J., and P.M. Howley: *Brd4-independent transcriptional repression function of the papillomavirus e2 proteins*. J Virol. 2007 Sep;81(18):9612-22.

Schweiger M, You J., and P.M. Howley: *Bromodomain Protein 4 Mediates the Papillomavirus E2 Transcriptional Activation Function*. J Virol.2006 May;80(9): 4276-85.

Selected invited talks

German Symposium on systems biology 2009: *Genome-wide alterations in colon cancer identified by next-generation sequencing techniques*

Deutscher Krebskongress 2008: *Systematische genomische Sequenzanalyse resistenz- und metastasierungsrelevanter Gene und deren regulatorische Sequenzen*

DNA Tumor Virus meeting 2006: *Brd4 separates the papillomavirus 16 E2 dependent transcriptional activation and repression functions.*

Awards

Michal Schweiger: Robert Koch Dissertation award, Charité 2008

Membership in professional societies

- Member of the German Society of Human Genetics
- Member of the American Society of Human Genetics
- Member of the ‚Studienstiftung des deutschen Volkes'

Teaching activities

Human genetics for medical students of the Charité

External funding

Österreichische Nationalstiftung für Forschung und Technologieentwicklung, IMGuS Project: *Systems biology of prostate cancer*

BMBF, Medical Systems Biology – MEDSYS: *PREDICT – a systems biology approach to pre-clinical testing in Cancer*

BMBF, NGFNplus: *IG Systems Biology of Genetic Diseases – Mutanom*

BMBF, NGFNplus: *IG Prostate Cancer*

BMBF, NGFNplus: *Modifiers of Intestinal Tumor Formation and Progression*

Technology Development

(Established: 06/2007)

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Alexey Davydov (since 07/08)
Alexandr Kirpiy (08/08 - 06/09)
Maria Banaru (11/07 - 05/08)
Anja Nowka (10/07 - 01/08)

Engineer
Jeannine Wilde (since 06/08)

Technicians
Michael Zinke (since 08/08)
Berit Lenz (since 11/09)
Sabine Thamm (09/07 – 01/08)

Scientific overview

Till June 2007, A. Soldatov's team was within Lehrach's group and has specialised in the area of SNP genotyping and sequencing. A new SNP-genotyping method was developed, patented (EP03019521) and successfully applied for genotyping of *Arabidopsis thaliana* and human DNA samples. We have also worked on the development of new sequencing technologies in the frame of the European MolTools project: (i) microbead based sequencing (EP05018950.5) and (ii) ligation based sequencing in collaboration with U. Landegren laboratory (Uppsala University, Sweden).

Since June 2007, our group specializes in technology development and bioinformatics related with second generation sequencing (SGS). SGS is one of the most perspective and quickly developing technologies in basic biological research. Currently, two short-read SGS platforms (synthesis-based Illumina GAII and ligation based ABI SOLiD 3) are available on the market and at the MPI for Molecular Genetics. Our group operates one Illumina GAII and three ABI SOLiD 3 sequencing platforms.

Both for ABI and Illumina platforms, we have set up the laboratory workflow in the department. Considerable efforts were invested in the improving and optimization of sequencing library preparation protocols: optimization of fragmentation step, increasing yield of particular enzymatic reactions, switching from commercial to cheaper home-made kits (Illumina), introduction of real-time library quality and concentration check, optimization of flowcell loading, improvement of magnetic bead separation (ABI platform) etc.
We have developed a SGS bioinformatics pipeline, which brings together the information about the whole sequencing process from sample handling to results analysis. The system enables each authorized user (also external collaborators) to track processing of samples, check the sequencing quality and view data in the genomic browser.

Now the group has a profound experience in application of SGS platforms for analysis of genome (resequencing) and transcriptome (gene expression profiling, splice junctions search, allele-specific expression, reverse transcription, etc.). We have performed RNA-Seq, ChIP-Seq, MedIP-Seq and genomic DNA sequencing (including sequencing library preparation, sequencing and sometimes data analysis) for several groups within the MPIMG (AG Yaspo, AG Nietfeld, AG Himmelbauer, AG Schweiger, AG Hoehe, AG Lange, AG Krobitsch, AG Ralser, dept. Herrmann) and for external collaborators (Y. Shiloh (U. Tel Aviv), C. Koncz (MPI for Plant Breeding Research, Cologne, J.Klose (Charite, Berlin)). In collaboration with ML Yaspo group (MPIMG) we have sequenced and analyzed human transcriptome from a human embryonic kidney and a B cell line. This work demonstrated, in particular, capacities of RNA-Seq if compared to microarray approach and showed exon skipping to be the most prevalent form of alternative splicing. In collaboration with MR Schweiger (MPIMG) we have successfully prepared libraries and sequenced DNA recovered from formalin-fixed and paraffin-embedded (FFPE) samples. This trial showed that SGS is applicable for analysis of stored clinical samples. In collaboration with H. Himmelbauer (MPIMG) we have sequenced two bacterial genomes (Haemophilus influenzae, Escherichia coli) to approbate the SHARCGS algorithm for the de novo sequencing applications.

Recently, we have developed, patented and published a convenient method for strand-specific sequencing of cDNA. The method is based on incorporation of uridine bases during first (or second) cDNA strand synthesis and subsequent destruction of this strand before sequencing. Knowledge of transcript orientation is important for transcriptome studies. It allows (i) to annotate novel genes correctly, (ii) to investigate antisense transcription, which plays an important regulatory role in all eukaryotes, (iii) to resolve colliding transcript overlaps, which are abundant in compact genomes of prokaryotes and lower eukaryotes, and (iv) to correctly determine gene expression levels in the presence of overlapping antisense transcription.

At present, main technology development activities of the group are the following:

- SGS based genotyping (wet procedure and accompanying analysis algorithm);
- SGS based methylation analysis (wet procedure and accompanying analysis algorithm);
- development of enrichment procedures for SGS.

We are also involved in several biological projects requiring SGS for analysis of genome and transcriptome:

- resequencing of the genome of the PWD mouse strain;
- 1000 Genomes project;
- GEUVADIS RNA-Seq;
- Treat 1000 project;
- IMGuS staetohepatitis project (in collaboration with ML Yaspo (MPIMG)) ;
- mouse transcriptional network studies (in collaboration with ML Yaspo (MPIMG).

From October 2009, we are involved in the FP7 HEALTH-2009-4.3.3-1 (SICA) project “Genomic variations underlying common neuropsychiatric diseases and disease-related cognitive traits in different human populations”. We will perform deep sequencing of genomics regions known to be associated with Alzheimer’s disease, alcoholism and schizophrenia.

Cooperation within the institute

- Marie-Laure Yaspo; Dept. Vertebrate Genomics
- Wilfried Nietfeld; Dept. Vertebrate Genomics
- Heinz Himmelbauer; Dept. Vertebrate Genomics
- Bodo Lange; Dept. Vertebrate Genomics
- Andreas Dahl; Dept. Vertebrate Genomics
- Michal Schweiger; Dept. Vertebrate Genomics
- Sylvia Krobitsch; Otto Warburg Laboratory
- Bernd Timmermann; Service group at MPI

General information

Selected publications

Parkhomchuk D, Amstislavskiy V, Soldatov A, Ogryzko V. *Use of high throughput sequencing to observe genome dynamics at a single cell level.* Proc Natl Acad Sci U S A. 2009 Nov 23. [Epub ahead of print]

Parkhomchuk D, Borodina T, Amstislavskiy V, Banaru M, Hallen L, Krobitsch S, Lehrach H, Soldatov A. *Transcriptome analysis by strand-specific sequencing of complementary DNA*. Nucleic Acids Res. 2009 Jul 20. doi:10.1093/nar/gkp596

Schweiger MR, Kerick M, Timmermann B, Albrecht MW, Borodina T, Parkhomchuk D, Zatloukal K, Lehrach H. *Genome-wide massively parallel sequencing of formaldehyde fixed-paraffin embedded (FFPE) tumor tissues for copy-number- and mutation-analysis*. PLoS ONE. 2009;4(5):e5548. Epub 2009 May 14.

Rosenkranz R, Borodina T, Lehrach H, Himmelbauer H. *Characterizing the mouse ES cell transcriptome with Illumina sequencing*. Genomics. 2008 Oct;92(4):187-94. Epub 2008 Aug 3.

Sultan M, Schulz MH, Richard H, Magen A, Klingenhoff A, Scherf M, Seifert M, Borodina T, Soldatov A, Parkhomchuk D, Schmidt D, O'Keeffe S, Haas S, Vingron M, Lehrach H, Yaspo ML. *A global view of gene activity and alternative splicing by deep sequencing of the human transcriptome*. Science. 2008 Aug 15;321(5891):956-60. Epub 2008 Jul 3.

Work as scientific referee

A. Soldatov serves as scientific referee for the following journals: Nucleic Acids Research, Biotechniques, PLoS Genetics.

Patents

Ligation-based method of analysis of single nucleotide polymorphisms on genomic DNA. EP03019521

Ligation-based synthesis of oligonucleotides with block structure. PCT/EP 2004/003921.

Parallel gel-loading procedure (appl. No. 05018950.5).

Method for differentiation of polynucleotide strands. EP 09008808.9

External funding

EU, FP 6: *MolTools,* 01/01 – 07/07

BMBF: *1000 Genomes,* since 07/08

IMGuS: *Systems biology of staetohepatitis*, since 09/08, with ML Yaspo, MPIMG

EU, FP7, HEALTH: *ADAMS*, since 10/09

Organization of scientific events

EMBO course on ChIP-Seq and RNA-Seq. 2-13 February, 2009 in the MPIMG (together with ML Yaspo group)

Cardiovascular Genetics

(Established: 2001)

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Secretary
Barbara Gibas (since 10/05)

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Andreja Brodarac* (03/08 - 03/09)
Alan Matthew Punnoose* (08/06 - 09/07)
Christina Grimm* (12/02 - 12/06)
Stefanie Hammer * (03/04 - 03/06)

PhD students
Markus Schüler* (since 01/07)
Jenny Schlesinger* (since 04/08)
Marcel Grunert* (since 07/08)
Zhang Qin* (since 10/08)
Lucas Rudigier* (since 04/09)
Martje Tönjes* (02/06 - 04/09)
Martin Lange* (04/03 - 07/08)
Jenny Fischer* (01/04 - 03/08)
Katharina Rost* (07/07 - 01/08)

Technician
Ilona Dunkel (since 01/02)

Engineer
Tammo Krüger* (03/06 - 12/07)

Scientific overview

The group consists of an interdisciplinary team with biologists, physicians and bioinformaticians and aims to analyse the molecular mechanisms underlying the cardiac developmental process in a systems biology approach. We focus on the transcriptional regulation process, which plays a key role for normal and abnormal cardiac development leading in the latter case to congenital heart diseases. We have a long-standing cooperation with Martin Vingron at the institute; additionally we have close cooperation in Berlin with Knut Reinert (Bioinformatics) and Felix Berger (Deutsches Herrzentrum Berlin) and abroad with Dinshaw Patel (NYH), and Christine Mummery (Leiden).

Current state of research

Cardiovascular disease is the leading cause of illness and death worldwide, and congenital heart diseases are the most common birth defects in humans. They arise during development of the embryo and affect one in every 100 live births and an even higher number in miscarriages. Most cardiovascular diseases have complex genetic and environmental origins and are only now becoming amenable to large-scale genetic analyses. This is in contrast to inherited single gene disorders, and in the somatic genetics of cancer, where important mechanistic insights have already been afforded.

* externally funded

The heart is the first functional organ during embryogenesis. A rapidly growing number of factors has been shown to be involved in regulating the pattern and timing of expression of genes responsible for the cardiac lineage determination, heart chamber formation, valvulogenesis and conduction-system development. Spatiotemporal and quantitative regulation of cardiac transcription factors must occur in a precise manner to ensure fine regulation of downstream targets. However, the ability of transcription factor binding to DNA is highly influenced by the chromatin status, and epigenetic mechanisms play an important role in establishing and maintaining transcriptional programs. This layer of control comprises post-translational modifications of histones, DNA methylation and chromatin remodelling. Thus to understand networks directing gene expression, the interplay between different transcription factors, co-regulatory elements and epigenetic factors has to be considered. Furthermore, recent studies have begun to unveil powerful roles for microRNAs in regulating and fine-tuning mRNA profiles largely by silencing target genes, *via* either translational repression or mRNA degradation.

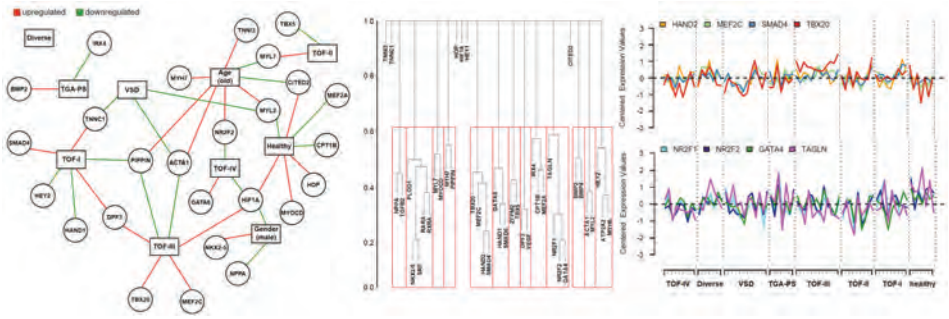

Figure 1: ***Left****, Network of deregulated genes and cardiac phenotypes. TOF – Tetralogy of Fallot; VSD – ventriclar septal defect; TGA-PS – transposition of great arteries and pulmonary stenosis.* ***Middle****, Cluster dendrogram showing 13 correlated gene groups (groups of genes showing correlated gene expression across 100 patients with different cardiac phenotypes).* ***Right****, Examples of two correlated gene groups.*

Our accomplishments

Over the last years we performed a panel of molecular studies and computational developments to gain insights into the transcription networks underlying myogenesis and congenital heart disease.

- Collection of biomaterial and detailed clinical phenotypes of congenital heart disease
- Set-up of a CardioVascular Genetics database and d-matrix as front-end/analysis tool
- Genome scale gene expression profiling of normal and malformed human hearts
- Analysis of cardiac transcription factor binding sites *in vivo* and their functional consequence
- Characterization of epigenetic marks, such as histone modification at a global scale
- Development of analysis tools for next-generation sequencing data

Finally, transcription networks based on the integration of obtained clinical, phenotypical and molecular data have been predicted.

During the course of these projects, three transcription factors raised particular interest and have been studied in more detail. CITED2 and TBX20 could be associated with congenital heart disease in human for the first time; mutations in CITED2 were shown to be potentially disease causative. DPF3 could be discovered as a

novel epigenetic transcription factor of particular importance for cardiac and skeletal muscle development and function. Moreover, functional studies showed that DPF3 represents the first plant-homeodomains known to bind histone acetylation marks, which displays a novel protein-domain function.

Ongoing and planned developments

The impact of microRNAs for cardiac development and disease

Considering the importance of miRNAs for the regulation of gene expression profiles in a given cell or tissue, we currently study miRNA expression profiles in a panel of congenital heart defects. Our preliminary data point already to disease-specific deregulation of a distinct set of miRNAs. Using 2nd generation sequencing technique (Solexa) we analyse miRNA profiles as well as mRNA profiles, where the latter can furthermore be studied in a spliceform-specific manner. We aim to integrate the obtained novel data with our previous results (e.g. transcription factor binding sites) to predict key disease nodes. This global approach will go along with functional studies in the mouse model, which is of particular importance as the human cardiac material is a cell-type mixture and therefore allows only limited conclusions.

Modular regulation of cardiac development and maturation by genetic and epigenetic factors

In the past, we investigated the relationship between gene expression profiles, five histone modifications (H3K4me1/2/3, H3ac and H4ac) and four cardiac transcription factors (Gata4, Mef2a, Nkx2.5 and Srf) in cardiac and skeletal myocytes. These data show frequent co-regulation of target genes and moreover a significant influence of epigenetic marks on the effect of transcription factor binding on gene expression. We currently focus on the dynamic changes of histone modifications, transcription factor binding and binding of histone modifying enzymes during cardiac development and maturation in mouse. The current technical advances enable the study of very limited material such as cardiac tissue obtained from E9.5 mouse embryos, which was a crucial requirement for the project.

Role of DPF3 and its signalling cascade in cardiac myogenesis

In 2008, we presented DPF3, a novel epigenetic key factor for heart and muscle development characterised by a double plant-homeodomain (PHD-finger). During development Dpf3 is expressed in the heart and somites of mouse, chicken, and zebrafish. Knockdown of dpf3 in zebrafish leads to incomplete cardiac looping and severely reduced ventricular contractility, with disassembled muscular fibers. DPF3 is associated with the BAF chromatin remodelling complex and binds methylated as well as acetylated lysine residues of histone 3 and 4. Thus, DPF3 represents the first plant homeodomains that bind acetylated lysine. It adds a further layer of complexity to the BAF chromatin remodelling complex by representing a tissue-specific anchor between modified histones and chromatin remodelling. Changes in chromatin structure and gene transcription are typically induced by external stimuli. In particular phosphorylation of chromatin-associated proteins is mediated by different kinases, such as p38, CaMK and CKII, and represents a powerful interface for the transmission of extracellular signals to chromatin. So far, the upstream signalling pathway of DPF3 and its role for myogenesis in mouse are undiscovered and therefore represent a particular focus of our research.

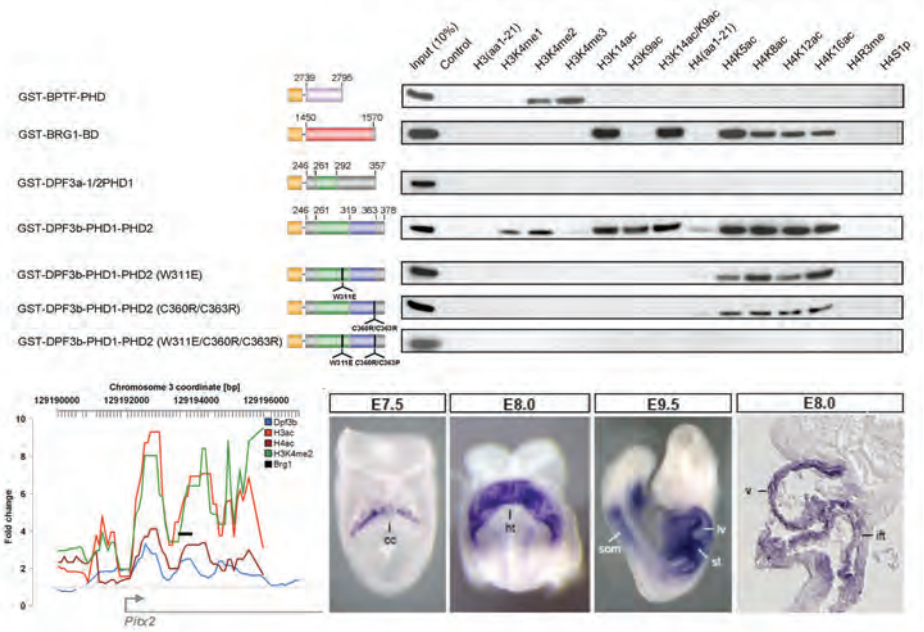

*Figure 2: **Top**, The PHD fingers of DPF3 interact with modified histones tails on histone 3 and 4. Western blot analysis of histone peptide pulldowns with indicated GST-DPF3 fusion proteins. **Bottom left**, Co-occurrence of DPF3, BRG1, H3K4me2 and H3ac/H4ac modifications on the murine* Pitx2 *locus. **Bottom right**, Expression pattern of* Dpf3 *during embryonic development analyzed by in situ hybridization.*

General information

Selected publications

Emde AK, Grunert M, Weese D, Reinert K, Sperling S. *MicroRazerS: Rapid alignment of small RNA reads*. Bioinformatics. Epub 2009 Oct 29.

Lange M, Kaynak B, Forster UB, Tönjes M, Fischer JJ, Grimm C, Schlesinger J, Just S, Dunkel I, Krueger T, Mebus S, Lehrach H, Lurz R, Gobom J, Rottbauer W, Abdelilah-Seyfried S, Sperling S. *Regulation of muscle development by DPF3, a novel histone acetylation and methylation reader of the BAF chromatin remodeling complex*. Genes Dev. 2008 Sep 1;22(17):2370-84.

Toenjes M, Schueler M, Hammer S, Pape UJ, Fischer JJ, Berger F, Vingron M, Sperling S. *Prediction of cardiac transcription networks based on molecular data and complex clinical phenotypes*. Mol Biosyst. 2008 Jun;4(6):589-98.

Fischer JJ, Toedling J, Krueger T, Schueler M, Huber W, Sperling S. *Combinatorial effects of four histone modifications in transcription and differentiation*. Genomics. 2008 Jan;91(1):41-51. Epub 2007 Nov 8.

Sperling, S., C.H. Grimm, I. Dunkel, S. Mebus, H.P. Sperling, A. Ebner, R. Galli, H. Lehrach, C. Fusch, F. Berger, and S. Hammer. 2005. *Identification and functional analysis of CITED2 mutations in patients with congenital heart defects*. Hum Mutat 26: 575-582

Selected invited talks

European Society of Cardiology, Annual Meeting, 08/2009

American Heart Association, Basic Cardiovascular Sciences Conference, 07/2009

Royal Dutch Academy of Sciences 200 year anniversary, Colloquium *Cardiac development, stem cells and disease*, 11/2008

EFS 2005 European Conference on Functional Genomics *Functional Genomics and Disease*, 09/2005

Memberships

ESHG Communications Committee (since 2008)

Board European Integrated Project *Heart Repair*, 2006-2010

Board European Society of Human Genetics, 2005-2010

European Society of Cardiology (since 2008)

German Society of Cardiology (since 2008)

German Society of Human Genetics (since 2003)

European Society of Human Genetics (since 2003)

Scientific referee

Silke Sperling serves as scientific referee for the following journals: Circulation, Circulation Research, Journal of Molecular and Cellular Cardiology, Nucleic Acid Research, Human Mutation, Molecular Medicine, Molecular BioSystems, Genomics, BMC Bioinformatics, Clinical Chemistry.

Membership in scientific advisory boards

Editorial Advisory Board of Molecular BioSystems (since 2008)

Scientific Advisory Board of Annual Meeting of the Association for European Paediatric Cardiology (since 2004)

Grant evaluation

FP7 EC, DFG, Heinrich Hertz-Stiftung, GIF, Estonia Genome Project, Austrian Science Fund FWF, Studienstiftung des Deutschen Volkes, ANR

Appointments of former members of the group (selected)

Stefanie Hammer - Group leader, Schering AG, Berlin

Martin Lange - Postdoc, Center for Genomic Regulation, Barcelona

Martje Tönjes - Postdoc, DKFZ, Heidelberg

Patent

Sperling S., Seelow D., Lehrach H.: *Verfahren und Vorrichtung zur graphischen Darstellung*. Deutsches Patent # 103 35 359.3

External funding

DAAD Sandwich PhD Stipendium für Qin Zhang, 2008-2010

European Commission FP7 Integrated Project *CardioGeNet,* Subprojekt *Analysis of T-box transcription networks in congenital heart disease*, 2009 – 2013

European Commission, FP6 Integrated Project *HeartRepair,* Subprojekt *Epigenetic and transcriptional networks of the heart*, 2006 – 2010

Deutsche Studienstiftung, *PhD Stipendium für Martje Tönjes*, 2006 – 2009

European Commission, FP6 Integrated Project: *Moltools, S*ubprojekt: *Development of tools for gene expression analysis*, 2004 – 2007

BMBF BioProfile: *Miniaturization of real-time PCR and gene expression analysis*, 2005 – 2007

Teaching

FU, Fachbereich Biologie, Chemie, Pharmazie, Biochemie, *Von der funktionellen Genomforschung zur Systembiologie*, since 2006

Organization of scientific events

Annual Meeting of the European Integrated Project *Heart Repair*, 2009

HeartRepair Workshop *Microarray Data Archive and Meta-analysis*, 2008

Retreat Huber group, EMBL/Sperling group, MPIMG, 2006

Public relations

Silke Sperling gave interviews for the following articles:

The Scientist „*A Planck Walk*" , 09/2008

Technology Review "*Von Nullen, Einsen und Genen*", 07/2008

Handelsblatt „*Das biologische Riesenpuzzle*", 06/2008

Deutschlandfunk „Ein Code jenseits der DNA", 11/2007

Systems Biology

(Established: 01/2009)

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Andriani Daskalaki*, MD (since 01/09)
Hendrik Hache*, PhD (since 01/09)
Elisabeth Maschke-Dutz* (since 01/09)
Alexander Kühn, PhD (since 08/09)

Scientific overview

Mathematical modeling and simulation techniques have turned out to be valuable tools for the understanding of complex systems in different areas of research and engineering. In recent years this approach came to application frequently also in biology resulting in the establishment of the research area systems biology. Systems biology tries to understand the behavior of complex biological systems by means of mathematical models. This requires computer-assisted integration of experimental data into coherent models. A challenging task is the development of comprehensive models that can be used in medical and pharmaceutical research for the establishment of a personalized medicine.

The systems biology group has its research interests in the mathematical modeling of cellular processes with respect to development and diseases. Within the group different systems biology resources and tools for the modeling and simulation of biological systems has been designed and implemented. These tools are used in current projects for the modeling of cancer-related signal transduction pathways and their subsequent gene regulatory network and the effect of mutations on the model behavior. Moreover, the group is working on the modeling of stem cell biology and host-parasite interaction. The research is driven by the integration of diverse 'omics data, as generated by current (high-throughput) technologies.

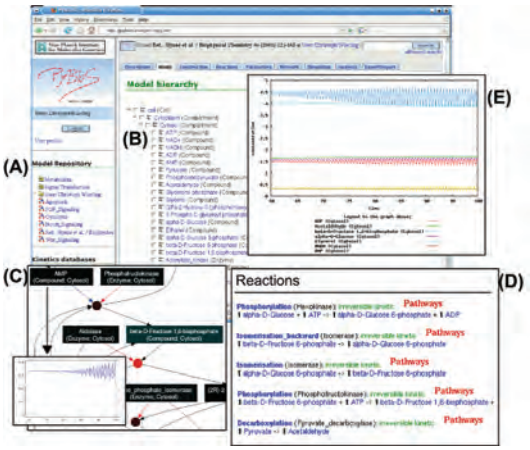

*Fig. 1: The PyBioS system acts as a repository for models **(A)** and provides functions for the modeling and simulation. Models are structured hierarchically according to subcellular localization **(B)**. Different tabs of the web interface provide functionalities for the visualization of the reaction network **(C)**, listing of reaction details **(D)**, and modeling and simulation **(E)**.*

* externally funded

The systems biology group is hosting the PyBioS modeling and simulation system (Wierling 2005, Wierling et al. 2007). PyBioS has a web-based user interface (Figure 1) and it makes use of well established methods for the mathematical description of biochemical reaction systems based on ordinary differential equation systems, and novel interfaces to biochemical pathway databases (e.g. Reactome, KEGG, ConsensusPathDB). In addition PyBioS provides several functionalities for model analysis and visualization.
Furthermore, a web application for the generation and analysis of gene regulatory networks (GRNs) called GeNGe has been developed (Hache et al. 2009). It provides an interactive user interfaces to generate GRNs and simulation data that can be used, e.g., as benchmark data for reverse engineering applications (Figure 2A). Furthermore, GeNGe offers features for the topological characterization of GRNs. The simulation results can be used to define critical network nodes and suitable candidates for perturbation experiments and thus guide future experimental work (Figure 2B).
Moreover, the systems biology group is involved in the development of the ConsensusPathDB database (Kamburov et al. 2009) that is hosted by the Bioinformatics group. Until December 2008 PyBioS, GeNGe and ConsensusPathDB have been developed in the Bioinformatics group (R. Herwig). Since 2009 support and development of PyBioS and GeNGe is continued by the newly established Systems Biology Group.

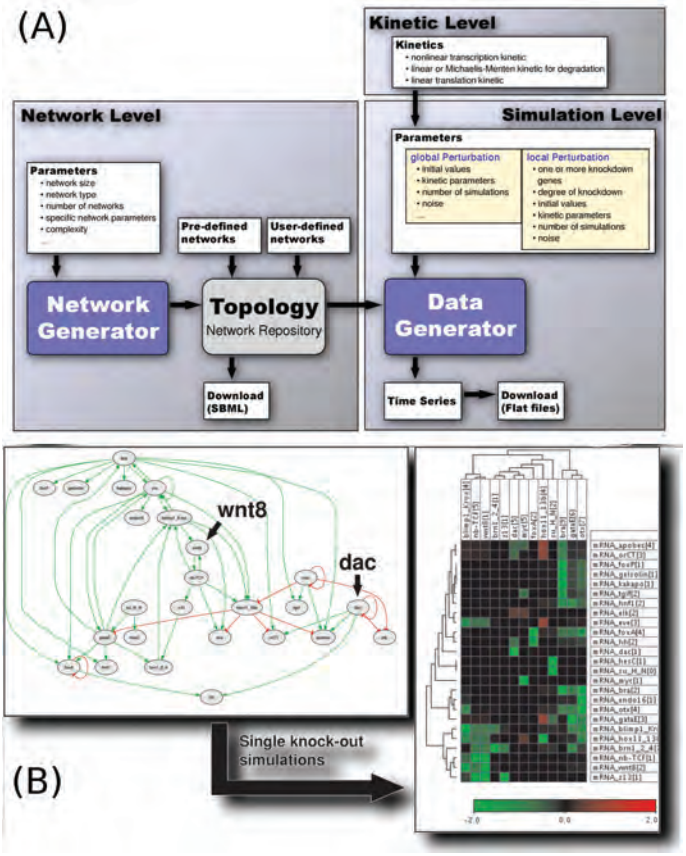

*Figure 2: **(A)** Flowchart of the simulation process in GeNGe. It is divided into three levels, the network level, to generate a network topology, the kinetic level, to select kinetic laws of the dynamic model, and the simulation level, to set the parameter values and simulate time series with local or global perturbation. **(B)** Large example of a gene regulatory network used for* in silico *knock-down experiments of each transcription factor (knock-down vs. control).*

Central research focus of the Systems Biology Group is the development of predictive models that can be used for *in silico* analysis of disease states and their treatment heading towards establishing a personalized medicine. For instance, progress in the treatment of tumors in individual patients will depend critically on being able to predict the effects of treatments in the context of the genome(s) involved. The development of predictive models is however complicated by the lack of information on many of the reaction kinetics needed. Information on the kinetics and kinetic parameters is either not available at all, or, at best, is based on experiments often carried out under conditions quite different from those in the living cell. Thus, computational modeling approaches must face the challenge of coping with this lack of information. One approach to overcome this limitation can be a rigorous analysis of the model's parameter space, e.g., by sampling unknown parameters from appropriate random distributions and a subsequent statistical evaluation. Such a kind of Monte Carlo-based approach requires to run thousands of simulations and thus can only be performed using distributed computing. This approach has been developed and implemented within the group (Figure 3A).

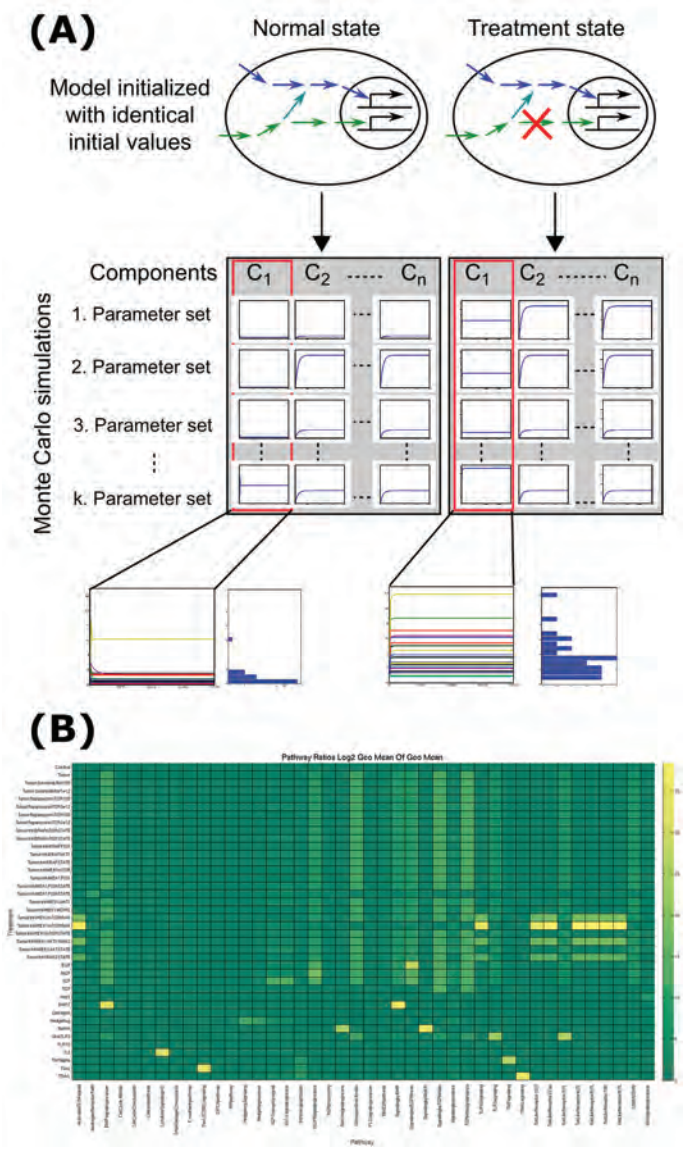

*Figure 3: **(A)** In the Monte Carlo approach multiple simulations with different sets of sampled kinetic parameters are performed and subsequently be used for the statistical evaluation of the model behavior (treatment vs. control). **(B)** This approach is used to study the effects of mutations and drugs on the cellular interaction network, e.g., in order to define a patient-specific treatment. The matrix shows* in silico *results of effected pathways (column) by different treatments (row).*

In the course of current research projects (Mutanom, MoGLI) the Systems Biology Group has established a large model of cancer-related pathways. Currently, the model comprises about 830 components and 1600 individual reactions and covers different signaling pathways, such as EGF-, IGF-, NGF-, Wnt-, Notch-, Hedgehog-, Fas-, Trail-signaling, etc. The model is used to study the effects of cancer-related somatic mutations and the identification of reasonable intervention points (Figure 3B). This approach will make it possible to define an individual treatment according to the patient's mutation pattern. Furthermore, within the MoGLI project we study the transcriptional program and the molecular circuitries regulated by Hedgehog (HH) and GLI proteins. The results will improve our understanding of the complex molecular networks regulated by oncogenic HH/GLI signaling and will accelerate the search for novel molecular targets that represent an opportunity for therapeutic intervention.

Future perspectives

Current tools of the systems biology group are going to be further developed and improved and they are going to be used in ongoing and new systems biology projects. A new project on stem cell reprogramming and differentiation related to steatohepatitis is going to start 2010. The established resources, tools, algorithms and models build a foundation for the application of systems biology strategies in medical and pharmaceutical research and, based on data from high-throughput genome, transcriptome, and proteome analysis, it enables the development of a personalized medicine.

Cooperation within the institute

- Ralf Herwig: Bioinformatics analysis and data integration
- Wilfried Nietfeld: Gene expression analysis
- Bodo Lange, Michal-Ruth Schweiger: Genetic and functional analysis of mutations
- James Adjaye: Stem cell reprogramming and differentiation
- Bernhard Herrmann: Annotation and modeling of pathways related to cancer and development

Special facilities

The systems biology group is hosting the PyBioS modeling system (http://pybios.molgen.mpg.de) and the GEne Network GEnerator (GeNGe, http://genge.molgen.mpg.de).

General information

Selected publications

Klipp E, Liebermeister W, Wierling C, Kowald A, Lehrach H and Herwig R (2009). *Systems Biology - A Textbook*, Wiley-VCH, Weinheim.

Kühn C, Wierling C, Kühn A, Klipp E, Panopoulou G, Lehrach H, Poustka AJ. *Monte-Carlo analysis of an ODE Model of the Sea Urchin Endomesoderm Network*. BMC Syst Biol. 2009 Aug 23;3(1):83.

Hache H, Wierling C, Lehrach H, Herwig R. *GeNGe: systematic generation of gene regulatory networks*. Bioinformatics. 2009 May 1;25(9):1205-7.

Kamburov A, Wierling C, Lehrach H, Herwig R. *ConsensusPathDB-a database for integrating human functional interaction networks*. Nucleic Acids Res. 2009 Jan; 37 (Database issue):D623-8. Epub 2008 Oct 21.

Wierling C, Herwig R, Lehrach H. *Resources, standards and tools for systems biology*. Brief Funct Genomic Proteomic. 2007 Sep;6(3):240-51.

Patent

H. Lehrach, C. Wierling, R. Herwig (2008) *Computer implemented model of biological networks* (MI-3905/P1832EP).

Work as scientific Referee

Christoph Wierling serves as scientific referee for the following journals: Database – Journal of biological databases and curation, PLoS Computational Biology.

External funding

BMBF projects: *Mutanom, MoGLI, METASTEM*

EU project: *SysCo*

Chromosome 21, Gene expression & Regulation

(Established: 1996)

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Hans-Jörg Warnatz* (10/07 - 04/09)
Marc Sultan* (07/05 - 07)

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Emilie Dagand, Emilie* (since 07/05)
Daniela Balzereit* (since 09/05)
Asja Nürnberger* (since 10/05)

Technicians
Sabine Thamm
Cindy Springer* (03/05 - 10/08)

Bioinformaticians
Meryem Avci* (since 09/09)
Alon Magen* (10/05 - 01/08)

Current state of research and scientific findings

The group, originally focused on human chromosome 21 (HSA21) and molecular genetics of Down syndrome (DS), is now involved in several studies aimed at understanding gene regulation networks. Besides high-resolution transcriptome profiling in DS and mouse models, current projects address structural analysis of gene promoters, characterization of transcriptional regulators, associated targets and regulatory networks modulating transcript expression.

Chromosome 21 and DS

(EU-ANEUploidy, "NGFN-promoter resource)
Trisomy 21 leads to DS, a developmental disorder characterized by a constellation of signs with a broad range of occurrence and severity among patients. It is likely that variation in gene expression in the population contributes to the heteroge-

* externally funded

neous clinical picture. Using a mouse model of DS (Ts65Dn), we monitored the activity of HSA21 gene orthologs in three brain regions by real-time PCR. We stratified the genes in three categories: 1) expression consistently higher in Ts65Dn than in euploids, (e.g *App* and *Cbr1)*; 2) expression displaying partial overlap between the two groups, and 3) genes with intermingled expression, precluding to differentiate trisomic from euploid (Sultan et al. 2007). We postulate that genes from the first category prioritize candidates for DS phenotypes. We are now investigating DS lymphoblastoid cell lines by RNAseq, allowing quantitative RNA profiles at a bp resolution, with a virtually unlimited dynamic range. In a pilot study, we compared digital transcriptomes obtained by RNAseq between two human cell lines, addressing differential gene expression, comparison to microarrays and prediction of alternative splicing events (Sultan et al. 2008; Richard et al, Nuc. Acid. Res, submitted).

We are analysing the binding properties of HSA21 transcription factors (Tfs) by chromatin immuno-precipitation and sequencing (ChIPseq). We generated >10 million reads for BACH1, GABPA, PKNOX1 and ERG, allowing a quantitative definition of binding sites (BS) at bp resolution. The action spectrum of HSA21 Tfs appeared very different, ranging between <100 to > 1000 targets. RNAi experiments (next §), showed that Tf knock-down has an effect on the expression levels for only a fraction of the targets, suggesting a pausing in regulatory regions in the absence of stimulatory signals (paper in prep.).

ChIPseq combined with comparative genomics can be powerful for tracing *cis*-evolutionary adaptations in gene regulation, which are likely to account for many of the phenotypic differences seen between closely related species. We search for the occurrence of BS for GABPA in orthologous regions of non-human primates. To assess the impact of a gain of BS in the human and hominid lineages, we performed promoter-reporter assays of wild type and mutated promoters. Reversion of human BSs to the ancestral states mostly resulted in lower gene activity, while mimicking the human BS in chimpanzee and rhesus led to increased gene activity (paper in prep).

We cloned most of the presumed gene promoters mapping to HSA21 and tested their capacity to drive transcription using transfected cell arrays. Promoter activities were correlated with the architecture of modular regulatory elements and with endogenous transcript levels (Warnatz et al. submitted).

Dissecting gene regulation networks

We are following two strategies:

1) In the RNAi project, the transcriptomes of HEK cells have been monitored on Affy arrays after the individual knock-down of ca. 180 human Tfs. Data are currently being analysed, and combined with ChIPseq, whenever available.

2) We are analysing the transcriptome of mouse chromosome-substitution strains (consomic) as readout for identifying factors modulating gene activity (see report 2006). Using a panel of C57BL/6-Chr#^{PWD} consomic mice (J. Forejt, Prague), we sequenced brain mRNAs of all consomic and parental strains (C57BL6/J and PWD/Ph). We identified 1,340 genes located on the substituted chromosomes (*cis*) whose expression levels change by at least two fold relative to B6. Besides, 3,783 genes showed *trans* effects. We observed that *cis* effects were generally stronger than *trans* effects, pointing out that cis-regulatory sequences were determinant in the control of gene expression. A large fraction of the genes showing *trans* effects showed changes in their expression levels in more than one consomic, likely reflecting the complexity in gene regulation orchestrated by multiple factors. Validation tests are performed by quantitative real time PCR (Fig.1).

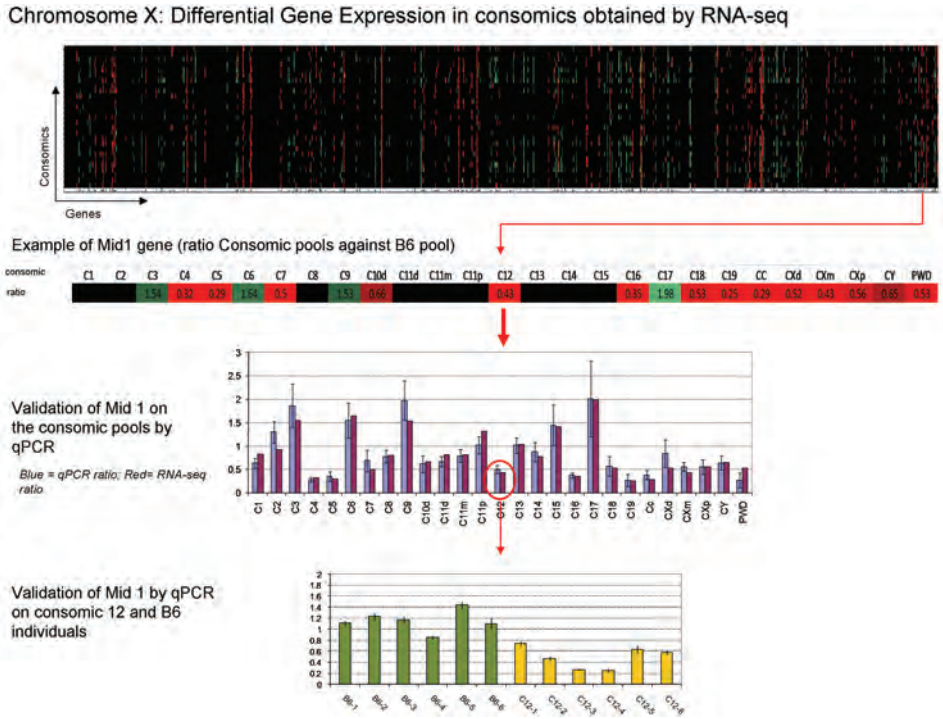

Figure 1: Variation of Mid1 gene expression in consomics

As partner of the Systems Biology of Steatohepatitis, we are performing RNAseq on consomic mice and parental strains for two consomic panels, "B6 x PWD" and "B6 x A/J" (Nadeau et al., 2000) for identifying factors involved in steatohepatitis, a metabolic liver disease representing a major cause of chronic liver damage in human. The phenotypic screen is based on the fact that B6, PWD and A/J respond differently to steatohepatitis inducing agents.
In a new EU project TRIREME (*Tackling the Response to Ionizing Radiation by Extensive Multilevel Exploration*) aimed at studying the DNA damage response, we are performing ChIP-seq for RelA (p65 subunit of NFkappaB signal transducer), activated upon irradiation in CAL51 cells. Binding properties prior and after irradiation will be compared and interpreted together with the corresponding mRNA profiles.

Mouse expression atlas

The EURExpress EU consortium generated mouse expression patterns on C57/Bl6 sagittal sections at E14.5 by non-radioactive ISH (see report 2006). Here, we co-manage the template design and built the ISH pipeline with the requested SOPs. We generated expression patterns for > 3,000 genes, representing ~50,000 images. The consortium has built an interactive digital transcriptome atlas ca. 18,000 genes (www.eurexpress.org); 40% of those show a restricted expression pattern (Fig. 2), and 950 genes are exclusively expressed in a single tissue (Diez-Roux et al. submitted).

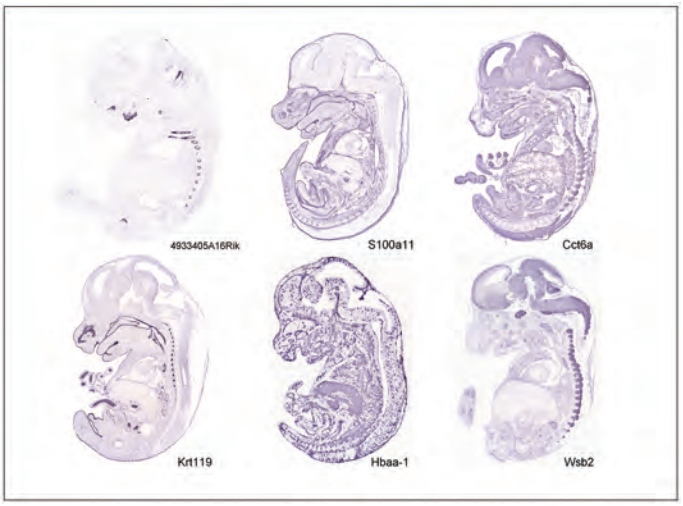

Figure 2: Examples of gene expression patterns at E14.5

Internal cooperations

- Alexey Soldatov, Dept. of Vertebrate Genomics - next generation sequencing (NGS) protocols and data analysis
- Ralf Herwig, Dept. of Vertebrate Genomics - microarray and NGS analysis
- Martin Vingron, Dept. of Computational Molecular Biology - NGS analysis
- Stefan Mundlos, Development & Disease Group - ISH

Special facilities

ISH pipeline, microtome and automated scanning microscope

Planned developments

Short term plans are 1) to analyse further the consomic systems; selected *cis* and *trans* effects will be mapped on F2 animals to narrow down the associated chromosomal region and ISH will be performed on consomics and parental strains. 2) To pursue ChIPseq/RNAi analyses for charting regulation networks. 3) to consolidate data on DS expression profiles. Longer terms plans will make use of the know-how gained in the current projects for stepping into the field of systems biology of cancer, involving the analysis of gene mutations and expression profiles for understanding affected pathways, and testing drug effects on patient-derived cell lines (e.g. funded project, ICGC, coordinated by P. Lichter, DKFZ).

General information

Selected publications

Sultan M, Schulz MH, Richard H, Magen A, Klingenhoff A, Scherf M, Seifert M, Borodina T, Soldatov A, Parkhomchuk D, Schmidt D, O'Keeffe S, Haas S, Vingron M, Lehrach H, Yaspo ML. *A global view of gene activity and alternative splicing by deep sequencing of the human transcriptome*. Science 2008 Aug 15;321(5891):956-60.

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Selected invited talks

The Biomedicum Helsinki Seminar Series: Biomedicum Helsinki Research Center, University of Helsinki, 12.10.2009

CRESCENDO: Consortium for Research into Nuclear Reports in Development and Aging. Workshop: O*n genome-scale approaches in nuclear receptor function,* Munich, 17.04.2008

Second Novo Nordisk Symposium: *From Genes to Endocrinology - New Challenges for Medical Care*, Kopenhagen, 01.12.2006

Work as scientific referee

Marie-Laure Yaspo serves as scientific referee for the following journals: Genome Research, PLoS Genetics, Nature methods, Genomics, Science, Human Genetics.

External funding

EU: *EURExpress - a European consortium to generate a web-based gene expression atlas by RNA in situ hybridization*, 01/05-10/09

EU: *ANEUPLOIDY - Understanding the importance of gene dosage imbalance in human health using genetics, functional genomics and systems biology*, 12/06-11/10

EU: *TRIREME - Systems-level, multi-layer understanding of cellular responses to ionizing radiation*, 01/09-12/11

BMBF, NGFN2: *SMP RNAi - Systematisch-Methodische Plattform "RNAi": Analyse von transkriptionsregulatorischen Netzwerken*, 01/05-10/08

IMGuS-*Systembiologie der Steatohepatitis*, 09/08-11/10

External collaborations

(additional to those in the grants)

Roger Reeves, Dpt. of Physiology, Johns Hopkins University School of Medicine, Baltimor, USA

Martin Seifert: Genomatix GmbH, Munich, Germany

Organisation of scientific events

M.-L. Yaspo has been main organizer of the following workshops:

EMBO Practical Course: Next Generation Sequencing: CHIP-seq and RNA-seq (co-organized with A. Soldatov and M. Vingron), 02.02.-13.02.2009

AnEUploidy Bioinformatics Workshop, 05.05.-07.05.2008

NGFN RNAi Workshop, 15.05.2006-18.05.2006

General information about the whole Department

Complete list of publications (2006-2009)

2009

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Zi Z, Klipp E. *SBML-PET: a systems biology markup language based parameter estimation tool*. Bioinformatics. 2006 Aug 22.

Selected invited talks (Hans Lehrach)

Keynote Lecture: Omics in the present and Future of Human Medicine, Workshop: Genomics in Cancer Risk Assessment,Italy, Venice, San Servolo, 28.08.2009

Genomic Tools and Systems Biology, READNA Symposium on Advanced Nucleic Acid Analysis Methods,Germany, Berlin, Harnackhaus, 06.07.2009

*Technological Advances & Systems Biology,*25th Anniversary Symposium on Genomics and Medicine, Fondation Jean Dausset - CEPH,France, Paris,22.03.2009

*From Functional Genomics to Systems Biology,*International Conference of the Institute of Molecular Genetics AS CR,Czech Republic, Prague,26.09.2008

Chair: Symposium:Omics: Genomics, Transcriptomics, Proteomics, XX. International Congress of Genetics in Berlin, Germany, 14.07.2008

*Targeted research Approaches: Presentation Scientific Research Interests,*EU-USA Workshop: How Systems Biology can Advance Cancer Research, Belgium, Brussels, 19.05.2008

*Keynote Lecture: Medical Systems Biology,*Systems Genomics 2008 Heidelberg, Germany,03.05.2008

*Vertebrate Genomics,*Symposium: From Systematic to Synthetic Biology,Scotland, Edinburgh,04.09.2007

Chair, Session "From Genomics to Function", Inflammattory Barrier Disease Meeting, Kiel, Germany, 13.07.2007

Functional Genomics, Systems Biology and Disease, Final MoolTools Meeting, Germany, Heidelberg, 05.06.2007

*Functional Genomics, Systems Biology and Disease,*European BioPerspectives Meeting 2007,Germany, Köln,31.05.2007

Invited Speaker: Genomics, Genome Sequences and Regulation of Gene Expression, World Immune Regulation Meeting, Swiss, Davos, 15.05.2007

*Opening Keynote Presentation: From Functional Genomics to a Systems Biology of Cancer,*Genomics and Cancer Conference 2006,Germany, Heidelberg,13.09.2006

*Chair und Keynote Lecture: From Functional Genomics to Systems Biology,*1st International Conference. Neurodegenerative Diseases: Molecular Mechanisms in a Functional Genomics Framework,Germany, Berlin, MDC,07.09.2006

*From Functional Genomics to Systems Biology,*20th IUBMB International Congress of Biochemistry and Molecular Biology and 11th FAOBMB Congress,Japan, Kyoto,21.06.2006

*Lecture,*European Workshop on European Database and Analysis Resources for Human Genetic Variation Research,Belgium, Brussels,02.03.2006

Membership in journal editorial boards (Hans Lehrach)

- Journal of Molecular Medicine, since 2002 (member of Advisory Board)
- Functional and Integrative Genomics, since 2005
- Neurodegenerative Diseases, since 2003
- BMC Genomics, since 2005 (associate editor)
- BMC Systems Biology, since 2007
- BMC Research Notes, since 2007 (associate editor)
- Journal of Biomedicine and Biotechnology, since 2009
- Genes, since 2009

Memberships in professional societies (Hans Lehrach)

Austrian Biochemical Society (until 2008)

Service to scientific community (Hans Lehrach)

Member of the Scientific Advisory Board, German Primate Center, Göttingen, 1998-2006

Chairman of the Scientific Advisory Board, Deutsches Ressourcenzentrum für Genomforschung GmbH, 2001-2006

Member of the Scientific Advisory Board Austrian Genome Research Project (GEN-AU), Austria, since 2001

Member of the Project Committee, National Genome Research Network, since 2001

Member of the Advisory and Evaluation Board of the Organisational Unit for Research Infrastructure, University Graz, since 2009

Member of the Advisory Board of Genome Biology, since 2000

Appointments of former members of the department

Prof. Edda Klipp: Faculty of Mathematics and Natural Sciences, Department of Biology, Humboldt University, Berlin

Dr. Heinz Himmelbauer: Head of the CRG Ultrasequencing Unit, Centre for Genomic Regulation (CRG), Barcelona, Spain

Dr. Konrad Büssow: Junior group leader, Helmholtz Zentrum für Infektionsforschung, Braunschweig

Dr. Sylvia Krobitsch: Head of Independent Minerva Group, OWL, MPIMG, Berlin

Dr. Sascha Sauer: Head of Independent Junior Research Group, OWL, MPIMG, Berlin

Dr. Michal Janitz: Senior Lecturer, School of Biotechnology and Biomolecular Sciences, University of New South Wales, Australia

Postdoctoral lecture qualification (Habilitation)

Silke Sperling (2009) *Discovering the transcriptional networks for cardiac development, function and disease with an systems biology approach*. *venia legendi* at Charité (Molecular Biology and Bioinformatics)

PhD theses 2009

Young-Sook Baek (2009) *Gene Expression Analysis of Differentiating U937 Cells*. Doctoral Thesis, FU Berlin, Germany

Thore Brink: *Transcriptional and signaling analysis as a means of investigating the complexity of aging processes in human and mouse*. Doctoral Thesis, Freie Universität Berlin, 02/2009 (supervisor: James Adjaye)

Hendrik Hache (2009) *Computational Analysis of Gene Regulatory Networks*. Doctoral Thesis, HU Berlin, Germany. (supervisor: Christoph Wierling)

Justyna Jozefczuk (2009) *Analysis of dynamic regulatory events during human embryonic stem cell differentiation into hepatocytes: new insights on downstream targets*. Doctoral Thesis submitted, FU Berlin, Germany (supervisor: James Adjaye)

Marc Jung (2009) *A data integration approach to mapping OCT4 gene regulatory networks operative in embryonic stem cells and embryonal carcinoma cells*. Doctoral Thesis submitted, FU Berlin, Germany (supervisor: James Adjaye)

Alexander Kühn (2009) *Functional analysis of dickkopf in the seaurchin Strongylocentrotus Purpuratus*. Doctoral Thesis, FU Berlin, Germany (supervisor: Georgia Panopoulou, Albert Poustka

Theam Soon Lim (2009) *Parameters affecting phage display library design for improved generation of human antibodies*. Doctoral Thesis, FU Berlin, Germany (supervisor: Zoltán Konthur)

Martje Tönjes (2009) *Transcription networks in heart development and disease with detailed analysis of TBX20 and DPF3*. Doctoral Thesis, FU Berlin, Germany FU Berlin (supervisor: Silke Sperling)

Hans-Jörg Warnatz (2009) *Systematic cloning and functional analysis of the proteins encoded on human chromosome 21*. Doctoral Thesis, FU Berlin, Germany (supervisor: Marie-Laure Yaspo)

Christoph Wierling (2009) *Theoretical Biology: Modeling and Simulation of Biological Systems and Laboratory Methods*. Doctoral Thesis, FU Berlin, Germany (supervisor: Ralf Herwig)

2008

Juliane Dohm (2008) *Physikalische Kartierung des Zuckerrübengenoms (Beta vulgaris) und Analyse genomischer Sequenzen aus Sanger- und Solexasequenzierung.* Doctoral Thesis, University Potsdam, Germany (supervisor: Heinz Himmelbauer)

Jenny Fischer (2008) *Regulatory Networks of Gene Expression in Heart and Skeletal Muscle Cells on the Level of Histone Modifications and Transcription Factors*. Doctoral Thesis, FU Berlin, Germany. (supervisor: Silke Sperling)

Peter Hurt (2008) *Genomic Sequencing, Annotation and Comparative Analysis of the Rat Major Histocompatibility Complex*. Doctoral Thesis, TU Berlin, Germany. (supervisor: Heinz Himmelbauer)

Martin Kerick (2008) *Development of an HLA microarray and its application in IBD*. Doctoral Thesis, FU Berlin, Germany (supervisor: Michal Schweiger)

Martin Lange (2008) *Studies on the role of transcription factor Dpf3 in epigenetic control of heart and skeletal muscle development*. Doctoral Thesis, FU Berlin, Germany (supervisor: Silke Sperling)

Florian Mertes (2008) *Ojoplano – Identifizierung und Charakterisierung eines neuen Gens in Säugetieren sowie Etablierung einer konditionalen Knockout-Maus für Untersuchungen zur Funktion*. Doctoral Thesis, TU Berlin, Germany (supervisor: Heinz Himmelbauer)

Hannah Müller (2008) *Proteomic and functional analysis of the Drosophila melanogaster centrosome identifies a novel role in cell cycle control.* Doctoral Thesis, FU Berlin, Germany (supervisor: Bodo Lange)

2007

Andreas Dahl (2007) *Development of a miniaturised platform for PCR based assays with application in gene expression analysis and SNP genotyping*. Doctoral Thesis, FU Berlin, Germany (supervisor: Lajos Nyarsik)

Jianping Liu (2007) *Towards rapid and cost-effective genome characterisation using LNA-modified oligonucleotide DNA hybridisation*. Doctoral Thesis, FU Berlin, Germany (supervisor: Michal Janitz)

Ute Nonhoff (2007) *Untersuchungen zur Rolle von Ataxin-2 im zellulären mRNA-Metabolismus*. Doctoral Thesis, FU Berlin, Germany (supervisor: Sylvia Krobitsch)

Mark Sultan (2007) *Taking a functional genomic approach to the study of down syndrome pathogenesis*, Doctoral Thesis, FU Berlin, Germany (supervisor: Marie-Laure Yaspo)

2006

Andrea Fiebitz (2006) *High-throughput Screening of Protein-Protein-Interactions in Mammalian Cells using transfected cell arrays*. Doctoral Thesis, FU Berlin, Germany (supervisor: Michal Janitz)

Yuhui Hu (2006) *Cell array-based functional analysis of human chromosome 21 proteins: Protein localization & programmed cell death (apoptosis)*. Doctoral Thesis, FU Berlin, Germany (supervisor: Michal Janitz)

Markus Ralser (2006) *Identification of Molecular Pathways Contributing to Spinocerebellar Ataxia Type 2*. Doctoral Thesis, University of Salzburg, Austria (supervisor: Sylvia Krobitsch)

Maryam Zadeh-Khorasani (2006) *Physical mapping of medaka (Oryzias latipes) genome*. Doctoral Thesis, FU Berlin, Germany. (supervisor: Heinz Himmelbauer)

Student theses

2009

Thomas Bergmann (2009) *Development of a Multi-Wavelength Fluorescence Reader for Nanoliter PCR Applications*. Diploma Thesis, TU Berlin, Germany (supervisor: Andreas Dahl)

Felix Bormann (2009) *Development of a Multiplex Assay for robust Determination of epigenetic Changes in genomic Regions, associated with the Formation of Colorectal Cancer.* Diploma Thesis, FU Berlin, Germany (supervisor: Andreas Dahl)

Lena Braun (2009) *Functional Analysis of conserved non-coding elements in zebrafish*. Diploma Thesis, TU Darmstadt, Germany (supervisor: Georgia Panopoulou, Albert Poustka)

Cornelia Dorn (2009) *Charakterisierung der Interaktion des epigenetischen Transkriptionsfaktors DPF3 mit dem Baf-Chromatin-Remodelingkomplex*. Diploma Thesis, HU Berlin, Germany (supervisor: Silke Sperling)

Udo Georgi (2009) *Comparison of the co-injection and Tol2 transposon based injection methods for the identification of regulatory elements in zebrafish*. Diploma Thesis, FU Berlin, Germany (supervisor: Albert Poustka, Georgia Panopoulou)

Nicole Hallung (2009) *Die Rolle des Origin Recognition Complex in Zellzyklus, Zentrosomenzyklus und Mikrotubuliorganisation in Drosophila SL2 Zellen*. Diploma Thesis, FU Berlin, Germany (supervisor: Bodo Lange)

Melanie Isau (2009) *Untersuchungen zur zellulären Funktion des Proteins Ataxin-2*. Diploma Thesis, FU Berlin, Germany (supervisor: Sylvia Krobitsch)

Christian Kähler (2009) *Funktionelle Charakterisierung von Ataxin-2-like: Untersuchungen zur Rolle desAtaxin 2-like Proteins im zellulären mRNA-Metabolismus*, Diploma Thesis, FU Berlin, Germany ((supervisor: Sylvia Krobitsch)

Michalina Mankowska (2009) *Untersuchung der Homo- und Heterodimerbildung des epigenetischen Transkriptionsfaktors DPF3*. Diploma Thesis, FU Berlin, Germany (supervisor: Silke Sperling)

Lina Milbrand (2009) *Untersuchung zur zellulären Funktion von Ataxin-2*. Diploma Thesis, University Greifswald, Germany (supervisor: Sylvia Krobitsch)

Lukas Mittermayr (2009) *Entwicklung einer array-basierten genetischen Karte für das Zuckerrübengenom*. Master Thesis, University Salzburg, Austria (supervisor: Heinz Himmelbauer)

Constanze Schlachter (2009) *Untersuchung der ligandenabhängigen strukturellen Dynamik repetitiver DNA-Elemente*. Master Thesis, FH Wildau, Germany (supervisor: Jörn Glökler)

Lisa Scheunemann (2009) *Development of a 'Quadro-TAG' Library Preparation for Gene Expression Profiling on Next-Generation Sequencing Platforms.* Diploma Thesis, FU Berlin, Germany (supervisor: Andreas Dahl)

Jana Tänczyk (2009) *Cloning and comparative analysis of the sea anemone (Nematostella vectensis) neural genes with their sea-urchin orthologs*. Diploma Thesis, FU Berlin, Germany (supervisor: Georgia Panopoulou, Albert Poustka)

Susanne Weber (2009) *Identifizierung funktioneller Intrabodies für die Untersuchung von Protein-Protein Wechselwirkungen in SCA2*. Bachelor Thesis, TU Braunschweig, Germany (supervisor: Zoltán Konthur)

2008

Stefan Börno (2008) *Molekularbiologische Überprüfung eines bioinformatischen Apoptosemodells*. Diploma Thesis, HU Berlin, Germany (supervisor: Michal Schweiger)

David Haselbach (2008) *Detektion einzelsträngiger DNA mit der Hilfe von DNAzymen*. Bachelor Thesis, University Potsdam, Germany

Armin Haupt (2008) *Die Rolle des 90 KDa heat Shock Protein in der DNA-Damage Response in Krebszellen*. Diploma Thesis, University Potsdam, Germany (supervisor: Bodo Lange)

Kamila Klamecka (2008) *Optimization of miniature format real-time PCR*. Master Thesis, Adam Mickiewicz University, Poznan, Poland (supervisor: Wilfried Nietfeld)

Stephan Klatt (2008) *Evaluation of the trypanosomatid protozoan host Leishmania tarentolae for recombinant protein expression*. Diploma Thesis, Technical University Braunschweig, Germany (supervisor: Zoltán Konthur)

Susanne Mathia (2008) *Evolution of the regulatory elements of the SoxB family from amphioxus and zebrafish*. Bachelor Thesis, FH Lausitz, Germany (supervisor: Georgia Panopoulou, Albert Poustka)

Konstantin Pentchev (2008) *Graph-based Simulation of Apoptosis using Petri Nets*. Bachelor Thesis, FU Berlin, Germany (supervisor: Ralf Herwig)

Florian Rubelt (2008) *Serological Evaluation of Putative Autoantigens in Coeliac Disease Patients by Protein Microarray Technology*. Diploma Thesis, FU Berlin, Germany (supervisor: Zoltán Konthur)

Jenny Schlesinger (2008) *Funktionsanalyse und Identifizierung von Interaktionspartnern des Transkriptionsfaktors DPF3*. Diploma Thesis, FU Berlin (supervisor: Silke Sperling)

Dominic Schmidt (2008) *ChIP-on-chip of the Chromosome 21 Transcription Factor BACH1*. Diploma Thesis, FU Berlin, Germany (supervisor: Marie-Laure Yaspo)

Andrea Wunderlich (2008) *Protein-Protein-Interaktionsnetzwerke des Zervix-Karzinoms*. Diploma Thesis, FU Berlin, Germany (supervisor: Michal Schweiger)

2007

Marcus Albrecht (2007) *Quality Control and Normalization of BeadArray Data*. Diploma Thesis, FH Berlin, Germany (supervisor: Ralf Herwig)

Hannes Hettling (2007) *SyBOP: A systems biology platform for simultaneous parameter and topology optimization*. Master Thesis, FU Berlin, Germany (supervisor: Ralf Herwig)

Doreen Janke (2007) *A CpG island array as a tool for studying the epigenetic basis of cellular differentiation*. Diploma Thesis, University Potsdam, Germany (supervisor: James Adjaye)

Atanas Kamburov (2007) *ConsensusPathDB - a database for matching pathway annotation for human. Department of Mathematics and Informatics*. Master Thesis, FU Berlin, Germany (supervisor: Ralf Herwig)

Clemens Kühn (2007) *Mathematical Modeling of a Sea Urchin Gene Regulatory Network*. Master Thesis, FU Berlin, Germany (supervisor: Georgia Panopoulou, Albert Poustka)

Anna Politis (2007) *Charakterisierung der Interaktion der humanen Transkriptionsfaktoren cJun und NFkappaB mit ihren Targetsequenzen*. Diploma Thesis, TU Berlin, Germany (supervisor: Harald Seitz)

Christina Röhr (2007) *Erstellung und Analyse von Genexpressionsprofilen in einem Mausmodell für Schlaganfall*. Diploma Thesis, University Kassel, Germany (supervisor: Wilfried Nietfeld)

Mareike Schnaars (2007) *Funktionelle Charakterisierung der Proteininteraktionen zwischen Ataxin-2, LSm12 und Chromogranin B*. Diploma Thesis, University Bremen, Germany

Anne-Kathrin Scholz (2007) *Isolation and Characterization of Protein Complexes*. FH Bonn-Rhein-Sieg, Germany (supervisor: Bodo Lange)

Dimitri Soumailakakis (2007) *Präparation und Charakterisierung des humanen VCP/AMFR-Komplexes für die Strukturaufklärung*. Diploma Thesis, TU Berlin, Germany

Katja Stirl (2007) *Mutationsanalyse von Kandidatengen angeborener Herzfehler*. Diploma Thesis, TU Bergakademie Freiberg, Germany (supervisor: Silke Sperling)

Anja Thormann (2007) *Topologische Analyse von Protein-Protein-Interaktionsnetzwerken*. Bachelor Thesis, FU Berlin, Germany (supervisor: Ralf Herwig)

Christopher Weidner (2007) *Identification and Characterisation of Ligands of the human Peroxisome Proliferator-Activated Receptor γ (PPARγ) by using Biophysical and Cell-based Methods*. Diploma Thesis, FU Berlin, Germany

2006

Arne Bittig (2006) *Combined Visualisation of Pathway and Protein-Protein Interaction Data*. Diploma Thesis, University Rostock, Germany (supervisor: Ralf Herwig)

Anselm Helbig (2006) *Modeling of the Yeast TOR pathway.* Diploma Thesis, FU Berlin, Germany

Desagani Naresh Kumar (2006) *Investigations into molecular pathways involved in spinocerebellar ataxia type 2*: Skövde University, Sweden

Markus Schüler (2006) *Organisation of transcriptomes – Searching for regulatory DNA elements involved in the co-regulated expression of genomic neighbours*. Master Thesis, FU Berlin Germany (supervisor: Silke Sperling)

Marvin Schulz (2006) *Dimension Reduction of Biochemical Network Models: Implementation and Comparison of Different Algorithms*. Bachelor Thesis, FU Berlin, Germany (supervisor: Ralf Herwig)

Philip Tomann (2006) *Investigations into molecular pathways involved in spinocerebellar ataxia type 2murinen Kardiomyozyten*. Diploma Thesis, FU Berlin, Germany (supervisor: Silke Sperling)

Jannis Uhlendorf (2006) *Development of a mathematical model for the occupancy of mRNA with ribosomes in Saccharomyces cerevisiae*. Bachelor Thesis, FU Berlin, Germany

Franziska Wegerich (2006) *Protein Microarray Analysen der Immunantwort gegen Neisseria meningitides*. Master Thesis, FH Wildau, Germany (supervisor: Zoltán Konthur)

Franziska Welzel (2006) *Funktionelle Charakterisierung der Interaktion von Ataxin-2 und DDX6*. Diploma Thesis, FU Berlin, Germany

Judith Wodke (2006) *Qualitative Modelling of the Human Cell Cycle*. Master Thesis, FU Berlin Germany (supervisor: Ralf Herwig)

Gina Ziegler (2006) *Validierung von Genexpressionsanalysen in einem Mausmodell für Hirnschlag*. Diploma Thesis, TU Berlin, Germany

Spin-offs

Atlas Biolabs GmbH, Köln, Germany, 2007

Alacris Pharmaceutical GmbH, Berlin, Germany, 2008

Teaching activities

EMBO Practical Course: *Next generation sequencing: ChIP-seq and RNA-seq*, MPIMG, Berlin, 01. - 13.02.2009

Lecture *From functional genomics to systems biology*, Freie Universität Berlin, since 1998

Organization of scientific events

Annual NGFN Meeting, Berlin, 26.-28.11.2009

EU-project meeting**:** *Genomic variations underlying common neuropsychiatric diseases and cognitive traits in different human populations*, 05. - 06.10.2009

1st International Conference Neurodegenerative diseases: *Molecular Mechanisms in a Functional Genomics Framework*, MDC, Berlin**,** 06. - 09.09.2006

Public relations (selection)

Dialog Direkt, Gesprächsforum der MPG, visit of a group of political decision-makers, 22.09.2009

Combining Deep Sequencing and Predictive Models to improve Cancer Therapy, Interview: In Sequence/GenomeWeb, 28.07.2009

Chemisch-genetische Profilierung mit Next Generation Sequencing, M. Ralser, H. Lehrach et al., article in „Laborwelt“ 3/2009, 19.06.2009

Systematische Analyse der genetischen Variabilität des Menschen, B. Timmermann, H. Lehrach et al., article in „Laborwelt“ 3/2009, 19.06.2009

1000-Genome-Project, Interview for the Europamagazin der Deutschen Welle, 04.04.2009

Statement about the *1000 Genomes Project*, BioTOP Report, 10.03.2009

Interview/Videostatement for the exhibition Science Express / Expedition Zukunft (04-11/2009), 22.12.2008

Sequenziersysteme der neuen Generation - Eine Perspektive, Yaspo, Sudbrak, Sultan, Lehrach, article in „GenomXpress“ 4.08, 10.12.2008

Die Offenbarung, interview for „Die Zeit“ about the Personal Genome Project of George Church, 30.10.2008

Was die Uni super macht, interview for "Der Tagesspiegel", 28.06.2007

Das Erbgut schnell durchleuchten. Wer den X-Prize gewinnen will, muss hundert Humangenome in zehn Tagen entziffern, interview for the „Berliner Zeitung", 08.11.2006

Guest scientists

Mireia Vilardell Nogales, Agencia de Gestión de Ayudas, Universitarias y de Investigación (AGAUR)/catalan government (Spain), 01.10.2009- 30.09.2011 (host: Herwig)

Gina Ziegler, TU Berlin, 01.10.- 31.12.2009 (host: Nietfeld)

Kathrin Trappe, FU Berlin, Institut für Informatik, 14.09.- 30.11.2009 (host: Bertram)

Mirjam Blattner, Veterinärmedizinische Universität Wien, Östereich, 01.- 30.09.2009 (host: Dahl)

Ulrike Giese, Universität Kassel, 24.08.- 02.10.2009 (host: Seitz)

Kirsten Heinecke, Universität Kassel, 24.08.- 31.10.2009 (host: Seitz)

Michelle Hussong Fachhochschule Kaiserslautern, Zweibrücken, 24.08.- 20.11.2009 (host: Schweiger)

Chen Hong, College of Animal Science and Technology, China, 12.08.- 16.09.2009 (host: Sperling)

Alexandra Wetzel, Universität Potsdam, 06.08.- 03.09.2009 (host: Glökler)

René Märker, FU Berlin, 03.08.- 31.10.2009 (host: Bertram)

Valko Petrov, Institute of Mechanics and Biomechanics, Bulgarian Academy of Sciences, Sofia, Bulgarien, 01.08.- 30.11.2009 (host: Herwig)

Dejan Gagoski, Universität Kassel, 21.07.- 31.07.2009 (host: Konthur)

Jenny Hoffmann, Uni Dresden, 14.07.- 14.08.2009 (host: Poustka/Panopoulou)

Lucia-Suzanne Postma, VU University Medical Center, Amsterdam, 13.07.- 10.10.2009 (host: Ralser)

Johannes Röhr, FU Berlin, 08.07.- 31.08.2009 (host: Bertram)

Thomas Sander, Cologne Center for Genomics (CCG), University of Cologne, 01.07.2009- 30.06.2010 (host: Höhe)

Katja Obst, Technische Fachhochschule Wildau, 26.06.- 31.07.2009 (host: Yaspo)

Jörn Bethune, FU Berlin, 15.06.- 30.09.2009 (host: Sperling)

Jana Tänczyk, FU Berlin, 12.- 30.06.2009 (host: Lehrach)

Matthias Linser, HU Berlin, 04.- 30.06.2009 (host: Höhe)

Thomas Bergmann, TU Berlin, 01.06.- 31.08.2009 (host: Dahl)

Frederick Klauschen, Charité Berlin, 01.06.- 31.12.2010 (host: Regenbrecht)

Yasmin Müller, TU Berlin, 01.06.- 01.12.2009 (host: Adjaye)

Peggy Motz FU Berlin 21.05.2009- 20.05.2010 (host: Poustka/Panopoulou)

Linda Deutscher, FU Berlin, 19.05.- 13.07.2009 (host: Konthur)

Oliver Herrmann, Fachhochschule Zittau/ Görlitz, 04.05.- 30.06.2009 (host: Poustka/ Panopoulou)

Hanah Kleyer, Universität Kassel, 04.05.- 14.06.2009 (host: Seitz)

Sandra Scharow, Universität Kassel, 04.05.- 14.06.2009 (host: Seitz)

Felix Bröcker, Universität Zürich, 01.05.- 31.10.2009 (host: Mölling)

Steve Michel, Johannes Gutenberg Universität Mainz, 03.04.- 14.06.2009 (host: Ralser)

Markus Bardua, Universität Stuttgart, 01.04.- 15.07.2009 (host: Glökler)

Michalina Mankowska, FU Berlin, 01.- 30.04.2009 (host: Sperling)

Eneida Franco Vencio, Universidade Federal De Goias Faculdade De Odontologia, 23.03.- 31.07.2009 (host: Adjaye)

Christian Börner, Technische Fachhochschule Berlin, 09.- 19.03.2009 (host: Sperling)

Rachel Cavill, Imperial College London, 09.- 24.03.2009 (host: Herwig)

Tonio Schütze, FU Berlin, 16.02.- 31.09.2009 (host: Konthur)

Ulrich Koestner, Georg August Universität Göttingen, 19.01.- 05.03.2009 (host: Poustka/ Panopoulou)

Christina Lill, Charité Berlin, 19.01.-31.12.2009 (host: Bertram)

Susanne Weber, TU Braunschweig, 19.01.-15.05.2009 (host: Konthur)

Delia Könnig, Charité - Julius-Wolff-Institut, 21.11.2008- 30.09.2009 (host: Adjaye)

Svetlana Mollova, TU Braunschweig, 03.11.-19.12.2008 (host: Konthur)

Lisa Scheunemann, FU Berlin, 23.10.2008-11.01.2009 (host: Nietfeld)

Wei Sun Southern, Medical University, China, 17.10.2008- 27.02.2009 (host: Nietfeld)

Zhang Qin, College of Animal Science and Technology, China, 01.10.2008- 31.10.2010 (host: Sperling)

Martje Tönjes, Studienstiftung des deutschen Volkes, 01.10.2008- 31.03.2009 (host: Sperling)

Katharina Wolfrum, Charité Berlin, 01.10.2008- 30.09.2011 (host: Adjaye)

Yongbo Wang, Shanghai University, China, 26.09.2008- 27.02.2009 (host: Nietfeld)

Nora Figueras, Universitat de Barcelona, Spanien, 22.09.2008- 28.02.2009 (host: Krobitsch)

Marie Louise Freverz, Trinity College, Cambridge, UK, 02.09.- 01.10.2008 (host: Konthur)

Dimitra Nousdili, University of the Aegean, Griechenland, 02.09.- 30.11.2008 (host: Poustka/Panopoulou)

Young-Sook Baek, 01.09.- 06.10.2008 (host: Seitz)

Zorica Buzasi, 01.- 30.09.2008 (host: Konthur)

Melanie Isau, FU Berlin, 01.09.2008-31.03.2009 (host: Krobitsch)

Steve Michel, Johannes Gutenberg Universität Mainz, 01.09.- 15.10.2008 (host: Ralser)

Anna-Lena Scherr, Uni Konstanz, 11.08.-19.09.2008 (host: Krobitsch)

Dejan Gagoski, Universität Kassel, 04.08.-12.09.2008 (host: Seitz)

Christian Hoffmann, TU Dresden, 04.08.-01.10.2008 (host: Poustka/Panopoulou)

Andreas Arendt, Uni Kassel, 04.08.-12.09.2008 (host: Seitz)

Ewelina Bakala, FU Berlin, 04.08.-12.09.2008 (host: Adjaye)

Anja Bauerfeind, MDC Berlin-Buch, 04.08.-31.12.2008 (host: Höhe)

Karolin Huckauf, 04.08.- 19.12.2008 (host: Krobitsch)

Birgit Gerisch, Universitätsklinikum Schleswig-Holstein, Kiel, 11.04.- 31.052008; 01.08.- 09.10.2009 (host: Lehrach)

Ali Katanforoush, University of Tehran, Iran, 25.07.- 31.12.2008 (host: Soldatov)

Christian Kähler, FU Berlin, 21.07.2008-31.03.2009 (host: Krobitsch)

Zorica Buzasi, 01.- 31.07.2008 (host: Konthur)

Stephan Starick, Uni Kassel, 30.06.-25.07.2008 (host: Seitz)

Antje Kammermeier, Uni Potsdam, 16.06.-01.08.2008 (host: Poustka/Panopoulou)

Stephan Klatt, TU Braunschweig, 01.06.-15.08.2008 (host: Konthur)

Florian Rubelt, FU Berlin, 01.06.-20.08.2008 (host: Konthur)

David Haselbach, Uni Potsdam, 07.04.-19.08.2008 (host: Seitz)

Lei Mao, Charité Berlin, 07.04.2008-07.04.2010 (host: Sauer)

Elvira Carrió Gaspar, Universitat de Barcelona, Facultat de Biologia, Spanien, 01.04.- 22.08.2008 (host: Krobitsch)

Julia Häfner, Universität Konstanz, 01.04.-08.06.2008 (host: Nietfeld)

Kyu-Hyeon Park, FU Berlin, 01.- 30.04.2008 (host: Poustka/Panopoulou)

Tatjana Schütze, FU Berlin, 01.04.2008-21.08.2009 (host: Glökler)

Sandra Catania, EMBL Heidelberg, 12.03.-31.08.2008 (host: Ralser)

Mirjam Blattner, Veterinärmedizinische Universität Wien, Östereich, 01.03.-30.06.2008 (host: Nietfeld)

Karin Mölling, Universität Zürich, 01.03.-31.08.2008 (host: Lehrach)

Simone Latkolik, Universität Wien, 04.-29.02.2008 (host: Ralser)

Martina Elisabeth Weigt, FU Berlin, 21.01.-15.02.2008 (host: Höhe)

Gerd Moe-Behrens, University College Oslo, Norwegen, 04.01.- 30.06.2008 (host: Adjaye)

Sandra Grodzicki, Universität Potsdam, 02.01.- 22.02.2008 (host: Poustka/ Panopoulou)

Daniela Amann-Zalcenstein, Weizmann Institute, Israel, 18.- 22.11.2007 (host: Sudbrak)

Katja Biens, TU Berlin, 01.11.2007- 29.02.2008 (host: Nietfeld/Dahl)

Karolina Janitz, 01.11.- 31.12.2007 (host: Lehrach)

Lina-Mareike Milbrand, Ernst-Moritz-Arndt-Universität, Greifswald, 12.02.- 06.04.2007; 01.11.2007- 31.07.2008 (host: Nietfeld, Krobitsch)

Mireia Vilardell Nogales, Agencia de Gestión de Ayudas Universitarias y de Investigación (AGAUR)/catalan government (Spain), 08.10.- 05.12.2007 (host: Herwig)

Michael Parsons, Oakhouse, Camberwell, London, UK, SES7TQ, 29.08.2007- 29.08.2008 (host: Nietfeld)

Silja Fuchs, Zoologisches Institut, Kiel, 06.- 17.08.2007 (host: Yaspo)

Robert Martin, Technische Fachhochschule Berlin, 06.08.- 09.09.2007 (host: Hoehe)

Kamila Klamecka, Adam-Mickiewicz-Universität, Poznan, 01.08.2007- 31.01.2008 (host: Nietfeld/Dahl)

Sascha Mohamed, Universität Kassel, 30.07.- 16.09.2007 (host: Seitz)

Kathrin Beutner, Universität Kassel, 23.07.- 16.09.2007 (host: Seitz)

Vincent Ramillon, Max-Planck-Institut für Wissenschaftsgeschichte, 06.06.- 15.09.2007 (host: Yaspo)

Valko Petrov, Institute of Mechanics and Biomechanics, Bulgarian Academy of Sciences, Sofia, Bulgarien, 30.04.- 01.06.2007 (host: Herwig)

Lena Linck, Institut für Biochemie, Berlin, 14.03.- 30.04.2007 (host: Sauer)

Felix Bormann, FU Berlin, 26.02.- 13.04.2007 (host: Sauer)

Elke Mayer-Enthart, Federal Institute for Materials Research and Testing (BAM), Berlin 12.- 28.02.007 (host: Seitz)

Detlef Groth, RZPD, Berlin, 01.- 28.02.2007 (host: Herwig)

Miriam Kaatz, TFH Wildau, 22.01.- 03.08.2007 (host: Kreutzberger)

Nina Kahlfeldt, FU Berlin, 12.12.2006- 09.02.2007 (host: Krobitsch)

Natascha Hill, Universität Potsdam, 20.11.2006 (host: Hoehe)

Mareike Schnaars, Universität Bremen, 01.11.2006- 31.08.2007 (host: Krobitsch)

Theam-Soon Lim, Inprom. Universität sains malaysia, Penang, Malaysia, 29.09.2006- 04.09.2009 (host: Konthur)

Johanna Gostner, Tiroler Krebsforschungsinstitut, Innsbruck/Österreich, 13.09.2006 (host: Krobitsch)

Martin Lorenz, Universität Kassel, 28.08.- 06.10.2006 (host: Seitz)

Michael Schelleckes, Universität Kassel, 28.08.- 06.10.2006 (host: Seitz)

Katrin Köhler, Johann-Wolfgang-Goethe-Universität, Frankfurt/Main 22.08.- 15.09.2006 (host: Klipp)

Claire Seror, Université Paul Sabatin, Toulouse, Frankreich, 01.- 31.08.2006 (host: Yaspo)

Christian Waltermann, Institut für Biologie, Humboldt-Uni Berlin, 01.08.2006- 01.08.2009 (host: Klipp)

Yvonne Baermann, RiNA GmbH, Berlin, 24.07.2006- 31.07.2007 (host: Krobitsch)

Guifré Ruiz Acero, Instituto Ouimico de Sarrai, Barcelona, 20.06.2006- 31.07.2008 (host: Adjaye)

Matteo Barberis, University of Milano-Bicocca, Dept. Biotechnology and Biosciences, 11.05.- 31.10.2006 (host: Klipp)

Xi Cheng, Institut für Klinische Pharmakologie, UKBF, Charité, Berlin, 07.03.2006- 06.10.2007 (host: Janitz)

Kerstin Pietsch, HU Berlin, 27.02.- 15.04.2006 (host: Yaspo)

Nadine Saul, HU Berlin, 27.02.- 15.04.2006 (host: Yaspo)

Franziska Wegerich, Technische Fachhochschule Wildau, 15.02.- 31.08.2006 (host: Büssow)

Kumar Naresh, 01.02.- 31.07.2006 (host: Lehrach)

Annika Andersson, Institut für Klinische Immunologie & Transfusionsmedizin Giessen 01.01.2006- 30.06.2007 (host: Seitz)

Kathrin Stoßberg, HU Berlin 06.12.2005- 06.12.2006 (host: Sperlin)g

Philip Rosenstiel, Universität Kiel 01.11.2005- 31.12.2007 (host: Lehrach)

Maria Sokolova, Genome Analysis Laborator, Institute of General Genetics RAS, Moskau 06.10.2005- 06.01.2006 (host: Soldatov)

Marion Noyer-Weidner, 01.09.2005- 28.02.2006 (host: Nietfeld)

Monica Hirsch-Kaufmann 01.07.2003- 31.01.2010 (host: Lehrach)

Manfred Schweiger 01.07.2003- 31.01.2010 (host: Lehrach)

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Dr. Tim Hucho (since 09/05)
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Introduction

For more than 10 years, genome research has focused on finding genetic risk factors for common disorders, based on the 'Common Disease – Common Variant (CDCV)' hypothesis – the assumption that for most of the common disorders like dementia, diabetes, coronary heart disease, autism and hypertension there are common genetic risk factors. In 2007, after many years of largely futile genome-wide association studies (GWAS), associated markers were identified for a wide variety of complex disorders, which was hailed as a decisive breakthrough in this field. However, these associations were only found after massively increasing cohort sizes and marker densities, meaning that the vast majority of the associated risk factors have small effects and that they are of no diagnostic and prognostic relevance. Therefore, it is now widely believed that for most common disorders, the CDCV hypothesis is wrong[1].

This certainly applies to mental retardation (MR) – the biggest unsolved problem of clinical genetics and the largest socio-economic burden of health care – where most severe forms are due to defined chromosomal abnormalities or single gene defects, instead of resulting from multifactorial inheritance, i.e. the interaction of many different gene variants and environmental factors. However, there is increasing evidence that single gene defects also play a significant, previously underestimated role in other complex disorders. This has led to growing uneasiness about the validity of the idea that GWAS is the preferred approach for identifying sequence variants in the human genome that predispose to, or cause, disease.

[1] Terwilliger & Hiekkalinna, Eur J Hum Genet 14:426-437, 2006; News Feature, Nature 456:18-21, 2008.

Moreover, it has raised serious doubts about the strategy, first proposed in the early nineties and uncritically adopted by leading genome centres worldwide, to focus exclusively on complex disorders.
After the introduction of massive-parallel next generation sequencing techniques, there are now indications for a paradigm shift in this field, with renewed attention on single gene disorders. At a recent meeting[2], two groups reported on their efforts to unravel the molecular basis of Mendelian disorders by sequencing all exons in the genomes of patients and their unaffected parents. Moreover, leading genome researchers expressed their belief that instead of GWAS, whole genome sequencing-based, large-scale elucidation of single gene disorders will be the strategy of choice for shedding more light on the molecular architecture of common disorders.

We were among the first to point out the inherent difficulties of GWAS in complex diseases and to stress that single gene disorders are important in their own right[3]. In line with this, our focus has been, and still is, on the elucidation of single gene disorders. Already in 1995, together with a Danish group, we had launched a project to study disease-associated balanced chromosome rearrangements, as a systematic way to identify disease genes. Almost in parallel, we and others founded the European MRX Consortium, a collaboration involving five European groups that soon became a leader in the search for gene defects causing X-linked mental retardation. In 2003, when it became clear that X-linked forms where not as common as previously thought, our group started a formalized collaboration with a potent group from Iran to study autosomal recessive forms of mental retardation. A year later, and in parallel with only four other groups world-wide, we generated whole genome-spanning tiling path BAC arrays, a novel tool for screening the entire genome for submicroscopic Copy Number Variants (CNVs), which had been implicated in MR and were suspected of playing a role in the aetiology of other diseases, too[4].

Developments since 2006

Next generation sequencing

Since our decision to become beta tester and first buyer of the Solexa/Illumina Genome Analyzer on the European continent, Solexa/Illumina has established itself as the leading manufacturer of next generation sequencing (NGS) systems world-wide, and NGS has become indispensable for our research into MR and related disorders. We were also among the very first users of novel genome partitioning methods, based on hybridization in solution (SureSelect, Agilent), array-based hybridization (Nimblegen and Agilent oligonucleotide arrays) and more recently, multiplex PCR amplification-based methods (Raindance)[5]. Moreover, we pioneered the combination of preparative chromosome sorting and NGS for mutation detection in X-linked and autosomal recessive disorders[6]. With substantial bioinformatics support from the Department of Computational Molecular Biology (head: Martin Vingron), these methods were successfully employed for mutation screening in defined genomic intervals, coding sequences and even in entire chromosomes. Together, they have become a valuable asset for gene finding, and ongoing efforts to generate a pipeline for the analysis of high-throughput sequenc-

[2] See News Feature, Nature 461:459, 2009.
[3] Ropers, Frankfurter Allgemeine Zeitung, 26.01.2001; Ropers, Am J Hum Genet 81:199, 2007; Ropers, OrphaNews, Dec.24th, 2008, http://www.orpha.net/actor/EuropaNews/2008/081224.html.
[4] For more details, see also Research Report 2006.
[5] Hu, Chen, Kalscheuer, Ropers et al, in preparation; see also report of Ullmann and Chen.
[6] See reports of Kalscheuer and Kuss.

ing data (S. Haas et al, Dept. Vingron) promise to overcome the residual limitations of this approach.

Identification of gene defects and genetic risk factors for MR and related disorders

Various complementary strategies were employed to map and identify genetic defects that underlie, or predispose to, mental retardation or related disorders, as outlined in the previous report. Given the many apparently relevant microdeletions and duplications observed in mental retardation, we extended the *search for copy number variants (CNVs)* to patients with autism, schizophrenia, attention deficit/hyperactivity disorder (ADHD) and several other complex diseases. Reasoning that these CNVs would only be relevant for diagnosis and prognosis if found in at least 1% of these patients, and in view of our budgetary constraints, cohort sizes were mostly limited to 100 well-characterized patients.

Among a wide variety of pathogenetically relevant CNVs, we identified a duplication on chromosome 16p13.1 as a risk factor for autism, and the reciprocal deletion in several patients with MR[7]. Follow-up studies[8] have shown that the del16p13.1 is one of the most common risk factors for MR known to date, and that it also predisposes for epilepsy[9]. Recently, other groups[10] found that the dup16p13.1 is also a risk factor for schizophrenia. Previously, other CNVs identified in our schizophrenia cohort had been implicated in autism. These observations point to pathogenetic links between MR, autism and schizophrenia, indicate that all three disorders are highly heterogeneous and have strengthened our belief that large-scale gene finding will eventually lead to the identification of novel regulatory pathways[11].

To speed up fine-mapping of breakpoints and gene finding in patients with *disease-associated balanced chromosome rearrangements (DBCRs)*, we have combined preparative sorting and next generation sequencing of derivative chromosomes from mentally retarded patients. In this way, we have identified several additional candidate genes for MR[12]. As pointed out by the reviewers of this article, this was the first application of NGS for finding novel disease genes. More recently, we have shown that genomic paired-read sequencing is sufficiently accurate for breakpoint mapping, i.e. that prior chromosome sorting is not necessary[13].

According to current estimates[14], approximately 10 percent of all genetic forms of MR are X-linked. To date (October 2009), about 90 genes have been implicated in *X-linked MR (XLMR)*, and mutations in one third of these have been reported in patients with non-syndromic forms of XLMR, with major contributions from our group and from other members of the European MRX Consortium. In 2006, when the Sanger-Wellcome Institute (Hinxton, UK) obtained funds for sequencing all X-chromosomal genes in 200 XLMR families, it was widely believed that this would lead to the identification of most missing XLMR genes, but in the course of this project, no more than 10 additional genes were found. Moreover, to confirm the status of candidate genes showing mutations in a single family, several hun-

[7] Ullmann et al, Hum Mutat 28.7 :674-682, 2007.
[8] Mefford et al, Genome Res 19:1579-1585, 2009.
[9] de Kovel et al, Brain 2009 online, doi:10.1093/brain/awp262.
[10] Ingason et al, Mol Psychiatry, Sept. 29th, 2009 [Epub ahead of print]; Ikeda et al, Biol Psychiatry Oct 30th, 2009 [Epub ahead of print].
[11] For details, see report Ullmann.
[12] Chen et al, Genome Research 18.7:1143-9, 2008.
[13] Chen et al, Eur J Hum Genet 2009, in press; for more details concerning DBCRs, see reports of Kalscheuer and Chen.
[14] Ropers & Hamel, Nature Rev Genet 6:46-57, 2005.

dred additional families had to be tested, which is why the Euro MRX Consortium was invited to join in before the results of this study were published[15]. In our opinion, the relatively meagre results of this study are primarily due to the incomplete coverage of coding regions on the X chromosome, which is due to the fact that only slightly more than 700 genes were screened for mutations, and on average, only 65 percent of the exonic nucleotides were screened. Moreover, this study focused on the detection of protein-truncating mutations, whereas about half of the intragenic sequence variants were non-polymorphic missense changes, and sequence variants outside exons and splice sites were not studied at all. Finally, many of the families studied were small, including also kindreds with only two affected males, where X-linkage is possible but not proven. This has encouraged us to embark on an even more ambitious project aiming to identify all sequence variants in the non-repetitive portion of the X-chromosome in >200 Consortium families with proven X-linked inheritance. For this project, which started in 2009 and is just reaching its production phase, we combine exon enrichment and chromosome sorting with state-of-the-art NGS[16].

Autosomal recessive MR (ARMR) has been largely disregarded in the past, probably because in Western populations, it is rarely familiar and often overlooked[17]. When we and our Iranian colleagues published the results of homozygosity mapping in the first 78 consanguineous ARMR families[18], we did not find any overlapping linkage intervals and concluded that this disorder must be extremely heterogeneous. In the meantime, we have studied more than 200 additional families, and this enabled us to identify several families with overlapping linkage intervals as well as several novel ARMR genes. In several of these, two or even three allelic mutations have been found. These observations appear to refute our earlier statements concerning the extreme heterogeneity of ARMR and argue for the existence of genes that are mutated in several percent of the patients, at least in Iran[19]. Moreover, at least two of these candidate genes seem to directly interact, thereby establishing the first step of a novel MR pathway[20].

From now on, novel genomic enrichment and NGS techniques will greatly accelerate these studies, and already today, the *recruitment and clinical characterization of patients and families* has become the most important rate-limiting factor (see also report Tzschach). Due to our long-standing international partnerships, we are very well positioned in this field. In 2007, we have initiated the MR-NET (co-ordinator A. Reis, Erlangen), which is part of the German National Genome Research Network (NGFN*plus*) and forms a new platform for recruiting German patients and families. Apart from the large and still growing number of XLMR families recruited by the EuroMRX Consortium, numerous additional families with XLMR have been identified in Iran[21] while recruiting familial cases for ARMR studies, and in collaboration with S. Mundlos (MPIMG and Charité Berlin), sporadic patients with MR are being recruited for CNV screening in the context of the MR-NET. So far, our Iranian partner has collected more than 500 consanguineous families with two or more mentally retarded children. In the majority of these, MR

[15] Tarpey et al, Nature Genetics 41.5:535-43, 2009.
[16] See also reports of Chen and Kalscheuer.
[17] See Ropers, Current Opinion in Genetics & Development **16**(3):260-269 (2006); id., Am J Hum Genet 81.2:199-207,2007; id., Current Opinion in Genetics & development 18:241-250, 2008..
[18] Najmabadi et al, Hum Genet 121:43-48, 2007.
[19] See report Kuss.
[20] Moheb, Kuss, Tzschach, Kahrizi, Najmabadi and Ropers, unpublished.
[21] Pouya et al, Eur J Med Genet 52:170-3, 2009.

should be due to autosomal recessive gene defects, and to our knowledge, this is by far the largest cohort of its kind.

Functional studies

During the past few years, the functional expertise available in various groups of our department has been a valuable asset for our research into MR and related disorders, as documented by numerous publications that are co-authored by several group leaders. Still, as predicted in the previous report, research into the function of disease genes can never keep track with their identification, and given the wide range of mechanisms leading to MR, the methodological infrastructure required for such studies will always be a problem.

A possible solution is to focus on the function of specific MR genes (such as PQBP1, CDKL5, ARHGEF9 and DYRK1A)[22] or pathways (e.g., the serotonin metabolism which has been implicated previously in behavioural disorders)[23]. Other groups deal with chromosome dynamics in mitosis and meiosis[24], mechanisms of pain sensitization and perception involving the brain[25] and chromatin modification, another important mechanism in MR[26]. The work of S. Schweiger is exceptional in that her previous investigation of a monogenic malformation syndrome has paved the way for ongoing research into drug therapies for cancer, Alzheimer and Huntington disease[27].

As a fast and (almost) general approach to shed more light on the function of novel MR genes, we have joined forces with Matthias Mann (MPIB, Martinsried) and Antony Hyman (MPICBG, Dresden), who have embarked on a large-scale project to study the interaction of all human proteins. This approach entails the transfection of BAC clones carrying GFP-tagged genes into HeLa and mouse ES cells, followed by GFP-mediated isolation and mass spectrometry of the relevant protein complexes[28]. In parallel, functional analyses of individual genes and proteins will be performed through collaboration with specialized groups.

Outlook

There is now increasing empirical and theoretical support for the notion that large scale identification of MR genes is a viable option for shedding light on their function, as mentioned above[29]: the more genes will be identified, the more likely it is that they will form functional clusters or pathways, and this will be a major asset for understanding their role in the brain and in the pathogenesis of MR. Therefore, and in view of the gradually diminishing resources of our department, which will be closed in Fall 2014, we will increasingly focus on the molecular elucidation of MR and related disorders, as a basis for reliable diagnosis, prevention and eventually, therapy. As of 2011, support from the EU and other sources will partially compensate the gradual loss of structural funding, and various possibilities are being explored to continue this research elsewhere if it is still competitive in 2014.

[22] See report Kalscheuer.
[23] See report Walther.
[24] See report Scherthan.
[25] See report Hucho.
[26] See report Ullmann.
[27] See report Schweiger.
[28] See also report Kuss.
[29] See also Outlook, Research Report 2006.

Signal Transduction in Pain and Mental Retardation

(Established: 09/2005)

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Juliane Schreier* (since 09)
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Technicians
Vanessa Suckow (since 05)

Introduction

The clinical approach to pain therapy is still driven by the pain concurrent symptomatic diseases such as *Herpes zoster* infection, diabetis and/or surgical intervention. The attempted leap to a mechanism based therapy by the BMBF funded "Deutsche Forschergruppe Neuropathischer Schmerz" has failed so far in the face of remaining confusion about the underlying molecular mechanisms. This is partially due to a very limited understanding of the intracellular signalling network responsible for peripheral sensitization, as past research has mostly focussed on the large numbers of extracellular mediators and ion channels involved in various pain states. Signalling has been rather inaccessible for research as no cell line system is established and because the sensory neurons are of a puzzling and ill defined heterogeneity.

Results since 2006

We established and since employ successfully a culture system of primary sensory neurons to study endogenous signalling underlying sensitization. Central to our research is the translocation of one protein kinase C isoform, PKCε, to the plasma membrane in the course of its activation. Based on our studies giving prove of principle for GPCRα-s/cAMP/Epac/PKCε crosstalk occurring only in a subpopulation of sensory neurons, we extended our knowledge about intracellular signalling events correlating with pain sensitization. Finishing a protein array approach, we identified 35 novel PKCε-substrate candidates. These results indicated a novel cellular organelle, the stress granule, to be centrally regulated by PKCε and thereby introduced a novel aspect to cellular pain sensitization beyond ion channel regula-

* externally funded

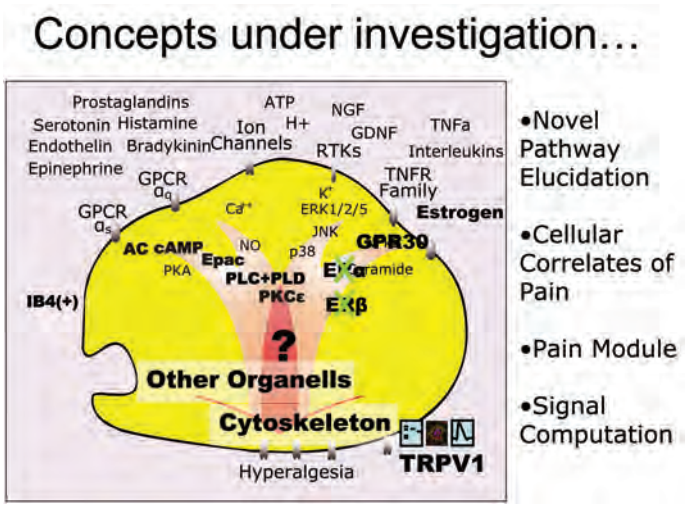
tion. Molecular and cellular details are under investigation, which we pursue in collaboration and technical exchange with Sylvia Krobitsch within the institute. In house collaboration on *in vitro* translation experiments will be established with Knud Nierhaus, and expression clones are shared with Zoltan Konthur.

In parallel, we investigated the action of PKCε signalling on a known phosphorylation target, the capsaicin receptor TRPV1. We describe for the first time that a TRPV ion channel can have a functionality beyond its ion channel properties. We describe PKCε phosphorylation to alter the direct biochemical interaction of TRPV1 with the ends of microtubules, which thereby get released to transfer the sensitizing signal to a nearby effector complex. These data are the very first to describe an ion channel cytoplasmic domain to be a mere signalling intermediate, and show on a cellular level that sensitization signalling results in cytoskeleton rearrangements (manuscript to be submitted by end of 2009).

Beyond the effect of sensitization signalling pathways on microtubules in a TRPV1-dependent manner, we also found TRPV1-dependent changes of the acting cytoskeleton. In addition, we found TRPV1-dependent actin reorganization and lamelipodia to filopodia transgression. The ramifications of these observations are presently under investigation.

Beyond the identification of novel cellular mechanisms of PKCε sensitization signalling we investigated if and how the peripheral sensory neuron integrates multiple sensitizing stimuli. Surprisingly we found, that the signalling outcome is less dependent on the input signal but rather on the signalling history of the cell. The signalling history was memorized for over 20 hours and resulted in inversion of the signaling outcome of successive stimuli. Thus, in primary cell cultures, single fibre electrophysiological recordings, animal behaviour as well as human behavioural studies the successive "sensitization" attempt resulted not in sensitization, but in complete erasure of already established sensitization. This opens a completely novel approach to therapeutical intervention. It is also the very first indication that the activation of endogenous signalling pathways can lead to memory deletion (manuscript currently under revision at PNAS).

The number of pain-inhibitory substances is very limited. We introduced two novel substances to the pain field: estrogen and EGF. We provided evidence for a first biological functionality for the novel estrogen receptor GPR30 *in vitro* as well as *in vivo*. This also gave a first rational for the second most common side effect, namely pain, in the course of breast cancer therapy with Fulvestrant, a now known GPR30 agonist (Kuhn et al. 2008).



EGF plays an important role during tissue regeneration after e.g. painfull mechanical tissue disruption. Establishing a quantitative single cell based immunfluorescence technique, we found different activation kinetics for the MAPK ERK1/2 if activated through EGF versus NGF exposure. Accordingly in behavioural experiments we did not find EGF to induce pain sensitization but to inhibit PGE2

induced sensitization. Indeed, increased pain sensitivity has been reported in clinical trials of cancer related EGFR-blocking therapy. This is the first investigation of the importance of signaling kinetics for pain sensitization (manuscript in preparation).

Being embedded into the Department of Human Molecular Genetics, we have been instrumental in the investigation of a mutation found to cause mental retardation. We described the mutation to result in a loss of function of the glutamate receptor Grik2, thereby giving the first evidence, that indeed glutamate receptors are in humans involved in higher brain functions (Motazacker et al. 2007).

Further, in collaboration with Andreas Kuss and Eckehard Friedel, we collected blood of fibromyalgia patients to identify in a first level of investigation expression markers for better categorization of this widespread phenotype with partial genetic background (prevalence about 5% of the European population). The underlying mutations have so far not been described, something we attribute first of all to the lack of a clear diagnostic marker. The study is ongoing.

Finally, we are involved in the functional analysis of a balanced chromosomal translocation that had been identified by Vera Kalscheur in the department to underlie mental retardation. The breakpoint was mapped to be close to the ion channel TRPV1. TRPV1 had so far mostly been investigated in the context of pain. We now identified TRPV1 to be a synaptic protein, to modulate strongly endo- as well as exocytosis, and to be an important regulator of dendritic spine like protrusions. Thereby we provided a first rational for the observed severe mental retardation phenotype (manuscript under revision at JCS).

We expanded our technical methodological abilites in the laboratory to viral techniques (lenti as well as adeno viruses), to life cell imaging (Calcium imaging as well as life observation of morphological changes), and to high content quantitative single cell microscopy. This allowed us to extend the focus of our work from PKCε to ERK as well as calcium and will facilitate further increase in the number of signaling components under investigation. Further techniques like behavioural experiments and single cell PCR are planned. I am proud to have been instrumental in formation of a BMBF consortium (including Harald Seitz at the MPIMG) around our attempt to identify intracellular signalling modules underlying pain sensitization. In that consortium, which I am coordinating, system biological approaches are initiated.

General information

Selected publications

Goswami C, Hucho T. *Submembraneous microtubule cytoskeleton: biochemical and functional interplay of TRP channels with the cytoskeleton*. FEBS J. 2008; 275:4684-4699

plus Editorial for this series about submembranous cytoskeleton, which we initiated and organised: Goswami C, Hucho T . *Novel aspects of the submembraneous microtubule cytoskeleton*. FEBS J 2008; 275:4653

Kuhn J, Dina OA, Goswami C, Suckow V, Levine JD, Hucho T. *GPR30 Estrogen Receptor Agonists Induce Mechanical Hyperalgesia in the Rat.* Eur J Neuroscience 2008; 27(7):1700-9

Motazacker M, Rost B, Hucho T, Garshasbi M, Kahrizi K, Ullmann R, Abedini S, Nieh S, Amini S, Goswami C, Tzschach A, Jensen L, Schmitz D, Ropers HH, Najmabadi H, Kuss AW. *A Defect in the Ionotropic Glutamate Receptor 6 Gene (GRIK2) is Associated with Autosomal Recessive Mental Retardation.* Am J Hum Genetics 2007; 81(4):792-8

Goswami C, Hucho T . *TRPV1 expression-dependent initiation and regulation of filopodia.* J Neurochem 2007; 103(4):1319-33

Hucho T, Levine JD. *Signaling Pathways in Sensitization: Toward a Nociceptor Cell Biology.* Neuron 2007; 55(3):365-376

Awards

Poster Prize GBM Herbsttagung 2007

PosterPrize Congress of the International Society of Gender Medicine 2006

Work as scientific referee

Tim Hucho serves as scientific referee for the following journals: PNAS, FEBS Journal, Cell Biochemistry and Function, Cell Biology International.

Tim Hucho serves as scientific referee for the following institution: DFG, University of Erlangen.

External funding

BMBF, MedSys: *Modeling of peripheral Pain Switches (MoPS)*, 02/09-01/12

DFG Hu 1636/2-1: *Identification of substrates of PKCε and their role in pain sensitization,* 05/08-04/11

Studienstiftungs-stipend with/for Julia Kuhn, max 3 years

Teaching activities

Lecture „Biochemie" at FU Berlin, each term since summer 07

Supervision of the practical „Radionuklidchemie" at FU Berlin, SS 2008/9, twice per semester one week each, WS 2009 one week

Lecture „Neurobiologische Grundlagen und Modelsysteme" in the course of the inhouse PhD Programm of the MPIMG (2008, 2009)

Organization of scientific events

Member of the organizing committee „Studiengruppe Neurochemie (GBM)" for the „GBM Herbstagung 2007"

Member of the organizing committee „Studiengruppe Molekulare Neurobiologie (GBM)" for the „GBM Herbstagung 2008"

Member of the organizing committee of the Workshop „Molecular Interactions" 2008

Member of the organizing committee of the Workshop „Molecular Interactions" 2009

Organization and chairing of the symposium „memory deletion" at the european neuroscience meetings of the FENS 2010 in Amsterdam

Public relations

Experimental lecture during the „Lange Nacht der Wissenschaften (Long night of science), 2006, 2009

Experimental lecture at the Marie Curie Gymnasium, Ludwigsfelde near Berlin, 11/08

Chromosome Rearrangements and Disease

(Established: 1995)

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Sarah Shoichet* (09/00-11/04)
Barbara Dlugaszewska* (10/00-10/04)
Jiong Tao (10/00-08/04)
Magdalena Mayer (02/02-10/04)
Luciana Musante* /12/00-02/03)

Technicians
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Astrid Grimme (since 02/04
Kirsten Hoffmann* (01/02-05/07)
Corinna Menzel (00-12/06)

Scientific overview

The Chromosome Rearrangements and Disease Group focuses on the genetic causes of human disorders of the brain, and on the pathogenetic mechanisms underlying known gene mutations.
For finding novel disease genes we apply three complementary approaches. One of these is to map the chromosome breakpoints of patients with a disease-associated balanced chromosome rearrangement. This endeavour was very successful and has resulted in the identification of numerous novel disease genes, both on the X chromosome and on autosomes. To expedite the process of translocation breakpoint mapping we use ultrahigh resolution oligonucleotide arrays and flow sorting of derivative chromosomes (in collaboration with R. Ullmann). For determining inversion breakpoints more rapidly and efficiently we use next generation sequencing (in collaboration with W. Chen). Recently, we have also embarked on systematic mutation screening in families with X-linked mental retardation (MR), employing next generation sequencing strategies (in collaboration with W. Chen). The third approach is to screen novel candidate genes, identified through establishing the protein-protein interaction network for known MR proteins, in patients with the corresponding clinical phenotype.
As a small side project we have continued our search for novel pathogenic mutations in patients with Noonan Syndrome or Noonan Syndrome-like phenotype.
In addition to mutation search, functional studies aiming to understand the pathology of known gene mutations include deciphering the molecular networks and protein complexes involving the “disease proteins”. These studies yielded new insights into the underlying molecular mechanisms, promise to reveal new disease genes and modifiers, and eventually might lead to potential therapeutic targets.

* externally funded

One recent example of successful novel disease gene finding by chromosome breakpoint mapping is *DYRK1A*, which lies on chromosome 21, within both the Down syndrome critical region and in the minimal region for partial monosomy 21. *DYRK1A* encodes a highly conserved dual-specificity tyrosine phosphorylation-regulated kinase. We have found that truncations of *DYRK1A* caused a clinical phenotype comprising prenatal onset of microcephaly, intrauterine growth retardation, feeding problems, developmental delay, and febrile seizures/epilepsy in two unrelated patients who both carried a *de novo* balanced translocation (Møller et al., Am J Hum Genet. 2008). Our results highlight the importance of a correct gene dosage of *DYRK1A* for normal brain development and strongly suggest that in humans the dosage of the associated kinase needs to be very tightly regulated.
Also recently, fine mapping of the X-chromosome breakpoint in a female patient presenting with a disturbed sleep-wake cycle, late-onset epileptic seizures, increased anxiety, aggressive behavior, and mental retardation indicated disruption of the collybistin gene (*ARHGEF9*). Expression of truncated collybistin proteins in cultured neurons interfered with synaptic localization of endogenous gephyrin and GABA(A) receptors. These results suggest that collybistin has a key role in membrane trafficking of gephyrin and selected GABA(A) receptor subtypes involved in epilepsy, anxiety, aggression, insomnia, and learning and memory (Kalscheuer et al., Hum Mut. 2009).

Using the same approach, we have found that truncations of the cyclin-dependent kinase-like 5 (*CDKL5*) gene are a significant cause of infantile spasms and early epileptic seizures in female patients (Kalscheuer et al., Am J Hum Genet. 2003, Córdova-Fletes et al., Clin Genet. 2009) and of an intractable seizure disorder, called atypical Rett syndrome (Tao et al., Am J Hum Genet. 2004 and unpublished results). In the meanwhile the link between atypical Rett syndrome and a mutation in *CDKL5* has been confirmed by several other groups. Likewise, we have shown that truncation of the *Netrin G1* gene caused a highly similar clinical phenotype in a patient with a balanced translocation involving chromosomes 1 and 7 (Borg et al., Eur J Hum Genet. 2005).

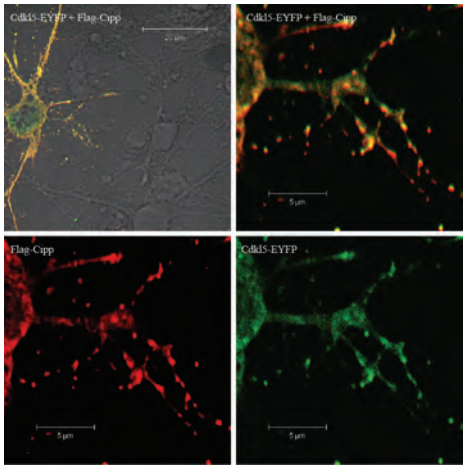

Figure 1: Colocalization of Cdkl5 and the PDZ-protein Cipp (InaD-like) in cortical mouse neurons (DIV 10). Primary neurons were transfected with Cdkl5-EYFP and Flag-Cipp expression constructs. Cdkl5 and Cipp colocalize in a dotted pattern along the neurites.

Along the same lines, our studies indicated that a *de novo* balanced chromosome rearrangement truncated the *FOXG1* gene (Shoichet et al., Hum Genet 2005). More recently, several other groups have confirmed that mutations in *FOXG1* cause atypical Rett syndrome.
To date, the functional role of CDKL5 is far from being understood and only two interaction partners have been discussed in the literature: the methyl CpG binding protein 2 (MeCP2) and the DNA methyltransferase1 (DNMT1). Mutations in *MECP2* are the major cause of typical Rett syndrome and CDKL5 has been proposed to regulate MeCP2 function in the nucleus. To gain more insights into the functions of the CDKL5 complex in the normal and diseased brain, we have searched for CDKL5 interaction partners and have investigated protein products that could act in the same signalling pathway. Interestingly, the majority of the newly identified components of the CDKL5 complex are associated with constitu-

ents of the cytoskeleton and have an established role in vesicle trafficking and protein turnover, respectively. Our findings suggest that CDKL5 is part of a protein network, which is involved in creating and regulating cell-cell contacts and may act as an upstream effector of MeCP2 (Rademacher and Kalscheuer, unpublished results).

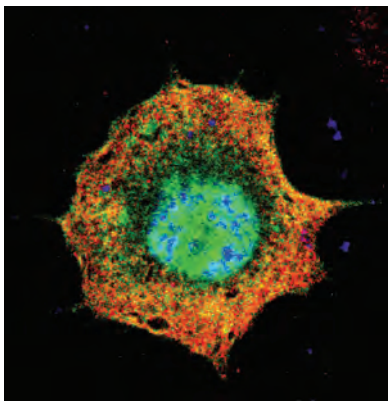

Figure 2: Colocalization of PQBP1 and one of the newly found interaction partners in the cytoplasma of neuronal F11 cells.

In addition, we have found that mutations in the X-linked polyglutamine binding protein 1 (*PQBP1*) gene cause mental retardation, microcephaly, short stature and other midline defects with variation in severity of the phenotypes, both within and between families (Kalscheuer et al., Nat Genet 2003; Cossee et al., Eur J Hum Genet 2006). The pathogenic nature of all but one mutations in the *PQBP1* gene is a premature termination codon (PTC) and we have evaluated the consequences on mRNA and protein expression. We could show that in the patients all frameshift mutations result in the production of a truncated PQBP1 protein (Musante et al., Hum Mutat 2009). Therefore, it is highly likely that the clinical phenotype is caused by the loss of wild-type PQBP1-associated functions and the presence of defect PQBP1 protein. RT-PCRs revealed mutation-specific reduction of *PQBP1* mRNAs carrying the PTCs that can be partially restored by blocking translation, thus indicating a role for the nonsense-mediated mRNA decay pathway. In addition, these mutations resulted in altered levels of *PQBP1* transcripts which skipped exon 4 probably as a result of affecting important splicing motifs. This hypothesis is supported by transfection experiments using wild-type and mutant *PQBP1* minigenes. These studies provide significant insight into the early events contributing to the pathogenesis of the *PQBP1* related XLMR disease. To get more insight into the possible functions of PQBP1, we have established a PQBP1 interaction network. Interestingly, our results suggest that PQBP1 might have diverse roles in gene or chromatin regulation and RNA metabolism, e.g. binding partners might play a role in transcription regulation, alternative splicing and mRNA stability/transport, particularly in the brain (Musante, Kunde, Kalscheuer, unpublished results). These findings support the hypothesis that PQBP1 protein scaffolds a variety of distinct multiprotein complexes *in vivo*. The role of normal and mutant PQBP1 in these processes is under study.

General information

Selected publications

Musante L, Kunde SA, Sulistio TO, Fischer U, Grimme A, Frints SGM, Schwartz CE, Martínez F, Corrado Romano, Ropers HH, Kalscheuer VM. *Common pathological mutations in PQBP1 induce nonsense-mediated mRNA decay and enhance exclusion of the mutant exon*. Hum Mutat. 2009 Oct 21, epub ahead of print.

Kalscheuer VM, Musante L, Fang C, Hoffmann K, Fuchs C, Carta E, Deas E, Venkateswarlu K, Menzel C, Ullmann R, Tommerup N, Dalprà L, Tzschach A, Selicorni A, Lüscher B, Ropers HH, Harvey K, Harvey RJ. *A balanced chromosomal translocation disrupting ARHGEF9 is associated with epilepsy, anxiety, aggression, and mental retardation*. Hum Mutat. 2009 Jan;30(1):61-8.

Møller RS, Kübart S, Hoeltzenbein M, Heye B, Vogel I, Hansen CP, Menzel C, Ullmann R, Tommerup N, Ropers HH, Tümer Z, Kalscheuer VM. *Truncation of the Down syndrome candidate gene DYRK1A in two unrelated patients with microcephaly*. Am J Hum Genet. 2008 May;82(5):1165-70.

Tao J, Van Esch H, Hagedorn-Greiwe M, Hoffmann K, Moser B, Raynaud M, Sperner J, Fryns JP, Schwinger E, Gécz J, Ropers HH, Kalscheuer VM. *Mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5/STK9) gene are associated with severe neurodevelopmental retardation*. Am J Hum Genet. 2004 Dec;75(6):1149-54.

Kalscheuer VM, Freude K, Musante L, Jensen LR, Yntema HG, Gécz J, Sefiani A, Hoffmann K, Moser B, Haas S, Gurok U, Haesler S, Aranda B, Nshedjan A, Tzschach A, Hartmann N, Roloff TC, Shoichet S, Hagens O, Tao J, Van Bokhoven H, Turner G, Chelly J, Moraine C, Fryns JP, Nuber U, Hoeltzenbein M, Scharff C, Scherthan H, Lenzner S, Hamel BC, Schweiger S, Ropers HH. *Mutations in the polyglutamine binding protein 1 gene cause X-linked mental retardation*. Nat Genet. 2003 Dec;35(4):313-5.

Selected invited talks

1st European Congress on Rett Syndrome, Milan Italy, 2009, *New insights into the pathomechanism of Rett-related disease entities*

Symposium on Molecular Medicine and Health, Hyderabad India, 2005, *Balanced chromosome rearrangements and disease*

3rd Iranian Congress of Genetic Disorders & Disabilities, Tehran Iran, 2004, *Balanced chromosome rearrangements and disease*

Annual meeting of the clinical geneticists of Quebec, Montreal Canada, 2004, *Progress in X-linked mental retardation*

European Society of Human Genetics Meeting, Munich, 2004, *Chromosome rearrangements and disease genes*

Awards

Award for the best poster presentation in "Molceular and biochemical basis of disease, developmental genetics, neurogenetics" at the German Society of Human Genetics Annual Meeting 2009. Kunde SA, Musante L, Müller EC, Ropers HH, Kalscheuer VM. *Studies on the cytoplasmic polyglutamine binding protein 1 (PQBP1) for understanding its role in mental retardation*. Med Gen (2009)21:288.

Award for the best poster presentation at the European Society of Human Genetics Annual Meeting 2009. Musante L, Kunde SA, Ropers HH, Kalscheuer VM. *Towards understanding the pathogenetic mechanism of PQBP1 mutations in X-linked mental retardation*.

Award of the 29th Blankenese conference Protein Processing Meets Synaptic Transmission 2009. Rademacher N, Kalscheuer VM. *New insights into the molecular pathomechanism of Rett-related disease entities*.

Award for the best poster presentation in "Molceular and biochemical basis of disease, developmental genetics, neurogenetics" at the German Society of Human Genetics Annual Meeting 2008. Kunde SA, Musante L, Müller EC, Ropers HH, Kalscheuer VM. *Towards understanding the role of the polyglutamine binding protein 1 (PQBP1) in mental retardation*. Med Gen (2008)20:248.

Membership in journal editorial boards

Review Editorial Board Member of Frontiers in Molecular Neuroscience

Scientific referee

Vera Kalscheuer serves as scientific referee for the following journals: Human Molecular Genetics, American Journal of Human Genetics, European Journal of Human Genetics, Journal of Medical Genetics.

In addition, Vera Kalscheuer serves as scientific referee for the Deutsche Forschungsgemeinschaft.

Memberships in professional societies

Founding member of the German Society of Human Genetics

Scientific advisor for the library of the MPIMG and member of the library committee

External funding

BMBF, NGFN2: *Identification of genetic risk factors for complex disorders by studying patients with associated balanced chromosomal rearrangements*. Joint with Dr. A. Tzschach, MPIMG, 2005-2008

DFG, SFB 577: *Analysis of Clinical Variability in Mendelian Disorders*, subproject *Search for modifier genes in X-linked mental retardation,* 2003-2009

Teaching activities

Lecture *Genetik für Bioinformatiker*, Free University Berlin, SS 2006, SS2007

Lecture, Seminar and Practical Course *Biologie für Mediziner*, Humboldt Universität Berlin, WS 2006/2007

Organization of scientific events

Member of the Dahlem Colloquium committee, an institute wide seminar series

Familial Cognitive Disorders

(Established: 06/2005)

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Dr. Masoud Garshasbi (since 05/08)

PhD students
Lia Abbasi Moheb (since 10/05)
Joanna Walczak Stulpa (since 10/05)
Sahar Esmaeli Nieh (since 11/06)
Lucia Püttmann (since 06/08)
Agnes Zecha (since 07/08)
Robert Weißmann (since 12/09)
Rui Tian[1] (09/05-09/09)
Masoud Garshasbi (02/04-05/08)
Mohammad Mahdi Motazacker (02/04-07/07)
Wei Chen (until 07/05)
Bartlomiej Budny (03-05)

Technicians
Bettina Lipkowitz (since 05)
Marianne Schlicht (since 05)
Georg Lienke (09/06-03/09)
Melanie Wendehack (08/02-12/06)
Marion Amende-Acar (until 03/06)

Scientific overview

X-linked forms of mental retardation (XLMR)

As member of the European MRX Consortium, our department has made important contributions to the elucidation of X-linked mental retardation (XLMR, for review see e.g. Gecz et al. 2009, Trends Genet 25:308; Ropers 2008, Curr Opin Genet Dev 18:241; Ropers 2006, Curr Opin Genet Dev 16:260) including the discovery that mutations in the histone demethylase *KDM5C (JARID1C)* gene are a frequent cause of XLMR [Jensen et al. 2005, Am J Hum Genet 76:227; Tzschach et al. 2006, Hum Mutat 27:389] or the identification of several mutations in the methyl transferase *FTSJ1* [Freude et al. 2004, Am J Hum Genet 75:305]. Both genes also feature prominently among the recent research activities of our group concerning functional aspects of MR genes. In this context, we have been able to describe a distinct gene expression fingerprint in lymphoblastoid cell lines as well as whole blood from male patients with mutations in *KDM5C* [Jensen et al., Pathogenetics 2009 (accepted)].
Using a murine embryonic stem cell line (RRD143, Baygenomics) with a genetrap in *Ftsj1*, for injection in C57BL/6 blastocysts we have produced mice lacking functional *Ftsj1* (collaboration with Diego Walther), and are presently investigating the effects of *Ftsj1* deficiency in this model.

[1]Supervised by S. Sigrist, FU Berlin

Furthermore, we have designed a resequencing array based on the Affymetrix 50K platform containing the coding and splice site regions of 17 XLMR genes (*ACSL4*, *ARX*, *ATRX*, *DLG3*, *FTSJ1*, *GDI1*, *IL1RAPL1*, *JARID1C*, *MECP2*, *NLGN4*, *PAK3*, *PHF6*, *PHF8*, *PQBP1*, *SLC6A8*, *TM4SF2* and *ZNF41*) in order to investigate the genetic basis of MR in unrelated sporadic male NS-MR patients from the cohort of the EURO-MRX consortium. Application of this array to DNA from 135 patients led to the identification of 6 previously unknown putative disease causing mutations in these genes.

Autosomal recessive forms of mental retardation (ARMR)

Most of our activities are focussed on the molecular causes of ARMR which is thought to be much more common than XLMR (see e.g. Ropers 2008, Curr Opin Genet Dev 18:241). However, until recently only three genes for non-syndromic ARMR (NS-ARMR) were known: *PRSS12* (neurotrypsin) [Molinari et al. 2002, Science 298:1779], CRBN (cereblon) [Higgins et al. 2004, Neurology 63:1927] and *CC2D1A* [Basel-Vanagaite et al. 2006, J Med Genet 43:203] due to insufficient family sizes and lack of consanguinity in western societies, which preclude successful mapping and identification of candidate loci. We have therefore joined forces with Prof. Dr. H. Najmabadi for a large-scale project, aiming at the systematic identification of genes that have a role in ARMR (see also Research Report 2006). Since the beginning of our collaboration in 2004 we have accumulated more than 200 families with NS-ARMR as well as many families with microcephaly and more than 10 with other syndromic forms of ARMR. Autozygosity mapping, based on whole genome SNP genotyping with various platforms in the majority of these families has so far enabled us to define 40 novel ARMR loci (Fig.1). For 10 of these we could identify the underlying gene defect.

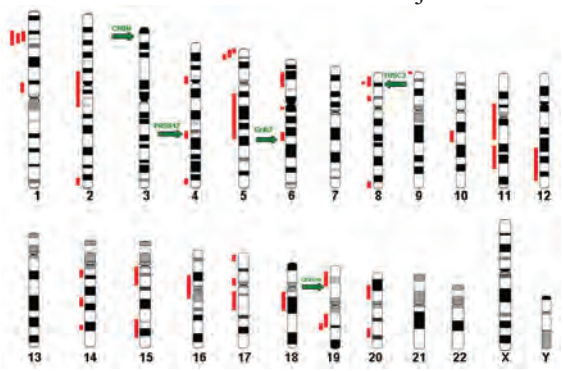

Figure 1. Distribution of ARMR loci throughout the genome. Single linkage intervals identified in 40 different families are represented by red bars. The locations of known genes for NS-ARMR are indicated by green arrows.

The first mutation we found was a deletion of approximately 150 to 200 kb, encompassing the promoter and the first 6 exons of the *MCPH1* gene [Garshasbi et al. 2006, Hum Genet 118:708]. We then identified a complex mutation affecting the kainate receptor subunit encoding gene *GRIK2* [Motazacker et al. 2007, Am J Hum Genet 81:792] and a c.1342C>T nucleotide substitution, which leads to a premature stop codon in exon 10 of *VLDLR,* a gene encoding a member of the low-density lipoprotein receptor (LDLR) superfamily [Moheb et al. 2008, Eur J Hum Genet 16:270]. This was followed by the discovery of a deletion in the *TUSC3* gene [Garshasbi et al. 2008, Am J Hum Genet 82:1158] which is thought to encode a subunit of the oligosaccharyl transferase complex that is involved in the key step of N-glycosylation.

The fifth mutation we found in a family with two branches and four female patients suffering from MR and oligomenorrhea. Here, a sequence change in the BOD1 gene [Esmaeeli-Nieh et al., presented at the annual ASHG conference, Hawai, USA, Oct 2009] leads to the loss of all isoforms of the gene product, a kinetochore protein that is involved in spindle attachment and mitotic chromosome segregation [Porter et al. 2007, J Cell Biol 179:187].

Furthermore, a stop mutation (R154X) was identified in the poly-A binding zinc finger protein gene *ZC3H14* in a family with three affected individuals with NS-

ARMR. ZC3H14 and its yeast ortholog NAB are thought to be important regulators of translation [Leung et al. 2009, Gene 439:71] and the functional impact of the stop mutation is being investigated (collaboration with A. Corbett).
Previously we had reported that ARMR is extremely heterogeneous and that common causes are unlikely to exist [Najmabadi et al. 2007, Hum Genet 121:43]. After having performed homozygosity mapping in many additional consanguineous families, this statement might need qualification. Recently we have identified several ARMR genes carrying mutations in more than one family (Fig.1).
The first two of these genes are *ZNF526* [Abbasi Moheb et al., presented at the Annual ASHG conference, Hawai, USA, Oct 2009], where two different mutations occurred in three different families and *NSUN2* with three different mutations in independent families. The ZNF526 protein has nucleic acid binding properties, and whole genome expression profiling in immortalized lymphoblasts from homozygous mutation carriers has enabled us to identify putative target genes may give a clue to the etiology of MR in these patients. In addition, we are performing chromatin immune precipitation experiments followed by next generation sequencing (ChIP-SEQ) to confirm these findings and to identify the DNA targets of the ZNF526 protein.
For ZNF526, NSUN2, BOD1 and an additional selection of >90 MR proteins we are currently performing a large-scale search for interaction partners by a systematic screen of the protein interactome (collaboration with A. Hyman, MPI-CBG, Dresden and M. Mann, MPI-BC, Munich). In these experiments, BACs containing the GFP-labelled murine orthologs of the genes are introduced into HeLa cells. The anti-GFP antibody is then used for pull-down assays followed by mass spectrometry. Functional follow-up studies will be performed in our group.
For the identification of novel MR causing mutations in large homozygous intervals we are currently applying genomic enrichment followed by next generation sequencing (collaboration with W. Chen and H. Hu). This has enabled us to identify apparently pathogenic allelic missense mutations in two families with overlapping linkage intervals on the short arm of chromosome 1 [Najmabadi et al., presented at the 14th X-linked mental retardation workshop, Bahia, Brazil, 2009] affecting the *ST3GAL3* gene. The protein encoded by *ST3GAL3* is a type II membrane protein that catalyzes the transfer of sialic acid from CMP-sialic acid to galactose-containing substrates. Functional investigations are in progress to verify the pathogenicity of these mutations. We expect that this strategy, which also enabled us to identify a plausible novel candidate gene on chromosome 6, will revolutionize the search for novel ARMR genes.

Other mendelian disorders

In a consanguineous Polish family with two patients suffering from Cranioectodermal Dysplasia (CED), a rare disorder characterized by craniofacial, skeletal, and ectodermal abnormalities, homozygosity mapping revealed a disease related locus on chromosome 3q21-q24. In the linkage interval from this family we identified a homozygous missense mutation in the IFT122 gene. As a component of the intraflagellar transport complex A, the IFT122 protein plays an important role in the assembly and maintenance of eukaryotic cilia and flagella [Pedersen & Rosenbaum 2008, Curr Top Dev Biol 85:23]. Sequencing of this gene in additional CED patients led to the identification of two further missense changes and a splicing mutation. Functional studies in patient fibroblasts as well as knockdown experiments in Zebrafish provide compelling evidence for the pathogenicity of these mutations and show that CED is a ciliopathy.

General information

Selected publications

Mir A, Kaufman L, Noor A, Motazacker MM, Jamil T, Azam M, Kahrizi K, Rafiq MA, Weksberg R, Nasr T, Naeem F, Tzschach A, Kuss AW, Ishak GE, Doherty D, Ropers HH, Barkovich AJ, Najmabadi H, Ayub M, Vincent JB. *Identification of mutations in TRAPPC9, which encodes the NIK and IKK-β binding protein (NIBP), in Non-Syndromic Autosomal Recessive Mental Retardation.* Am J Hum Genet 2009; 85: 909-915

Cízková A, Stránecký V, Mayr JA, Tesaøová M, Havlíèková V, Paul J, Ivánek R, Kuss AW, Hansíková H, Kaplanová V, Vrbacký M, Hartmannová H, Nosková L, Honzík T, Drahota Z, Magner M, Hejzlarová K, Sperl W, Zeman J, Houštìk J, Kmoch S. *TMEM70 mutations cause isolated ATP synthase deficiency and neonatal mitochondrial encephalocardiomyopathy.* Nature Genetics 2008; 40(11): 1288-90.

Garshasbi M, Hadavi V, Habibi H, Kahrizi K, Kariminejad R, Behjati F, Tzschach A, Najmabadi H, Ropers HH, Kuss AW. *A defect in the TUSC3 gene is associated with autosomal recessive mental retardation.* Am J Hum Genet 2008; 82(5): 1158-64

Motazacker MM, Rost BR, Hucho T, Garshasbi M, Kharizi K, Ullmann R, Abedini SS, Esmaeli-Nieh S, Amini SH, Goswami C, Tzschach A, Jensen LR, Schmitz D, Ropers HH, Najmabadi H, Kuss AW. *A Defect in the Ionotropic Glutamate Receptor 6 Gene (GRIK2) is Associated With Autosomal Recessive Mental Retardation*. Am J Hum Genet 2007; 81(4):792-798.

Najmabadi H, Motazacker MM, Garshasbi M, Kahrizi K, Tzschach A, Chen W, Behjati F, Hadavi V, Nieh SE, Abedini SS, Vazifehmand R, Firouzabadi SG, Jamali P, Falah M, Seifati SM, Gruters A, Lenzner S, Jensen LR, Ruschendorf F, Kuss AW, Ropers HH. *Homozygosity mapping in consanguineous families reveals extreme heterogeneity of non-syndromic autosomal recessive mental retardation and identifies 8 novel gene loci*. Hum Genet 2007; 121(1): 43-48.

Selected invited talks

Autosomal recessive intellectual disability: elucidating the molecular basis of a heterogeneous genetic disorder, Emory University, Atlanta GA, USA, 15.10.2009

Unravelling the molecular background of autosomal recessive mental retardation, Institute for Human Genetics, University of Würzburg, Würzburg, Germany, 19.05.2008

Work as scientific referee

Andreas Kuss serves as scientific referee for the following journals: Human Genetics, Molecular Biology Reports.

Teaching activities

Lecture *Homozygosity Mapping* in the course of the inhouse PhD Programm of the MPIMG (2008, 2009)

Public relations

Experimental lecture during the *Lange Nacht der Wissenschaften* (Long night of sciences), 2006

Clinical Genetics

(Established: 10/2001)

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Maria Hoeltzenbein, MD (10/01-07/07)

Technicians
Susanne Freier
Nadine Nowak (since 01/08)

Scientific overview

The main objective of the "Clinical Genetics" group is the recruitment, evaluation and clinical characterization of patients and families for the various research groups of the "Human Molecular Genetics" department. Access to suitable families and individual patients is crucial for human genetic research that aims at elucidating novel disease genes. Although mental retardation (MR) – the focus of this department's interest - is not a particularly rare condition (it has a prevalence of approximately 1-2%), only a small subset of these patients qualify as starting points for promising investigations. Generally, this concerns either familial forms of MR in which linkage analysis can be performed, or carriers of chromosome aberrations.

Autosomal recessive mental retardation

In a large collaboration with the Genetic Research Centre in Tehran/Iran (Prof. Hossein Najmabadi) we are being provided with DNA, blood samples and clinical information of consanguineous families with multiple mentally retarded children. The "Familial Cognitive Disorders" group (AW Kuss) performs homozygosity mapping in these families. The majority of the patients from Iran suffer from unspecific (non-syndromic) mental retardation, but several families also have additional clinical problems. Many of these syndromes are apparently novel and do not resemble other published MR syndromes (e.g. Kahrizi et al., Eur J Hum Genet. 2009 Jan;17(1):125-8.; Tzschach et al., Br J Dermatol. 2008 Sep;159(3):748-51.). Apart from the clinical evaluation (frequently including proposals for specific investigations such as MRI scans, ophthalmologic examinations and others), we screen the regions of homozygosity for promising functional candidate genes according to morphological similarity or (putative) functional links to other syndromes based on several databases.

In a collaborative project with Dr. A. Rajab (Muscat, Oman), we obtained access to several consanguineous families with syndromic forms of MR. The spectrum of disorders included a form of albinism, ectodermal dysplasia, a muscle disorder

and a large family with MR and epilepsy. We also received material from families with syndromic forms of mental retardation from our partners in Poznan/Poland (Prof A Latos-Bielenska).

X-linked mental retardation

The elucidation of novel genes involved in X-linked mental retardation (XLMR) continues to be a major research focus of our department. Our group recruits families with putative X-linked MR by collaboration with clinical geneticists in Germany and abroad (e.g. Tzschach et al Hum Mutat. 2006 Apr;27(4):389., Budny et al Hum Genet. 2006 Sep;120(2):171-8.). We also keep clinical data of formerly submitted families updated and communicate the results of mutation analysis to the respective physicians or families. Apart from families with non-syndromic XLMR, we also investigate families with syndromic XLMR. Linkage analysis and mutation analysis in these families is being performed in the "Familial Cognitive Disorders" group (AW Kuss).

Disease associated chromosome aberrations

Breakpoint analysis of disease-associated balanced chromosome rearrangements combining array CGH (R. Ullmann's group), FISH and recently also next-generation sequencing techniques (V. Kalscheuer's and W. Chen's groups) is a fast and efficient strategy to identify novel disease-causing genes (e.g. Chen et al Genome Res. 2008 Jul;18(7):1143-9.; Kalscheuer et al Hum Mutat. 2009 Jan;30(1):61-8.). Comprehensive clinical characterisation is a prerequisite for the selection of patients suitable for analysis. The identification of a disrupted gene or genes at the breakpoints often raises new questions concerning specific phenotypic details. The "Clinical Genetics" group obtains these data through collaboration with referring doctors or by contacting the patients or their families.
Our group recruits additional patients with *de novo* disease associated balanced chromosome rearrangements by maintaining and extending a network of clinical geneticists and other specialists.

Unbalanced chromosome aberrations

Both large, cytogenetically visible unbalanced chromosome aberrations and small, submicroscopic aberrations which are only detectable by array CGH (R. Ullmann's group) are an important cause of congenital malformations and mental retardation, and they can point to single genes responsible for a specific phenotype. Our group characterises such patients clinically and aims to establish genotype-phenotype correlations (e.g. Tzschach et al, Eur J Hum Genet 2009 (in press)). Our department is a member of the "German Mental Retardation Network (MRNet)" which performs array CGH analysis in a large patient cohort.

Cell culture facility

We establish permanent cell lines from peripheral blood lymphocytes by EBV transformation after obtaining informed consent from patients in whom molecular cytogenetic or molecular genetic investigations are planned. Our cell culture lab performs EBV transformation, stores the cell lines and provides ready-to-use DNA, RNA or metaphase chromosome spreads for FISH investigations to the respective research groups.

Genetic counselling

Andreas Tzschach, who is a board-certified clinical geneticist ("Facharzt für Humangenetik"), offers genetic counselling at the Institute of Medical Genetics (director: Prof. S. Mundlos) of the Charité - Universitätsmedizin Berlin, and he regularly takes part in clinical genetics, dysmorphology and cytogenetics meetings.

External collaborations

Autosomal recessive mental retardation

- Hossein Najmabadi, Genetic Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
- Anna Rajab, MD, Genetic Unit, DGHA, Ministry of Health, Muscat, Sultanate of Oman

Balanced chromosome rearrangements

- Niels Tommerup, Wilhelm Johannsen Centre, Copenhagen, Denmark

X-linked mental retardation

- EUROMRX consortium (www.euromrx.com)

Mental retardation in Germany

- German Mental Retardation Network (MRNet, www.german-mrnet.de)

We also collaborate with numerous clinical geneticists in Germany and abroad.

General information

Selected publications

Tzschach A, Bisgaard AM, Kirchhoff M, Graul-Neumann LM, Neitzel H, Page S, Ahmed A, Müller I, Erdogan F, Ropers HH, Kalscheuer VM, Ullmann R. *Chromosome aberrations involving 10q22: Report of three overlapping interstitial deletions and a balanced translocation disrupting C10orf11*. Eur J Hum Genet. 2009, Oct 21. [Epub ahead of print]

Kahrizi K, Najmabadi H, Kariminejad R, Jamali P, Malekpour M, Garshasbi M, Ropers HH, Kuss AW, Tzschach A. *An autosomal recessive syndrome of severe mental retardation, cataract, coloboma and kyphosis maps to the pericentromeric region of chromosome 4*. Eur J Hum Genet. 2009 Jan;17(1):125-8.

Tzschach A, Bozorgmehr B, Hadavi V, Kahrizi K, Garshasbi M, Motazacker MM, Ropers HH, Kuss AW, Najmabadi H. *Alopecia-mental retardation syndrome: clinical and molecular characterization of four patients.* Br J Dermatol. 2008 Sep;159(3):748-51.

Garshasbi M, Hadavi V, Habibi H, Kahrizi K, Kariminejad R, Behjati F, Tzschach A, Najmabadi H, Ropers HH, Kuss AW. *A defect in the TUSC3 gene is associated with autosomal recessive mental retardation.* Am J Hum Genet. 2008 May;82(5):1158-64.

Tzschach A, Lenzner S, Moser B, Reinhardt R, Chelly J, Fryns JP, Kleefstra T, Raynaud M, Turner G, Ropers HH, Kuss A, Jensen LR. *Novel JARID1C/SMCX mutations in patients with X-linked mental retardation.* Hum Mutat. 2006 Apr;27(4):389.

Work as scientific referee

A. Tzschach serves as scientific referee for the following journals: American Journal of Medical Genetics, Journal of Medical Genetics, Obesity, European Journal of Medical Genetics.

External funding

BMBF, NGFN2, *Identification of genetic risk factors for complex disorders by studying patients with associated balanced chromosomal rearrangements*. (Joint with V. Kalscheuer, MPIMG).

Teaching activities

Seminar "Human Genetics" for medical students, Humboldt University Berlin/ Charité-Universitätsmedizin Berlin, Institute of Medical Genetics (7 seminars of 90 minutes each term)

Co-organiser (together with HH Ropers) and lecturer at an educational workshop ("Genetik der geistigen Behinderung") of the "Akademie Humangenetik", Würzburg, 27.-28.2.2009

Molecular Cytogenetics

(Established: 2004)

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Anne Steininger (since 04/06)
Grit Ebert (since 10/09)
Artur Muradyan (01/05-05/09)

Technicians
Ines Müller (since 04)
Alisho Ahmed (since 06/08)
Hannelore Madle (11/07-08/09)
Jan Jurkatis (05/07-03/08)
Melanie Wendehack (until 12/06)
Marei Schubert (04/05-04/06)
Ralph Schulz (intil 01/05)
Barbara Meinck (until 10/04)

Scientific overview

Analysis of DNA copy number variants

The Molecular Cytogenetics Group was founded in 2004. The first goal of the group was the creation of a high resolution BAC array platform enabling the genome wide identification of submicroscopic DNA copy number changes. We have accomplished this task in early 2005. In the same year we have also published CGHPRO, a program dedicated to the analysis and visualization of array CGH data. The next years were dominated by our efforts to exploit this array platform in the most efficient way. In cooperation with several partners from the clinics we set out to investigate the impact of copy number variants (CNVs) on the aetiology of various diseases. For most of the bigger screening projects we have invited guest scientists from the partner institution to work in our laboratory in order to get familiar with the array CGH methodology. In this way we have not only increased the manpower in our laboratory, but at the same time accelerated the transfer of this technology to the clinics. This strategy turned out to be very productive also in terms of publications, leading to 57 articles since 2006 alone.

Several of our publications have questioned the current understanding of genotype-phenotype correlation. In one paper published in 2007, for example, we have described a microdeletion/duplication of 16p13.1, which predisposes to mental retardation and autism, respectively. In the meantime the relevance of both the deletion and the duplication has been confirmed by independent groups. Actually, as stated in one of these confirmatory reports, with a frequency of 1.1% in patients

with MR, the 16p13 duplication appears to represent one of the most frequent causes of intellectual disability. Meanwhile the 16p13 paper is cited also for another reason. The duplication has turned out to be a paradigm for the genetic relationship of distinct diseases such as autism, mental retardation and schizophrenia. Up to now four independent studies have linked the very same 16p13 duplication also to schizophrenia.
The paper with the most citations within this year is our report on the first comprehensive screen of CNVs in patients with schizophrenia. In this study we have implicated the mental retardation gene *NRXN1* in the aetiology of schizophrenia and revealed that a direct interaction partner of this gene, *APBA2*, was located in a genomic region lost in another patient. Already one year after publication three independent studies have replicated our findings. In addition the technical quality of the underlying data set was evaluated favourably by an independent group of bioinformaticians in an inter-platform comparison (LaFrambiose, 2009).

MRNET: a German wide initiative to investigate the genetic causes of mental retardation

For many CNVs disease association has been established by identifying recurrent aberrations in unrelated patients. In order to identify the less frequent and maybe low penetrant pathogenic CNVs the number of patients to be investigated has to increase considerably. To cope with this problem in MR research our group is actively participating in a network called the MRNET. Aim of this German wide initiative is to collaboratively analyse around 1200 patients with MR. Up to now our group has already screened 185 patients in the course of this project and has established a pipeline for the high throughput verification of CNVs based on custom designed oligonucleotide arrays. We have also develeoped the software for filtering and displaying the data, which is used by all participants of this network.

Integrative analysis of genetic and epigenetic changes and their consequences on gene expression

One goal of our current work is to gain more insights into the consequence of DNA copy number changes by considering additional levels of information such as data on gene expression or epigenetic modifications. For that purpose we have established ChIP on Chip and MeDIP, two methods enabling the genome wide analysis of histone modifications and DNA methylation, respectively. Yet, in contrast to others, we have not focused on transcription start sites and CpG Islands, but instead studied epigenetic changes genome wide by means of our BAC array platform. Our results indicate that, at least in tumor cell lines, the correlation of moderate DNA copy number changes with gene expression is surprisingly low and that only a few genes are regularly deregulated when comparing different cases with the very same chromosomal change. Strikingly, according to our data global hypomethylation leads to downregulation of gene expression. We have also observed that regions sharing the same trend of chromatin modifications can extend to several megabases and are recurrent in many cell lines. Some of these recurrent intervals overlap with regions involved in genomic disorders such as the Williams Beuren syndrome. We are currently investigating this unexpected connection in more detail. One aspect under study in this context is the dynamics of large scale chromatin modifications during differentiation.

Collaborations within the Institute

The Molecular Cytogenetics group participates in the project “Balanced Chromosome Rearrangement and Disease”, which currently involves four groups of the department. In this project we are responsible for the array based fine mapping of

chromosomal breakpoints and the exclusion of CNVs elsewhere in the genome. The high-throughput sequencing projects of the department are supported by our group in two ways. Firstly, we perform CNV analysis in patients prior to sequencing and secondly, we are actively involved in the array-based sequence enrichment for specific chromosomal intervals.

Outlook

In the recent years the commercial availability of DNA microarrays has promoted the rapid introduction of array CGH into clinical routine. Consequently, the systematic search for CNVs is no longer confined to specialized research laboratories and we are able to shift our focus from the mainly descriptive analysis of CNVs to studies dedicated to an improved understanding of their phenotypic consequences. We will continue our multi-dimensional analysis of genetic and epigenetic modifications. However, given the growing competition in this field, we will refrain from a purely descriptive genome-wide inventory of epigenetic marks. Instead we will focus on specific aspects such as the dynamics of epigenetic modifications during certain phases of differentiation.

General information

Selected publications

Kirov G, Gumus D, Chen W, Norton N, Georgieva L, Sari M, O'Donovan MC, Erdogan F, Owen MJ, Ropers HH, Ullmann R. *Comparative genome hybridization suggests a role for NRXN1 and APBA2 in schizophrenia*. Hum Mol Genet. 2008 Feb 1;17(3):458-65.

Ullmann R, Turner G, Kirchhoff M, Chen W, Tonge B, Rosenberg C, Field M, Vianna-Morgante AM, Christie L, Krepischi-Santos AC, Banna L, Brereton AV, Hill A, Bisgaard AM, Müller I, Hultschig C, Erdogan F, Wieczorek G, Ropers HH. *Array CGH identifies reciprocal 16p13.1 duplications and deletions that predispose to autism and/or mental retardation.* Hum Mutat. 2007 Jul; 28(7):674-82.

Chen W, Erdogan F, Ropers HH, Lenzner S, Ullmann R. *CGHPRO — a comprehensive data analysis tool for array CGH.* BMC Bioinformatics. 2005 Apr 5;6:85.

Selected invited talks

Array CGH in tumor cytogenetics. Tumor Cytogenetics Meeting, Semmering, Austria 2005

The variable consequences of chromosomal aberrations. Cytomics Symposium, Tokyo, Japan; 11/2007

Technical aspects of array CGH. Modern methods in cancer research; Vilnius, Lithuania, 2008

Grant evaluation

R. Ullmann serves as referee for the following institutions/calls: Netherlands Genomics Initiative, Horizon Breakthrough project grant application 2008; Jubilee Funds of the National Bank/ Austria, single grant application 2008; GIS-Institute for Rare Diseases in France, Rare diseases and structural variation 2007.

Work as scientific referee

R. Ullmann serves as scientific referee for the following journals: American Journal of Human Genetics, American Journal of Medical Genetics, Bioinformatics, BMC Bioinformatics, BMC Genomics, European Journal of Human Genetics, Gene, Human Genetics, Human Molecular Genetics, Journal of Medical Genetics, Nature Protocols.

External funding

Jose Carreras Stiftung: *Einsatz von Array-CGH und anderer molekularzytogenetischer Techniken zum Nachweis kryptischer genetischer Imbalancen und Translokationen bei akuten lymphatischen Leukämien (ALL) im Kindesalter,* 2006-2009 (application together with Prof. Dr. Karl Seeger, Charite Berlin)

Teaching activities

Teaching at Universities

Molecular Cytogenetics in Tumor Biology, University of Salzburg, Austria, 2005, 2006

Human Genetics for Bioinformaticians, Freie Universität Berlin, Germany (together with Ropers, Schweiger, Kalscheuer), 2004, 2005, 2006

Molecular Biology and Genetics II, Freie Universität Berlin, Germany (together with Ropers, Schweiger & Kalscheuer, Wittig, Huber, Hinderlich, Klein, Peiser, Dobrinski), 2007

Modern methods for the genome wide analysis of genetic and epigenetic changes in tumor biology and human genetics, University of Salzburg, Austria, 2008, 2009

Summerschools/Courses

Principles of array CGH, International Summer School in Functional Genomics, Wilhelm Johannsen Centre for Functional Genome Research, Copenhagen, Denmark, 2007, 2008

Principles of arrayCGH & DNA copy number variation, Postgraduate Course in Cytogenetics, Wilhelm Johannsen Centre for Functional Genome Research, Copenhagen, Denmark, 2007

How next generation sequencing can benefit from molecular cytogenetic techniques, course in integration of cytogenetics, microarrays and massive sequencing in biomedical and clinical research, European School of Genetic Medicine/ EuroMediterranean University Centre of Ronzano. Bologna, Italy, 2008, 2009

The role of DNA copy number changes in congenital disorders and tumorigenesis, PhD program of the Max Planck Institute for Molecular Genetics, 2008, 2009

Public relations

Up to now the Molecular Cytogenetics group participated four times in the "Long Night of Science", which takes place once a year in Berlin. This year´s title of our presentation was: "*What makes tumor cells so dangerous*?"

Neurochemistry Group & Mouse Lab; Monoamine signalling and disease

(Established: 02/2003)

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Dr. Maik Grohmann (since 03/08, guest scientist)
Dr. Nils Paulmann (since 04/08)

PhD students
Jakob Vowinckel (since 07/06)
Paul Hammer (since 01/07)
Silke Stahlberg (since 07/08)
Jens-Uwe Peter (02/03-12/08)
Maik Grohmann (04/03-03/08)
Nils Paulmann (09/03-03/08)

Technicians
Angela Lüttges
Sabine Otto
Monika Dopatka (until 12/08)

Scientific overview

Figure 1. Sugar and Serotonin-deficient Mice helped identify a link between Diabetes mellitus and serotonin.

Our group is focused on the elucidation of molecular causes of human diseases by the generation, analysis, and rescue experiments of transgenic and knockout mouse models. The use of transgenic mice is one of the most straightforward tools to study gene function. Our facility offers the generation of transgenic and knockout mice to all groups in the Department, the Max-Planck-Institute and other interested laboratories. Our facility constituted in part the Central Facility for Animal Model Generation of the SFB577: "Molecular Basis of Clinical Variability in Mendelian Disorders". Currently we work on animals with defined genetic dysfunctions in genes involved in mental retardation (e.g. *Mcph1* and *Ftsj1*) and in monoaminergic systems (e.g. *Tph1* and *Tph2*) aiming the elucidation of the numerous hormonal and neurotransmitter effects of serotonin (5-HT), histamine (HA), and the catecholamines dopamine (DA) and norepinephrine (NE). Furthermore, we closely cooperate with the Department of Computational Molecular Biology in order to combine bioinformatics with experimental verification of computational results.

The dichotomy of the serotonergic system

Nine low-molecular weight neurotransmitters have been identified in the central nervous system of vertebrates and four of them are the above-mentioned evolutionary ancient primary monoamines. 5-HT, one of these nine neurotransmitters, is not only the messenger of some thousands of neurons [*Science* 299 (2003) 76; *Biochem. Pharmacol.* 66 (2003) 1673], but an ubiquitous substance in peripheral tissues and fluids, from which 5-HT was first isolated six decades ago identifying it as the vasoconstrictor compound in serum, which appears in conjunction with platelet degranulation [*Cell* 115 (2003) 851; *Mol. Cell. Biochem.*, 295 (2007) 205]. Tryptophan hydroxylase (TPH) catalyzes the rate-limiting step in the biosynthesis of 5-HT biasing the serotonergic system in its whole.

A dichotomy of the serotonergic system consisting of two 5-HT-synthesizing TPH isoforms was recently characterized by us, using *Tph* gene-targeted (*Tph1*-/-) mice. TPH1, the enzyme known for decades, is broadly expressed in non-neuronal tissues and the novel TPH2 is almost restricted to neurons [*Science* 299 (2003) 76; *Biochem. Pharmacol.* 66 (2003) 1673]. Thus, TPH2 catalyses the rate-limiting step of 5-HT biosynthesis in the central nervous system [*Science* 299 (2003) 76; *Biochem. Pharmacol.* 66 (2003) 1673]. The neurotransmitter 5-HT is involved in multiple facets of mood control and the regulation of sleep, anxiety, alcoholism, drug abuse, food intake, and sexual behaviour. For these reasons, we work on the biochemical properties of TPH2 in order to understand the central nervous system 5-HT biosynthesis [*J. Biol. Chem.*, 281 (2006) 28105; *J. Neurochem.* 2007 102 (2007) 1887]. Furthermore, we and our collaborators are working on the elucidation of TPH2-dependent human psychiatric disorders [*Mol. Psychiatry* 9 (2004) 980; *Biol. Psychiatry*, 62 (2007) 1288].

A novel intracellular mechanism of 5-HT signalling was discovered in the viable *Tph1*-/- mice, a mechanism depending on the 5-HT transporter (SERT)-driven entry of 5-HT into cells in conjunction with Ca^{2+} mobilization, which culminates in the constitutively activating covalent modification of small GTPases of the Rho and Rab families with 5-HT in a transglutaminase-mediated reaction [*Cell* 115 (2003) 851; *PLoS Biol.* 7 (2009) e1000229]. We termed this post-translational protein modification 'serotonylation'.

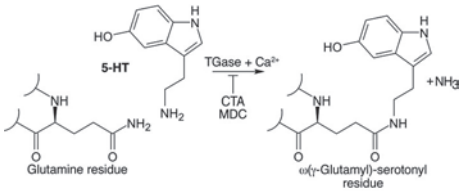


Figure 2: Scheme of protein serotonylation of glutamine residues by transglutaminase.

Extraneuronal serotonin is involved in primary haemostasis [*Cell* 115 (2003) 851], mammary gland involution [*Dev. Cell* 6 (2004) 193], liver regeneration [*Science* 312 (2006) 104], and insulin secretion [*J. Endocrinol.* 200 (2009) 23; *PLoS Biol.* 7 (2009) e1000229]. Our collaborators and we are working on these items and also on the analysis of tissue-specific expression of splicing isoforms of TPH1.

5-HT also functions as a growth factor, particularly in early embryonic development [*Mol. Brain Res.* 68 (1999) 55]. Mitogenic effects of 5-HT in adult tissues are also gaining attention, for example, in the pathological hyperplasia of the pulmonary artery smooth muscle cells (PA-SMCs) in pulmonary hypertension [*Hypertension* 49 (2007) 232]. However, the underlying mechanisms for such processes have remained elusive, while a growing body of evidence points to a crucial involvement of the SERT in the aetiology of this disease. Finally, the functional analysis revealed an involvement of RhoA serotonylation in the aetiology, as previously postulated [*Cell* 115 (2003) 851].

Platelets contain large amounts of 5-HT and can be easily obtained from peripheral blood. Washed platelets are an accepted model for synaptic vesicle metabolism mechanisms. Therefore, the platelets of our *Tph1*-/- mice deliver the first

opportunity to study transmitter-devoid vesicles. We are cooperating with G. Ahnert-Hilger to elucidate vesicular trafficking mechanisms. We have reported that the vesicular monoamine content regulates VMAT2 activity through Gaq in mouse platelets based on evidence for autoregulation of vesicular transmitter uptake [*J. Biol. Chem*. 278 (2003) 15850]. Similar autoregulation is also given in other neurotransmitter storage systems [*J. Neurosci.* 25 (2005) 4672]. In addition, under physiological conditions, platelets are crucial players in delivering serotonin to a variety of target organs and play a central role for instance in the immune response and in liver regeneration [*Science* 312 (2006) 104].

Primary monoamines and protein monoaminylation

Recently, we have identified a novel signalling mechanism in platelets, the 'serotonylation' of small GTPases [*Cell* 115 (2003) 851]. In addition, the other biogenic monoamines HA, DA, and NE can cause an analogous transglutaminase-mediated activation of signalling proteins by 'hisaminylation' and 'catecholaminylation', wherefore we coined the generic term 'monoaminylation' [*Cell* 115 (2003) 851].

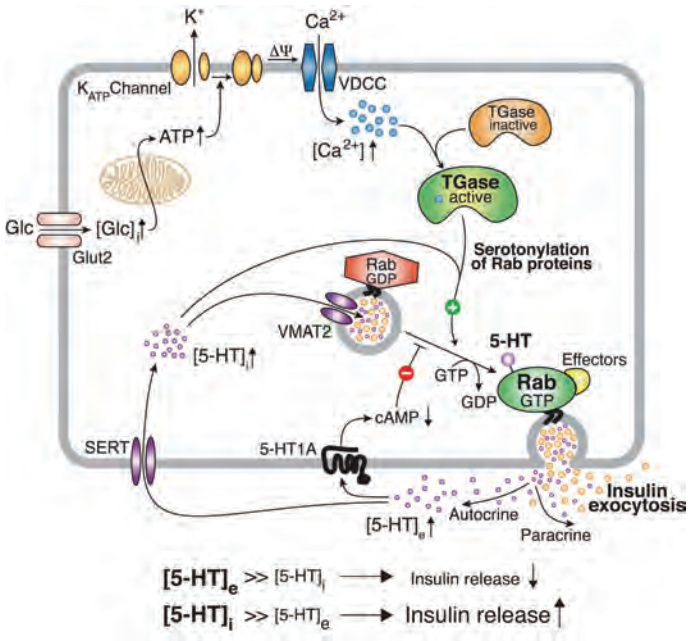


*Figure 3. Proposed model of 5-HT-induced exocytosis of β-granules from glucose-stimulated β-cells. The classic mechanism of glucose induced Ca^{2+} influx is represented in the upper part of the scheme. This Ca^{2+} activates transglutaminases that serotonylate central signalling proteins, which promote insulin secretion [*PLoS Biol. 7 (2009) e1000229].*

We now identified this mechanism also in β-cells of the pancreas [*PLoS Biol.* 7 (2009) e1000229]. As in platelets, serotonylation regulates the secretion of storage granules from these cells. Under normal conditions 5-HT controls the release of insulin. When the 5-HT levels are low like in 5-HT-deficient mice, proper insulin secretion is hampered and blood glucose concentration rises to noxious levels after a meal, a hallmark of diabetes. Thus, the identification of the insulin-releasing action of 5-HT may open new avenues for intervention in diabetes, a main research objective for further studies.

The pancreas is the third disease-associated context of serotonylation identified since the first description of this mechanism in bleeding disorders by us [*Cell* 115 (2003) 851]. In addition to the finding's contribution to the understanding of 5-HT's role in the widespread disease diabetes, the special case of serotonylation highlights the general physiological relevance of protein monoaminylation exemplarily. Like the phosphorylation of proteins, also the monoaminylation has a deep impact on several cellular processes. In contrast to what was assumed for a long time, water-soluble hormones like 5-HT, HA, and catecholamines act not only at the cell's face *via* surface receptors, but also within cells *via* monoaminylation.

Internal cooperations

- Vera Kalscheuer, MPIMG
- Susann Schweiger, Charité Berlin/ MPIMG
- Stefan Mundlos, Charité Berlin/ MPIMG
- Martin Vingron, MPIMG
- Stefan Roepcke, MPIMG

External cooperations

- Prof. Gudrun Ahnert-Hilger, Charité Berlin
- Prof. Michael Bader, Max-Delbrück-Center (MDC) for Molecular Medicine, Berlin
- Prof. Peter Beyerlein, TFH Wildau
- Prof. Pierre-Alain Clavien, Universitätshospital Zürich, Switzerland.
- Prof. Heidrun Fink, Freie Universität Berlin
- Prof. Christian Gachet, EFS Strasbourg, INSERM, France
- Dr. Katrin Hoffmann, Charité Berlin
- Prof. Joachim Klose, Charité Berlin
- Prof. Josef Priller, Charité Berlin
- Prof. Marjan Rupnik, University of Maribor, Slovenia
- Prof. Margharet McLean, University of Glasgow, UK
- Prof. Annette Schürmann, DIFE Potsdam
- Prof. Karl Sperling, Charité Berlin
- Prof. Kent Vrana, Penn State University, Pennsylvania, USA
- Prof. Erich Wanka, Max-Delbrück-Center (MDC) for Molecular Medicine, Berlin
- Priv.-Doz. Peter Zill, Ludwig-Maximilians-Universität (LMU) Munich

General information

Selected publications

Paulmann N, Grohmann M, Voigt J-P, Bert B, Vowinckel J, Bader M, Skelin M, Jevšek M, Fink H, Rupnik M, Walther DJ. *Intracellular serotonin modulates insulin secretion from pancreatic βcells by protein serotonylation*. PLoS Biol 2009;7: e1000229

Scheuch K, Lautenschlager M, Grohmann M, Stahlberg S, Kirchheiner J, Zill P, Heinz A, Walther DJ*, Priller J*. *Characterization of functional promotor polymorphisms of the human tryptophan hydroxylase-2 gene in serotonergic raphe neurons*. Biol. Psychiatry 2007; 62:1288-1294 (*shared authorship)

Lesurtel M, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, Gachet C, Bader M, Clavien P-A. *Platelet-derived serotonin mediates liver regeneration*. Science 2006; 312:104-107.

Walther DJ, Peter J-U, Winter S, Höltje M, Paulmann N, Grohmann M, Vowinckel J, Alamo-Bethencourt V, Wilhelm CS, Ahnert-Hilger G, Bader M. *Serotonylation of Small GTPases is a Signal Transduction Pathway that Triggers Platelet α-Granule Release.* Cell 2003; 115:851-862.

Walther DJ, Peter JU, Bashammakh S, Hörtnagl H, Voits M, Fink H, Bader M. *Synthesis of serotonin by a second tryptophan hydroxylase isoform.* Science 2003;299:76.

Work as scientific referee

Since 2003, D. Walther serves as scientific referee for the following journals: Journal of Histochemistry & Cytochemistry, Cephalalgia, Biological Psychiatry, Behavioural Brain Research, and Molecular Psychiatry.

In addition, D. Walther served as referee for the following institutions: Higher Education Authority (HEA) of Ireland (evaluation of animal facilities, 09/06), county court Munich (expert assessment about the use of 5-hydroxytryptophan as antidepressive compound, 10/08)

Teaching activities

Lectures on „Trangenic Animals in Research and Production – Overview and Perspectives" at the Freie Universität Berlin, since 04/07

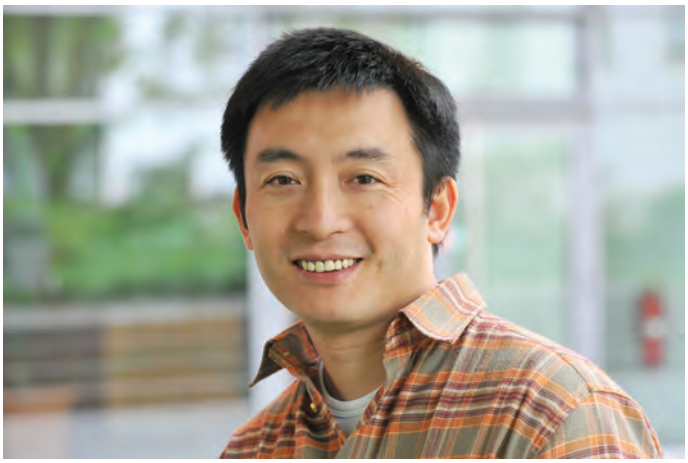
University lectureship at the Technische Fachhochschule Berlin, FB Biotechnologie, since 07/08

External funding

DFG, SFB 577: *Analysis of the clinical variability in Mendelian disorders (*subprojects B1 and Z1)

Associated Group: Applied Bioinformatics

(Established: 01/2007)



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Hui Kang (since 09/00)
Raghu Bhushan (02/00 - 06/09)

Technicians
Corinna Menzel (since 01/07)
Melanie Bienek (since 06/09)

Scientific overview

The recent introduction of massive parallel sequencing technology has revolutionized genomic research. These so-called next generation sequencing platforms, such as Roche/454, Illumina/solexa and ABI/SOLiD system can sequence DNA orders of magnitude faster and at much lower cost than conventional Sanger method. With their incredible sequencing capacity, my lab has been focused on developing and implementing various genomic assays based on this new generation of sequencers. We are now applying the assays in identifying genetic factors underlying human diseases as well as studying transcriptional and posttranscriptional regulation of miRNA genes.

Characterisation of breakpoints in disease-associated balanced chromosome rearrangements

The frequency of *de novo* balanced translocations in patients associated with congenital malformations and/or developmental delay is twice that among the general population, suggesting a causative link between the rearrangements and the observed phenotype in at least half of the disease-associated balanced translocations (DBCRs). The phenotype in these cases can be caused by the disruption or inactivation of specific gene(s) at the translocation breakpoints. Therefore, characterisation of the breakpoints in DBCRs has often been a promising starting point in molecular elucidation of early-onset Mendelian disorders. Recently such a strategy has also been applied to search for genetic risk factors for complex and late-onset diseases.

Mapping translocation breakpoints using conventional methods, such as *in situ* hybridization with fluorescent dye-labeled bacterial artificial chromosome clones (BAC-FISH), is rather laborious, time consuming and often provides limited reso-

lution of breakpoint positions. With the development of array painting techniques, which combine DNA array and chromosome sorting technologies, the efficiency has been greatly improved and the ultra-high resolution was achieved by the sequential painting with two arrays, a tiling path large insert array and a region-specific, ultra-high-resolution oligonucleotide array. Using 'next generation' massive parallel sequencing technology, we have introduced a novel and rapid method to map translocation breakpoint by shotgun sequencing flow-sorted derivative chromosomes. The coverage attained by this method was sufficient to bridge the breakpoints by PCR amplification, and the procedure allows to determine their exact nucleotide positions in a short time frame. Recently, to further improve the method and in order to characterise the breakpoints in all types of balanced chromosome rearrangements more efficiently and more accurately, we performed massively parallel sequencing using Illumina 1G analyser and ABI SOLiD systems to generate short sequencing reads from both ends of DNA fragments. By identifying read pairs spanning the breakpoints, we were able to map the breakpoints to a region of a few hundred base pairs that could be confirmed by subsequent PCR amplification and Sanger sequencing of the junction fragments. Collaborating with Vera Kalscheuer and Andreas Tzschach, we are now implementing our method in large-scale breakpoint mapping and gene finding.

Molecular elucidation of genetic factors underlying mental retardation by genome partitioning and large-scale next generation sequencing

Using Sanger sequencing, mutation screening in genomic intervals defined by linkage analysis or in a large number of candidate genes is often extremely tedious. Recently advance in massive parallel sequencing technology has dramatically improved the efficiency and reduced the cost. However, the cost of sequencing the complete genome using presently available instruments is still too high to apply to a large number of human patients. Therefore, robust methods to isolate relevant genomic regions for targeted sequencing are required. In this project, we evaluated different genome partitioning strategies including droplet-based PCR from RainDance Technologies, solution hybrid selection from Agilent Technologies and chromosome sorting. With different strengths, they are eventually combined to identify genetic factors underlying mental retardation.

Transcriptional and posttranscriptional regulation of miRNA genes

miRNAs are small non-coding RNAs that control the expression of target genes at the posttranscriptional level. Recently, more and more miRNAs have been implicated in a variety of biological processes including brain development and function. Whereas much attention has been focus on finding the target genes regulated by miRNAs, little is known about the system which regulates miRNA expression. One major focus of the lab is to study transcription and posttranscriptional regulation of miRNA genes. In the study of transcriptional regulation, we are involved in genome wide discovery of miRNA promoters in pre-B cells using ChIP-seq, mRNA-seq and small RNA sequencing methods.

It has been demonstrated that the Drosha or Dicer processing of individual miRNA can be regulated. Though, it is yet not known how generalized the phenomena are. We are therefore interested in studying the posttranscriptional regulation of miRNAs, especially regulation of Dicer processing at the genomic level by genome-wide profiling of miRNA precursor (pre-miRNA) and mature miRNA from the same sample and comparing their relative abundance across different samples. Currently, we are developing a novel assay to efficiently profile pre-miRNA based on new sequencing technology.

De novo transcriptome sequencing using 454 pyrosequencing

New sequencing technologies are not only robust tools for the investigation of transcriptome in model organisms, but also manifest great potential in studying non-model organisms. To facilitate comprehensive transcriptome characterization, we are developing methods for transcriptome sequencing using 454 pyrosequencing. With long read length and high accuracy, it is particularly suitable for *de novo* sequence assembly. To be fit for 454 sequencing, our methods consist of steps to remove poly A+ tails and cDNA library normalization. The sequencing data would provide a comprehensive reference resource for further functional studies.

General information

Selected publications

Fu X, Fu N, Guo S, Yan Z, Xu Y, Hu H, Menzel C, Chen W*, Li Y, Zeng R, Khaitovich P* (2009). *Estimating accuracy of RNA-Seq and microarrays with prote-omics*. BMC Genomics 10:161-169 (* shared corresponding authors)

Stoeckius M, Maaskola J, Colombo T, Rahn HP, Friedländer MR, Li N, Chen W, Piano F, Rajewsky N (2009). *Large-scale sorting of C. elegans embryos reveals the dynamics of small RNA expression*. Nat Methods. 2009 Sep 6. [Epub ahead of print]

Kuss AW, Chen W (2008). *MicroRNAs in brain function and disease*. Curr Neurol Neurosci Rep 3:190-197.

Friedländer MR, Chen W, Adamidi C, Maaskola J, Einspanier R, Knespel S, Rajewsky N (2008). *Discovering microRNAs from deep sequencing data using miRDeep.* Nat Biotechnol. 26(4), 407-415.

Chen W, Kalscheuer V, Tzschach A, Menzel C, Ullmann R, Schulz M, Erdogan F, Li N, Kijas Z, Arkesteijn G, Pajares IL, Goetz-Sothmann M, Heinrich U, Rost I, Dufke A, Grasshoff U, Glaeser BG, Vingron M, Ropers HH (2008). *Mapping translocation breakpoints by next-generation sequencing*. Genome Res. 18: 1143-1149.

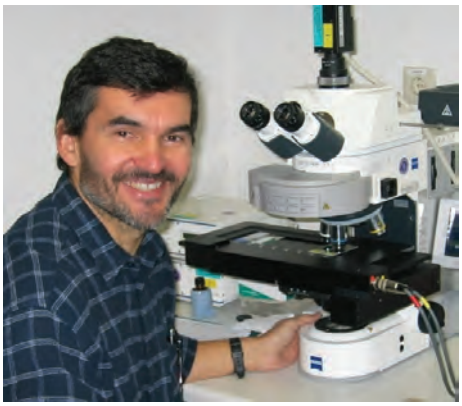
Teaching activities

22nd course in Medical Genetics, 04/2009, Bertinoro di Romagna

Integration of Cytogenetics, Microarrays and Massive Sequencing in Biomedical and Clinical Research, 10/2008 Bologna

Associated Group: Meiosis & Chromosome Dynamics

(Established: 01/2004)



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Scientist
Dr. Caroline Adelfalk (06/03-12/05, guest scientist since 01/06)

Scientific overview

The germ line is particularly vulnerable to genotoxic agents. DNA damage and recombinational misrepair can lead to mutation & chromosomal aberrations and amongst others to mental retardation and congenital genetic defects. A major interest and focus of our group is to understand chromosome behavior in meiotic prophase and the genesis of chromosomal aberrations. Besides genotoxic influences, homologous recombination at illegitimate sites is thought to fuel the chromosome rearrangements, those seen in patients as well as in an evolutionary context. It is thus imperative to understand the nature of DNA double strand break repair, break points and recombinogenic sequences in the context of genome architecture and nuclear topology. Genes expressed in brain are also expressed in testis and given our interest in meiosis, we have established tools for high resolution analysis of first meiotic prophase progression in the genetic model systems budding yeast and mouse.

Since 2006 we moved on with our comparative analysis of the impact of mutations in genes involved in DNA repair and telomere stability in the mouse and yeast and developed a highly sensitive live cell imaging microscope system (TILL) for live cell analysis in the light sensitive prophase I stage of the model species *S. cerevisiae*. By this, we pioneered work that for the first time showed in live meiocytes exceeding meiotic movements of telomeres and entire nuclei, and that these dynamics required the expression of a functional telomere complex, cohesin and an actin network independent of recombination. We continued this line of research and showed that the telomere mobility translates to whole chromosomes (Fig. 1) much surprisingly during all of prophase I. This gave new impetus to the perception how meiotic telomere and chromosome be-

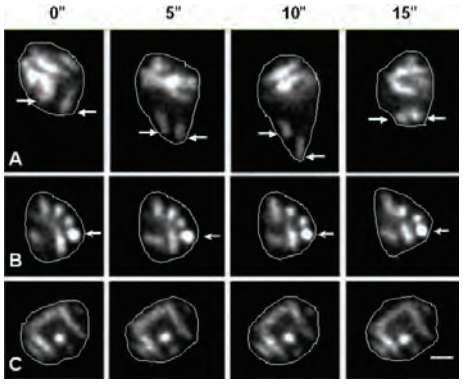

Figure 1: Zip1-GFP-marked chromosomes in live yeast pachytene meiocytes. Frames in 5s intervals and nuclear border outlines are shown. ***(A)*** *In the wild type the nucleus undergoes constant shape changes and often "maverick" chromosomes (arrows) rapidly move from and back to the mass of chromosome.* ***(B)*** *Nucleus of a ndj1Δ cell whose SCs and nucleus undergo only limited mobility due to defective telomeres, with the arrow marking a stationary polycomplex as reference.* ***(C)*** *Chromosome & nuclear mobility is paralyzed in a WT nucleus treated with the actin poison LatB. Bar: 1µm.*

havior is regulated and linked to recombination. These observations have sparked new hypotheses and work in the meiosis field.
We also investigated a number of mouse strains with mutations in DNA-damage-responsive genes and telomere genes for alterations in meiotic chromosome behavior. Our efforts have established a first integrated view of telomere and chromosome mobility phenotypes in relation to mutations with altered prophase I and recombination progression, and provide a first circuitry around the meiotic telomere, which will aid analysis of failure of germ cell differentiation in infertile patients and mice. Furthermore, our analysis will help to understand why up to one third of fertilized human eggs are aneuploid, the leading genetic cause of developmental failure and pregnancy loss.
Currently, we are cooperating with A. Kuss on the role of Bod1 protein in meiosis, whose gene has been found to be mutated in a family with two branches and four female patients suffering from mental retardation and oligomenorrhea, where all isoforms of the BOD1 gene product have been lost. Since BOD1 appears to be a kinetochore protein likely involved in spindle attachment and mitotic chromosome segregation, we work to localize the BOD1 protein in meiotic cells. In a cooperation with R. Ullmann and A. Muradyan we study the genomic signature left by acute high dose radiation in single cell survivor clones derived from irradiated A549 tumour cells by array-CGH, expression profiling and molecular cytogenetics to highlight genomic damage elicited by ionising radiation, to better understand the signatures elicited by genotoxic agent that may alter a cells fate towards misdifferentiation.

General information

Selected publications

Adelfalk C, Janschek J, Revenkova E, Liebe B, Göb E, Alsheimer M, Benavente R, de Boer E, Novak I, Höög C, Scherthan H and Jessberger R. *Cohesin SMC1ß protects telomeres in meiocytes*. *J Cell Biol* 2009; 187:185-99.

Erenpreisa J, Cragg MS, Salmina K, Hausmann M, Scherthan H. *The role of meiotic cohesin REC8 in chromosome segregation in g irradiation-induced endopolyploid tumour cells*. *Exp Cell Res* 2009; 315:2593-2603.

Scherthan H, Trelles-Sticken E. *Absence of yKu/Hdf1 but not myosin-like proteins alters chromosome dynamics during prophase I in yeast*. Different 2008; 76:91–98.

Scherthan H, Wang H, Adelfalk C, White EJ, Cowan C, Cande WZ, Kaback DB. *Chromosome mobility during meiotic prophase in Saccharomyces cerevisiae.* Proc Natl Acad Sci USA 2007; 104:16934-16939.

Liebe B, Petukhova G, Barchi M, Bellani M, Braselmann H, Nakano T, Pandita TK, Jasin M, Fornace A, Meistrich ML, Baarends WM, Schimenti J, de Lange Z, Keeney S, Camerini-Otero RD, Scherthan H. *Mutations that affect meiosis in male mice influence the dynamics of the mid-preleptotene and bouquet stages*. Exp Cell Res 2006; 312:3768-3781.

Open access activities

There are 22 free full-text articles in PubMed Central as of 10/2009

Selected invited talks

DNA-repair and chromosome dynamics in meiosis. Invited lecture at the Heinrich-Pette-Institute, Hamburg, Germany, 02/2009

Meiotic chromosome dynamics. Chair and invited plenary lecture at the 16th Int. Chromosome Conference. Amsterdam, NL, 08/2007

Modulation of meiotic telomere dynamics. Invited plenary lecture at the joint meeting of the Biochemical Society and the Genetics Society "Meiosis and the causes and consequences of recombination". 28.-31.03.2006, University of Warwick, UK

Telomeres in space and time, evolutionary and germ cell aspects. Invited plenary lecture, Minisymposium, Biocenter, Vienna, Austria, 10/2006

Scientific honors / awards

Rank 48 among the top 50 cited German Molec. Biologists (Labor J. 5/2008).

1st place, CNRS Concours Chercheurs no. 21/01, DR2, 2006

Work as scientific referee

H. Scherthan serves as scientific referee for the following journals: BOR, Dev. Cell, Dev. Biology, J. Cell Biol., J. Cell Sci., Eur. J. Cell Biol., Chromosoma, Chromos. Res., Health Physics.

In addition, H. Scherthan serves as referee for the following institutions: DFG, The Wellcome Trust, Deutsche Krebshilfe.

Membership in journal editorial boards

Editorial Advisory Board of *Chromosome Research*, since 2009

Teaching activities

Special Practical Course *Molekulare Cytologie/Cytogenetik*; Lecture: *Grundlagen der Molekularen Cytologie/Cytogenetik*. Lecture and seminar series *Chromosome Biology* (since 2007) each term; Technical Univ. of Kaiserslautern.

Organization of scientific events

Workshop organizer & Chair of the session *Meiosis and the regulation of recombination*, 16th Int. Chromosome Conference, Amsterdam, The Netherlands, 2007.

Associated Group:
Clinical Genetics and Biochemistry

(Established: 2000)

Head
Professor Dr. Susann Schweiger
Email: schweiger@molgen.mpg.de

Scientists
Dr. Sybille Krauss* (since 00)
Prof. Rainer Schneider (since 07, associate fellow)

PhD student
Eva Kickstein* (since 06)

Technician
Melanie Kunath* (since 05)

Scientific overview

Scientific achievements

Some time ago we had identified mutations in the *MID1* gene to be causative for the X-linked form of Opitz BBB/G syndrome (OS, Quaderi et al., 1997, *Nature Genetics*). Since then the scientific focus of my group has been at the characterization of the physiological function of the *MID1* gene product and the analysis of the mechanisms of disease development in OS patients. Our work has led to the identification of a microtubule-associated protein complex that plays a central role in insulin receptor and mTOR signalling and is a translation control unit for the regulation of compartmentalized protein translation.

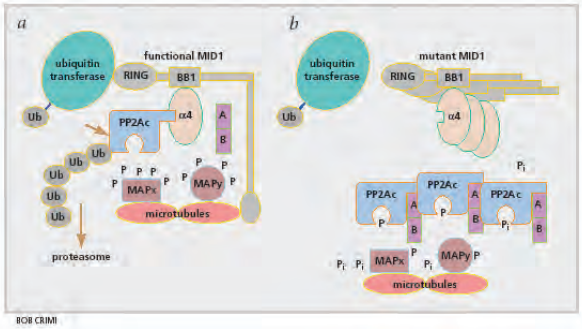

Figure 1: ***(a)*** *The microtubule-associated protein MID1 together with the regulatory PP2A subunit α4 targets the catalytic subunit of PP2A towards ubiquitin dependent modification and degradation by the proteasome.* ***(b)*** *In OS patients MID1 loses its contact to the microtubules, the microtubule-associated pool of PP2A can no longer be ubiquitinated and accumulates at the microtubules. This leads to the hypophosphorylation of microtubule-associated proteins.*

The MID1 protein is a multidomain protein with a RING finger domain and two zinc-binding B-Boxes in its N-terminus. We have shown that the C-terminus of the MID1 protein associates to microtubules (Schweiger et al. 1999, *PNAS*) and *via* the two B-Boxes it binds to the α4 protein, which is a regulatory subunit of protein phosphatase 2A (PP2A). The close vicinity of the ubiquitin ligase MID1 to PP2A results in the ubiquitin dependent modification and degradation of the microtubule-associated pool of the catalytic subunit of PP2A. In OS patients

* externally funded

this mechanism is disrupted and the catalytic subunit of PP2A accumulates at the microtubules (Fig. 1, Trockenbacher et al. 2001, *Nature Genetics*).
We have further found that the MID1/α4/PP2A protein complex assembles a microtubule-associated ribonucleoprotein (RNP) complex, that, in addition to the three proteins, contains (i) active ribosomes, (ii) several translation factors and (iii) mRNAs that bind to the complex *via* G-rich RNA motifs (Aranda-Origillés et al., 2008, *Human Genetics*, Fig. 2). Unpublished results show that the MID1/α4 protein complex regulates the translation efficiency of mRNAs that are attached to it and that up-regulation of its activity by, for example, over-expression of MID1 result in an increase in protein synthesis from these mRNAs (manuscript in preparation).

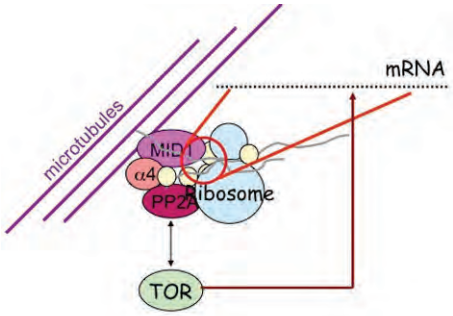

Figure 2: MID1, α4 and PP2A assemble a microtubule-associated protein complex that contains active polyribosomes, translation factors and mRNAs, that associate to the complex via G-rich RNA motifs. PP2A and its counteracting enzyme, mTOR regulate the translation efficiency of these mRNAs.

Interestingly, it seems to be a set of mRNAs with very specific functions that bind to the MID1/α4/PP2A RNP complex. In a database search with MID1 binding RNA motifs we could identify close to 100 mRNAs, all of which are coding for proteins either involved in protein translation, functioning at the synapse or playing central roles in polarizing and migrating cells. Interestingly, translation efficiencies of several of these mRNAs are impaired in cell lines from OS patients. This observation together with FRAP data showing that the MID1 protein complex is actively transported along the microtubules (Aranda-Origillés et al., 2008, *PLOSone*) led us hypothesize that the MID1 protein complex would function as a translation regulator for compartmentalized protein synthesis in either neurons or cells comprising protein gradients such as polarizing and migrating cells.

Furthermore, by comparing clinical genetic phenotypes we were able to establish a novel and unexpected connection of the MID1/α4/PP2A complex and shh signalling. The shh pathway is involved in body patterning and limb and brain development. It also is an oncogenic pathway, gain of function of which seems to be a critical factor during carcinogenesis. Loss of function of components of the shh signalling cascade have been linked to syndromes that significantly overlap clinically with the phenotype seen in OS patients. We could show that PP2A activity and the MID1 protein complex regulate the transcriptional activity of GLI3, one of the three transcriptional effector molecules of the shh pathway (Krauss et al. 2008, *Cancer Research*; Kraus et al., *PLoS One*). Interestingly, increase of the activity of PP2A *via* the MID1 protein complex can significantly decrease the activity of the shh signalling cascade, which makes the MID1/α4/PP2A protein complex a very promising target for an anti-shh cancer therapy.

Future plans - the MID1/alpha4 ubiquitin ligase complex, a promising drug target for cancer and neurodegenerative disorders

PP2A is not only a very potent tumour suppressor, but it is also by far the most important enzyme to de-phosphorylate microtubule-associated tau protein. Hyperphosphorylated tau is the major component of paired helical filaments, a pathological hallmark in brains from Alzheimer's Disease (AD) patients. In addition to that, our data show that the MID1 / α4 / PP2A influences the synthesis of several proteins, gain of function of which are the pathogenic factors driving the development of other neurodegenerative disorders such as amyotrophic lateral sclerosis, Parkinson's Disease and Huntington's Disease. Taken together this suggests that a

mechanism that influences the activity of a specific pool of PP2A would be a very promising drug target for several cancer entities as well as neurodegenerative disorders. Although this is widely accepted in the literature and the development of inhibitory molecules for kinases has been very successful, until now it has not been possible to accelerate phosphatase activity in small molecule drug discovery approaches.
The MID1/α4 ubiquitin ligase complex seems to regulate the turn over and the activity of the microtubule-associated pool of PP2A quite specifically (Trockenbacher et al. 2001, *Nature Genetics*). In several validation approaches using knockdown technology we could show that a decrease of MID1/α4 ubiquitin ligase activity indeed leads to an increase of PP2A activity and influences several of the mentioned pathogenic processes including tau phosphorylation and the production of pathological protein in neurodegenerative disorders. From mutation and deletion analysis data of the MID1 protein and the NMR structures of the B-Box domains of the MID1 protein and the α4 protein we have concluded that the interphase between MID1 and α4 would be targetable by small molecules. Together with our collaboration partners from the University of Innsbruck and the Lead Discovery Unit in Dortmund we have set up an alpha screen to screen for small molecules that disturb the interaction of MID1 and α4 and thereby inhibit ubiquitin ligase activity and increase PP2A activity. In addition we are currently developing several cell free and cell based assays and establish animal models to validate potential hits that will be produced in the alpha screen.

In summary, the discovery of a microtubule-associated protein complex that controls microtubule-associated PP2A activity has become a promising drug target for a potential therapy of cancer and neurodegeneration. In addition, the nature of the interaction of the MID1 protein and the α4 protein seems to be ideal for a small molecule approach targeting a protein-protein interaction.

General information

Selected publications

Aranda-Orgillés B, Aigner J, Kunath M, Lurz R, Schweiger S. *Active Transport of the Ubiquitin Ligase MID1 along the Microtubules Is Regulated by Protein Phosphatase 2A*. PLoS One 2008; 3(10): e3507

Winter J, Roepcke, S, Krause S, Mueller EM, Otto A, Vingron M, Schweiger S. *Comparative 3'UTR analysis allows identification of regulatory clusters that drive Eph/ephrin expression in cancer cell lines*. PLoS One 2008;3(7): e2780

Krauß S, Foerster J, Schneider R, Schweiger S. *PP2A and rapamycin regulate the nuclear localization and activity of the transcription factor GLI3*. Cancer Research 2008; 68(12): 4658-65

Aranda-Orgillés B, Winter J, Aigner J, Köhler A, Jastrzebska E, Stahl J, Müller EC, Otto A, Wanker EE, Schneider R, Schweiger S. *The Opitz syndrome gene product MID1 assembles a microtubule-associated ribonucleoprotein complex*. Hum Genet 2008; 123(2):163-76

Winter J, Kunath M, Roepcke S, Krause S, Schneider R, Schweiger S. *Alternative polyadenylation signals and promotors act in concert to control tissue-specific expression of the Opitz Syndrome gene MID1*. BMC Mol Biol 2007; 8:105.

Work as scientific referee

S. Schweiger serves as scientific referee for the following journals (selection): Journal of Neuroscience, Human Molecular Genetics, Experimental Cell Research and Cancer Research.

Special achievements

S. Schweiger was board certificated for the Speciality of Human Genetics in November 2006 (Fachärztin für Humangenetik)

Appointments of former members of the group

Jennifer Winter: Group leader at the Max Planck Institute of Immunobiology, Freiburg / Breisgau

Susann Schweiger - Chair for Molecular Medicine and Head of the Centre for Oncology and Molecular Medicine at the University of Dundee (Medical School)

Patents

US-preliminary patent # 60/380,590: *Intervention in intracellular PP2A levels via its interaction with the a4 protein: implications for Alzheimer and cancer treatment*.

German patent office invention disclosure: *Strategies to enhance production of proteins in eukaryotic cells*.

External funding

Volkswagen Foundation Germany: *The renaissance of the human model: monogenic phenotypes as gateways to signaling networks in development and disease*, 04/05 - 03/10

AtaxiaUK: *Translation control of mRNAs with CAG repeat expansions and its implication for pathogenesis and therapy of SCA2 and other spinocerebellar ataxias*, 08/08-07/11

Tenovus Scotland: *The microtubule-associated MID1/PP2A mRNP and its role in the pathogenesis of Chorea Huntington*, 04/08-03/11

Chief Scientist Office: *The MID1/PP2A protein complex: a novel tool to develop therapies for Spinocerebellar Ataxias*, 10/09- 09/10

General information about the whole Department

Complete list of publications (2006-2009)

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Adelfalk, C., Janschek, J., Revenkova, E., Liebe, B., Göb, E., Alsheimer, M., Benavente, R., de Boer, E., Novak, I., Höög, C., Scherthan, H., and Jessberger, R. *Cohesin SMC1β protects telomeres in meiocytes.* J Cell Biol 187:185-99 (2009).

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Garcia-Cruz, R., Roig, I., Robles, P., Scherthan, H., and Garcia, M. *ATR, BRCA1 and γH2AX localize to unsynapsed chromosomes at the pachytene stage in human oocytes. Reprod.* BioMedicine Online 18:37-44 (2009).

Graul-Neumann, L.M., Stieler, K.M., Blume-Peytavi, U., Tzschach, A. *Autosomal dominant inheritance in a large family with focal facial dermal dysplasia (Brauer-Setleis syndrome).* Am J Med Genet A. Mar16;149A(4): 746-750 (2009).

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Hu, H.Y., Yan, Z., Xu, Y., Hu, H., Menzel, C. Zhou, Y.H. Chen, W., Khaitovich, P. *Sequence features associated with microRNA strand selection in humans and flies.* BMC Genomics. Sep4;10(1):413 [Epub ahead of print] (2009).

Kahrizi, K., Najmabadi, H., Kariminejad, R., Jamali, P., Malekpour, M. Garshasbi, M., Ropers, H.H., Kuss, A.W., Tzschach, A. *An autosomal recessive syndrome of severe mental retardation, cataract, coloboma and ky-*

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Krauß, S., So, J., Hambrock, M., Köhler, A., Kunath, M., Scharff, C., Wessling, M., Grzeschick, K.H., Schneider, R., Schweiger, S. *Point Mutations in GLI3 Lead to Misregulation of its Subcellular Localization.* PLoS One Oct 15;4(10):e7471 (2009).

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Tzschach, A. *Genetik der nichtsyndromalen geistigen Behinderung.* Medgen 21:231-236 (2009).

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Selected invited talks (H.-Hilger Ropers)

Molecular elucidation of MR: Strategies and progress report, XXIII Spanish Genetic Congress, Valladolid, 15.06.2006

Genetic dissection of MR by high resolution array CGH and other approaches, Lecture at Dept. of Genetics and Evolutionary Biology, Institute of Biosciences, Univ. Sao Paulo, Brasil. 01.08.2006.

High resolution array CGH, AP Echo conference 2006, Shanghai, 28.08.2006

Frequent genomic imbalances in Mendelian and complex diseases: why association studies often fail. Nederlands Anthropogenetische Vereniging, Utrecht, 24.11.2006.

Nieuwe perspectieven voor het ophelderen van erfelijke ziekten, The Royal Netherlands Academy of Arts and Sciences, Amsterdam, 26.02.2007

Speaker at the *3rd International Meeting on Cryptic Chromosomal Rearrangements, MR and autism*, Troina, Italy, 13.04.2007

Evangelischer Hochschuldialog : "Gott in den Genen?, Albert-Ludwigs-Universität, Freiburg, 07.07.2007 (public discussion)

New perspectives for the elucidation of human diseases, Berlin Brandenburg Academy of Sciences and Humanities, 22.06.2007

Lecture at the 1st International Congress on Health, Genomics, and Biotechnology, Teheran, 24.11.2007

Disease related genome research: novel opportunities for developing countries, HGM 2008, Hyderabad, India, 30.09.2008

Lecture at the Deutsch-Koreanisches Symposium on Biotechnology, Seoul, January 15-16, 2009

Ethical and social Issues of the Human Genome Project, Annual Meeting of the Indian Society of Human Genetics, New Delhi, India, 17.03.2009 (plenary lecture)

Cognitive impairment: what have we learned, what lies ahead, University of Nijmegen, 03.04.2009

Lecture at the Regpot Conference (EU 7th Framework), Ankara, 20.04.2009

Vom Mütterchen die Frohnatur..' : neue Perspektiven für die Aufklärung der Funktion des menschlichen Genoms und Konsequenzen für die Krankenversorgung, 11. Gaterslebener Begegnung, Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, 08.05.2009

The sequencing revolution: Implications for genome research and health care, Rudbek Seminar Series, Uppsala, 08.10.2009

Selected memberships (H.-Hilger Ropers)

- Elected member of HUGO Council (since 2003) and member of HUGO Scientific Program Committee
- Elected member of the Royal Netherlands Academy of Arts and Sciences
- Elected member of the Berlin-Brandenburg Academy of Sciences and Humanities
- Elected Secretary of the Biomedical class of the Berlin-Brandenburg Academy of Sciences and Humanities, 2007
- Honorary medal of the Society for Human Genetics (GFH) 2009

Membership in journal editorial boards (H.-Hilger Ropers)

- Genome Medicine
- Human Genetics
- Clinical Genetics

Service to the scientific community (H.-Hilger Ropers)

- Evaluation of Telethon, Italy 2009
- Member of the scientific advisory board Biozentrum Würzburg, since 2003

Appointments of former members of the department

Susann Schweiger - Chair for Molecular Medicine (2006) and Head of the Centre for Oncology and Molecular Medicine (2007) at the University of Dundee (Medical School)

PhD theses

2009

Masoud Garshasbi (2009) *Identification of 31 genomic loci for autosomal recessive mental retardation and molecular genetic characterization of novel causative mutations in four genes*, PhD Thesis, Freie Universität Berlin (supervisor: Andreas Kuss)

Stella-Amrei Kunde (2009): *Untersuchung zur Funktion des PQBP1-Komplexes,* PhD thesis submitted in 06/09, Freie Universität Berlin (supervisor: Vera Kalscheuer)

Artur Muradyan (2009) *SPON2 and its implication in epithelial-mesenchymal transition*, Freie Universität Berlin (supervisor: Reinhard Ullmann)

Nils Rademacher (2009) *Untersuchungen zur Funktion der Kinase CDKL5 / STK9,* PhD thesis submitted in 09/09, Freie Universität Berlin (supervisor: Vera Kalscheuer)

2008

Maik Grohmann (2008) *Die Rolle der Tryptophan-Hydroxylase 2 bei der Entstehung psychiatrischer Erkrankungen,* PhD Thesis, Freie Universität Berlin (supervisor: Diego Walther)

Mohammad Mahdi Motazacker (2008) *Identification of novel genetic loci for non-syndromic autosomal recessive mental retardation and molecular genetic characterization of a causative GRIK2 mutation*, PhD Thesis, Freie Universität Berlin (supervisor: Andreas Kuss)

Nils Paulmann (2008) *Die Serotonylierung kleiner GTPasen moduliert die Insulinsekretion,* PhD Thesis, Freie Universität Berlin (supervisor: Diego Walther)

Joyce So (2007): *Molecular and phenotypic analysis of Opitz syndrome patients and characterization of the relationship between the RBCC protein MID1 and the tumour suppressor CYLD*. PhD Thesis, Freie Universität Berlin (supervisor: Susann Schweiger)

2006

B. Budny (2006)*Molecular background of X-linked mental retardation*. PhD Thesis, Karol Marcinkowski University, Poznan, Poland.

Beatriz Aranda-Origillés (2006) *Characterization of the MID1/a4 multiprotein complex*. PhD Thesis, Freie Universität Berlin (supervisor: Susann Schweiger)

Wei Chen (2006) *Development and application of CGHPRO, a novel software package for retrieving, handling and analysing array CGH data*. PhD Thesis, Freie Universität Berlin

Sebastian Haesler (2006) *Studies on the evolution and function of FoxP2, a gene implicated in human speech and language, using songbirds as a model*. PhD Thesis, Freie Universität Berlin (supervisor: Constanze Scharff)

Olivier Hagens (2006) *Search for genes involved in Human Cognition. Molecular characterization of two novel genes, FBXO25 and KIAA1202, disrupted by a translocation in a mentally retarded patient.* PhD Thesis, Freie Universität Berlin (supervisor: Vera Kalscheuer)

Jens-Uwe Peter (2006) *Molekularbiologische und pharmakologische Manipulation der Tryptophanhydroxylasen*. PhD Thesis, Freie Universität Berlin 2006 (supervisor: Diego Walther)

Student Theses

2009

C. Bäth (2009) *Monoaminylierung von Transkriptionsfaktoren am Beispiel des CLOCK*, Bachelor Thesis, Freie Universität Berlin (supervisor: Diego Walther)

B. Behrendt (2009) *Modellierung der serotonininduzierten Ausschüttung densegranulärer Botenstoffe aus Thrombozyten,* Bachelor Thesis, Technische Fachhochschule Wildau (supervisor: Diego Walther)

M. Bienemann (2009) *Modellierung der serotonininduzierten Ausschüttung des Insulins aus β-Zellen des Pankreas,* Bachelor Thesis, Technische Fachhochschule Wildau (supervisor: Diego Walther)

René Buschow (2009) *Quantitative Analyse neuronaler Subgruppen mit Hilfe automatisierter Immunfluoreszenz*, Diploma Thesis, Institut für Biologie, Universität Kassel (supervisor: Tim Hucho)

Grit Ebert (2009) *Veränderungen der Genexpressionsmuster im Verlauf der Retinsäureinduzierten Zelldifferenzierung,* Diploma Thesis, Humboldt Universität zu Berlin (supervisor: R. Ullmann)

A. Funke (2009) *Identifikation von BRN2-regulierten Genkandidaten und Untersuchung der Auswirkung des SNPs rs1696 7794 auf die Bindungsaffinität von BRN2,* Bachelor Thesis, Technische Fachhochschule Wildau (supervisor: Diego Walther)

S. Mucha (2009) *Studien zum Einfluss von Serotonin auf die Glucose-induzierte Insulinsekretion,* Bachelor Thesis, Technische Fachhochschule Wildau (supervisor: Diego Walther)

F. Roske (2009) *Die Veränderung der Aufnahme und Inkorporation von biogenen Monoaminen durch Adipogenese in 3T3-L1-Zellen, Bachelor Thesis,* Universität Bayreuth (supervisor: Diego Walther)

B. Schoder (2009) *Vergleich viraler Promotoren mit dem humanen ribosomalen RPL12-Promotor in eukaryotischen Expressionssystemen*, Bachelor Thesis, Technische Fachhochschule Wildau (supervisor: Diego Walther)

C. Schwarzer (2009) *Etablierung eines immunologischen Nachweissystems zur Erfassung des Aktivierungszustandes der kleinen GFTPase Cdc42*, Bachelor Thesis, Universität Bayreuth (supervisor: Diego Walther)

C. Technau (2009) *Monoaminylierung von Transkriptionsfaktoren am Beispiel des CREB,* Bachelor Thesis, Freie Universität Berlin, 2009 (supervisor: Diego Walther)

Lars Theobald (2009) *Untersuchung und Charakterisierung transaktivierender Eigenschaften monoaminylierter Transkriptionsfaktoren,* Diploma Thesis, Technische Fachhochschule Berlin (supervisor: Diego Walther)

Zofia Wotschofsky (2009) *Translocation breakpoint mapping by Illumina/Solexa technology*, Diploma Thesis, Technische Universität Berlin (supervisor: Wei Chen)

2008

Christine Andres (2008) *Relative Immunofluoreszenz - Eine Mikroskop basierte Methode zur Untersuchung der Aktivierung von Signalwegen in heterogenen Zellsystemen wie DRG-Neuronen*, Diploma Thesis, Freie Universität Berlin (supervisor: Tim Hucho)

Vivien Boldt (2008) *Chromosomale Veränderungen in Präneoplasien und Karzinomen der Brust und deren Auswirkungen auf die Genexpression,* Diploma Thesis, Freie Universität Berlin (supervisor: R. Ullmann)

Barbara Glowacka (2008) *Funktionelle Unter-suchungen zur Rolle von PQBP1 (Polyglutamin-Bindungsprotein1) bei der Genexpression*, Diploma thesis, Freie Universität Berlin (supervisor: Vera Kalscheuer)

Melanie Krüger (2008) *Die Rolle der TPH1 in der Ätiologie von Alkoholismus*, Diploma Thesis, Freie Universität Berlin (supervisor: Diego Walther)

Ann Schöler (2008) *Die Bedeutung der Monoaminylierung in neuronalen Differenzierungsprozessen*, Diploma Thesis, Technische Universität Berlin (supervisor: Diego Walther)

Juliane Schreier (2008) *Untersuchungen der Östrogen-Effekte auf die MAP-Kinase ERK in primären sensorischen Neuronen und F-11 Zellen,* Diploma Thesis, Freie Universität Berlin (supervisor: Tim Hucho)

Karsten Sollich (2008) *Establishment of a lenti-virus-based gene knock-down system in rat sensory neurons,* Diploma Thesis, Technische Universität Berlin (supervisor: Tim Hucho)

Anne Steininger (2008) *Genetische und epigenetische Veränderungen bei kutanen T-Zell-Lymphomen,* Diploma Thesis, Freie Universität Berlin (supervisor: R. Ullmann)

Tina Sulistio (2008) *Determination of transgene integration sites in mutant PQBP1 transgenic mice and characterization of the expression pattern of various PQBP1 mutations*, Diploma thesis, Mannheim University of Applied Sciences (supervisor: Vera Kalscheuer)

Anke Walther (2008) *Untersuchungen zur Rolle von CDKL5 Interaktionspartnern beim atypischen Rett-Syndrom*, Diploma thesis, Technische Universität Berlin (supervisor: Vera Kalscheuer)

2007

A. von Bock (2007) *Untersuchung von RNA-Editierungen in der Tryptophan Hydroxylase 2.* Master Thesis, Freie Universität Berlin (supervisor: Diego Walther)

F. Baumkötter (2007) *Monoaminylierung in der Hyperproliferation humaner pulmonärer glatter Muskelzellen.* Diploma Thesis, Freie Universität Berlin (supervisor: Diego Walther)

S. Gohlke (2007) *Monoaminylierung von Huntingtin.* Diploma Thesis, Freie Universität Berlin (supervisor: Diego Walther)

Paul Hammer (2007) Molekulare *Untersuchungen zur Genregulation von TPH2.* Master Thesis, Technische Fachhochschule Wildau (supervisor: Diego Walther)

M. Mehnert (2007) *Einfluss von Monoaminen auf Differenzierungs- und Proliferationsprozesse von embryonalen und adulten neuronalen Stammzellen.* Diploma Thesis, Freie Universität Berlin (supervisor: Diego Walther)

A. Nauman (2007) *Herabregulierung der Expression muriner Transglutaminasen durch RNA-Interferenz. Master Thesis,* Technische Fachhochschule Wildau (supervisor: Diego Walther)

S. Pohl (2007) *Serotoninkataboliten in der Ätiologie des Alkoholismus*. Diploma Thesis, Albert-Ludwigs-Universität Freiburg (supervisor: Diego Walther)

Silke Stahlberg (2007) *Funktionelle Charakterisierung des M1-Motivs in Promotoren der Gene humaner ribosomaler Proteine*. Diploma Thesis, Freie Universität Berlin (supervisor: Diego Walther)

A. Walter (2007) *Identifizierung von monoaminylierten Proteinen in neuronalen Zellen. Diploma Thesis,* Freie Universität Berlin (supervisor: Diego Walther)

2006

K. Albers (2006) *Development of a laboratory information management system (LIMS) for medical genetic investigations.* Bachelor Thesis, Freie Universität Berlin

T. Döser (2006) *Monoaminylierung in der zellulären Immunantwort*. Diploma Thesis, Technische Fachhochschule Berlin (supervisor: Diego Walther)

S. Grabow (2006) *Welche Proteine sind Ziel der Monoaminylierung in T-Lymphozyten?* Diploma Thesis, Freie Universität Berlin (supervisor: Diego Walther)

Dejan Ninkovic (2006) *Untersuchungen zu PQBP1 und einigen wahrscheinlichen Proteininteraktionspartnern*, Diploma Thesis, Freie Universität Berlin (supervisor: Vera Kalscheuer)

A. Salamon (2006) *Functional Aspects of a Mutation in the PLP2 Promoter Region of Patients with Non-Syndromic X-Linked Mental Retardation*. Technische Universität Berlin

Jakob Vowinckel (2006) *Monoaminylierung von Signalproteinen*. Diploma Thesis, Freie Universität Berlin (supervisor: Diego Walther)

M. Walther (2006) *Der Einfluß von Ethanol auf den Metabolismus von Serotonin.* Diploma Thesis, Technische Fachhochschule Berlin (supervisor: Diego Walther)

Guest scientists

Prof. Klaus Wrogemann, MD, PhD, Dept. of Biochemistry & Medical Genetics,University of Manitoba,Canada, 02-08/09

Roxana Karaminejad, MD, Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran, 03-05/07; 10-12/07; 07/08

Anne Thorwarth, MD, Institute for Experimental Pediatric Endokrinology, Charité University Medicine Berlin, 09/06-12/07

Markus Pisecker, PhD, St. Anna Kinderkrebsforschung, CCRI, Children's Cancer Research Institute, Vienna, Austria, 01-04/07

Dilihan Gumus, MD,Msc, PhD Student Cardiff University, Cardiff, UK, 01-07/06

Sandra Selch, PhD student, University of Würzburg, Germany, 04-06/06

Rikke Moeller, MD, The Wilhelm Johannsen Centre for Functional Genome Research, University of Copenhagen, Denmark

Department of Computational Molecular Biology

(Established: 10/2000)

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Computer systems administrator
Wilhelm Rüsing

Group leaders of the Department
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Dr. Stefan Haas (since 01/01)
Dr. Sebastiaan Meijsing (since 09/09)
Dr. Rainer Spang (09/01-12/06)
Dr. Alexander Schliep (05/02-06/09)
Dr. Eike Staub (09/03-02/06)
Dr. Roland Krause (01/05-05/08)

Introduction

Computational biology studies biological questions with mathematical and computational methods. In the area of molecular biology and genomics, the possibility to apply such formal methods, of course, comes from the availability not only of genome sequences, but also of large amount of functional data about biological processes. Computational molecular biology encompasses both development and adaptation of methods in the areas of mathematics, statistics, and computer science, as well as pursuing biological questions applying these tools and close collaborations with experimentalist. In the context of the MPI for Molecular Genetics, computational approaches have become an integral part of most of the research projects pursued.

The research interest of the Computational Molecular Biology Department lies in understanding gene regulatory mechanisms as well as structure and evolution of the eukaryotic genome. To this end, mathematical, computational, and also experimental approaches are being developed and employed. The department is struc-

tured into several research groups, the largest of which is the *Transcriptional Regulation Group* headed by Martin Vingron. The work of this group focuses on theoretical concepts in the prediction of cis-regulatory elements, gene regulatory networks and epigenetic aspects of regulation. Peter Arndt heads the *Evolutionary Genomics Group* which works on developing models how the DNA in primates has evolved. Stefan Haas is heading the *Gene Structure and Array Design Group*. The focus of this group is on transcriptomics and gene structure. As of September 2009, Sebastiaan Meijsing has been building up the *Mechanisms of Transcriptional Regulation Group*, a new, experimental group that will work on transcription factors and protein-DNA interaction.

The current structure and focus of the department differs from what it used to look like a few years ago. In the beginning – the department was founded in 2000 – the groups in the department worked on a broad spectrum of questions ranging from protein evolution to microarray data analysis. The latter was the topic of the *Computational Diagnostics Group* of Rainer Spang, who left end of 2006 for a professorship in Regensburg. His group dealt with microarray data analysis in the context of cancer profiling. The *Algorithmics Group* headed by Alexander Schliep developed machine learning algorithms for pattern recognition in a number of biological problems. Alexander Schliep left in 2009 to take a professorship at Rutgers University, New Jersey. From January 2005 until May 2008 Roland Krause led a group on *Microbial Virulence*, linking the MPI for Molecular Genetics and the MPI for Infection Biology. The *Protein Families and Evolution Group* was headed by Eike Staub who left for industry in February 2006. For the Algorithmics Group this report contains a summary of its activities, while the other groups had been described in earlier research reports.

In contrast to this diversified structure of the department, the last couple of years have brought about an increased focus and concentration. The existing groups share a general interest in mechanisms and evolution of gene regulation. In fact, the groups of Arndt, Haas and Vingron now cooperate very closely and have recently been joined by the experimental lab headed by Meijsing, who also works on transcriptional regulation. The general goal of the department is to elucidate biological processes and mechanisms, albeit based more on theoretical and computational methods than on experimental ones, and, to this end, apply the most adequate and state-of-the-art mathematical and computational techniques.
Some significant research results of the last years are:

- Development of biophysically motivated prediction methods for transcription factor binding sites and determination of tissue-specific transcription factors;
- Development of an analysis pipeline for next generation sequencing data;
- Comprehensive description of the statistics of transcription factor binding site prediction;
- Identification of strand-specific patterns of mutagenesis due to the process of transcription;
- Evolutionary model explaining long-range correlations in the genome.

The study of gene regulation and of structure and evolution of the genome is currently under rapid development. In particular, it has become increasingly clear that in addition to transcripton factors, chromatin structure plays an important role for regulation and for understanding the genome in general. We are currently in the process of extending in the direction of theoretical studies of this so-called epigenetic regulation. At the same time, evolution is shaping the genome and the

regulatory networks, just as it is shaping the protein world. Thus, we are also working on extending our earlier studies on evolution of gene families to the study of evolution of gene regulation.

Members of the department come from various backgrounds, ranging from mathematics, statistics and computer science *via* physics to biology and genetics. The largely theoretical type of work of course relies heavily on powerful computers. The *computer equipment* of the department comprises PCs under Linux, a cluster of 32-bit processors and a cluster of 64-bit Opteron processors, largely for sequence analysis and typical bioinformatics applications. Recently, two new 32-processor, 64-bit computers with large memory were acquired for numerical computations and for processing the new sequencing data. Several RAID arrays with together more than 95 TB of disk space are available. The computer set-up is maintained by the department system administrator, Willi Rüsing, in close cooperation with the institute computing unit.

Department members contribute substantially to the *bioinformatics curriculum* at Free University of Berlin. We teach a number of courses and offer students to do internships, practical courses, and thesis work with us. This brings many bright, young students to the department and at the same time allows the university to show the students a much larger spectrum of bioinformatics than would normally be possible in the university framework. In cooperation with the university we have established an *International Max Planck Research School on Computational Biology and Scientific Computing* (IMPRS-CBSC, http://www.imprs-cbsc.mpg.de/). In 2009, this IMPRS was reviewed and recommended for an extension. With funding from the Max Planck Research Award we have initiated the *International Otto Warburg Summer Schools*. The schools bring together international lecturers with a select group of national and international students and combine lecture-style teaching and research seminars to give an overview of new important areas in computational biology. This initiative is being continued in spite of the expiration of the funds from the award.

During the reporting period we have been involved in a number of *national and international projects* and collaborations. On a national level, we are part of BMBF funded project in the context of NGFN, the National German Network on Genome Research, of an SFB and a graduate school funded by DFG, and of a project funded by Volkswagenstiftung. On an international level, we are participating in several EU projects. A number of research visitors are financing their stay at the department from fellowships from Alexander von Humboldt Foundation or DAAD (German Academic Exchange Service). From 2001 to 2006, significant funding to the department came from the BMBF-funded Berlin Center for Genome Based Bioinformatics (BCB), a large network made up of several Berlin bioinformatics groups and coordinated by Martin Vingron. Since 2006 Martin Vingron has also acted as one of the directors at the newly founded CAS-MPG Partner Institute for Computational Biology in Shanghai, China. He spends around six weeks a year there, spread over six individual visits roughly every other month. In terms of service to Max Planck Society, Vingron has also been a member of the BAR commission on purchase of large computing equipment in MPG and of several other ad-hoc committees. He has acted as managing director of MPIMG from 2003-2008. In 2004, Vingron was elected to the National German Academy Leopoldina and, in the same year, also received the Max Planck Research Award. In 2009 he became chair of the steering committee of the RECOMB conference series, a renowned international conference on computational biology.

Evolutionary Genomics Group

(Established: 10/2003)

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Scientist
Dr. Brian Cusack (since 06/08)

PhD students
Federico Squartini (since 10/05,IMPRS)
Paz Polak (since 10/06, IMPRS)
Yves Clement (since 10/08, IMPRS)
Philipp Messer (02/05 – 03/08)

Scientific overview

Unraveling the evolutionary forces responsible for variations of neutral substitution patterns among taxa or along genomes is a major issue for detecting natural selection within sequences. The genomes of many species (and of individuals within a species) have been sequenced today. This gives us the unprecedented opportunity for a quantitative analysis of this data with respect to evolutionary aspects. Due to advances in next generation sequencing technologies this is possible with more power and precision than before.

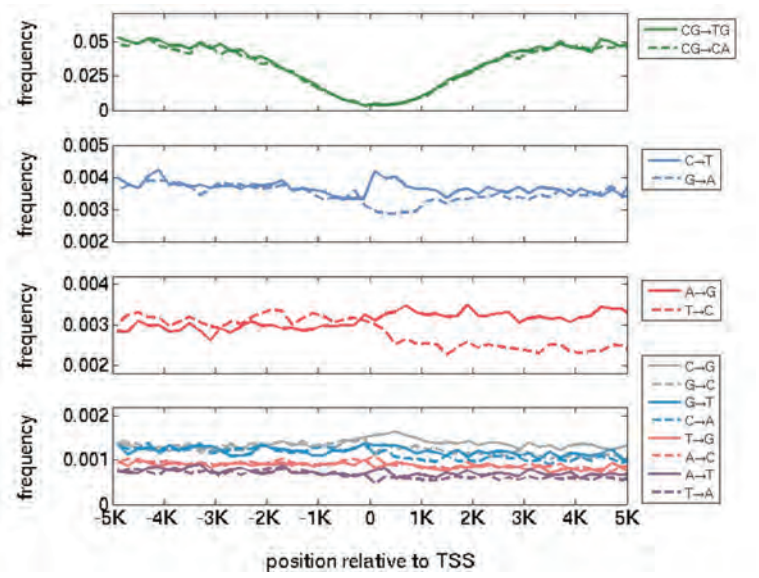
We use both comparative genomics and the study of genomic fossils (e.g. retroviral sequences, pseudo genes) to learn more about the processes that shape the human genome and the genomes of other species. We investigate processes on short length scales, e.g. nucleotide substitutions, insertions and deletions and long length scales, e.g. insertions of repetitive elements and duplications. Our analysis is complemented by studies of the mathematical underpinnings of models for nucleotide substitutions and phylogeny as well as experimental approaches to study selection *in vitro*.

Comparative analysis of nucleotide substitutions

[Clement, Polak, Arndt]

Mammalian genomes show large-scale regional variations of GC-content (the isochors), but the substitution processes at the origin of this structure are poorly understood. It has been shown that meiotic recombination has a major impact on substitution patterns in human, driving the evolution of GC-content.

But also other cellular processes have an influence on nucleotide substitutions. A regional analysis of nucleotide substitution rates along human genes and their flanking regions allowed us to quantify the effect of mutational mechanisms associated with transcription in germ line cells. Our analysis revealed three distinct patterns of substitution rates. First, a sharp decline in the deamination rate of methylated CpG dinucleotides, which is observed in the vicinity of the 5' end of genes. Second, a strand asymmetry in complementary substitution rates, which extends from the 5' end to 1 kbp downstream from the 3' end, associated with transcription-coupled repair. Finally, a localized strand asymmetry, an excess of C->T over G->A substitution in the non-template strand confined to the first 1-2 kbp downstream of the 5' end of genes. We hypothesize that higher exposure of the non-template strand near the 5' end of genes leads to a higher cytosine deamination rate.



Substitution rates in introns and in intergenic regions in the vicinity of 5' end of human genes. The panels show the estimated 12 single-nucleotide substitution rates and the CpG deamination rates in non-overlapping 200-bp-long windows along the nontemplate strand.

Recently we also established that the presence of CpG Island has an asymmetric influence on nucleotide substitution up and downstream indicative of a cellular process that starts at a CpG Islands and moves outwards beyond its 5' and 3' end.

Models of genome evolution

[Arndt, Bielow, Engleitner, de la Chaux, Messer]

In the recent past it became clear that besides nucleotide substitutions also the insertion and deletion of short pieces of DNA as well as the insertion of repetitive elements have a substantial influence on the evolution of GC isochors in mammals.

We have shown that simple expansion randomization systems (ERS) are able to generate long-range correlation of the GC content, which is one of the hallmarks of isochors. A wide range of such ERSs fall within one universality class and the characteristic decay exponent of the correlation function can easily be calculated from the rates of the underlying processes. This result gives us also a simple method to simulate long-range correlated sequences and recently we were able to quantify the influence of such correlations on the alignment statistics of sequence, which turned out to be quite substantial. Corresponding corrections should be taken into account when calculating p-values for the alignment of genomic sequences. At the basis of expansion randomization systems are processes that duplicate, insert, or delete segments of a sequence.

Comparing the genome of humans to the ones of its closest relatives, the chimpanzee and rhesus monkeys, gave us the opportunity to investigate instances of nucleotide insertions and deletions on small scales and quantify their rates in the genomic context. In the future we also want to extend our analysis and also include

insertion of repetitive elements into the vertebrate genomes. In particular we want to understand the quantitative differences in variations of the GC-content between hominoids and rodents. At the end we will generate a much richer null model of genomic evolution, especially for the evolution of promoter regions, which often include multiple binding sites for the same transcription factors.

In vitro selection

[Arndt]

The advancements of next generation sequencing technologies give us a novel tool for the quantitative analysis of Systematic Evolution of Ligands by Exponential Enrichment (SELEX) experiments. Such experiments are conducted in close collaboration by the Glökler group (Dept. Lehrach). Starting from a highly divers pool of DNA sequences ligands to particular molecules, e.g. transcription factors or other cellular relevant molecules are enriched through subsequent rounds of selection. In-house sequencing capabilities give us the opportunity to sequence the DNA pools after each round of selection. This way we are going to study the dynamics of selection for strong binding ligands in lieu of a highly diverse background of unspecific ligands. Since very high diversities can be charted using Illumina sequencing we will also be able to study non-dominant secondary clones and follow the dynamics of their frequency in the population during rounds of selections. New approaches to cluster and analyze the clonal structure of synthetic sequence pools have to be developed.

Mathematics of evolutionary models

[Arndt, Squartini]

Markov models describing the evolution of the nucleotide substitution process, widely used in phylogeny reconstruction, usually assume the hypotheses of stationarity and time reversibility. Although these models give meaningful results when applied to biological data, it is not clear if the two assumptions mentioned above hold and, if not, how much sequence evolution processes deviate from them. To this aim, we introduced two sets of indices that can be calculated from the nucleotide distribution and the substitution rates. The stationarity indices (STIs) can be used to test the validity of the equilibrium assumption. The irreversibility indices (IRIs) are derived from the Kolmogorov cycle conditions for time reversibility and quantify the degree of non-time-reversibility of a process. Computations of these indices for genomic nucleotide substitutions in *Drosophila simulans* and *Homo sapiens* reveal statistically significant deviations from the ideal case of a process that has reached stationarity and is time reversible.

Phenotypic mutations

[Arndt, Cusack]

Recent studies have hinted at the importance of “phenotypic mutations” (errors made in transcription and translation) in molecular evolution. These are thought to facilitate positive selection for adaptations that require multiple-substitutions but the generality of this phenomenon has yet to be explored.

Our research in this area focuses on the importance of phenotypic mutations to negative selection and to the maintenance of genomic robustness by selective constraint. We initially approached this in the context of Nonsense Mediated Decay (NMD)-based surveillance of human gene transcription. We have discovered a pattern of codon usage in human genes that compensates for the variable NMD efficiency by minimizing nonsense errors during transcription. Our future work will focus on whether phenotypic mutations due to other types of mis-transcription constitute a similar selective force.

Selected information

Selected publications

Polak P. and Arndt P.F. (2008): *Transcription induces strand-specific mutations at the 5′ end of human genes*. Genome Research 18 (8), 1216-23

Duret L. and Arndt P.F. (2008): *The impact of recombination on nucleotide substitutions in the human genome*. PLoS Genet 4(5), e1000071

Squartini F. and Arndt P.F. (2008): *Quantifying the stationarity and time reversibi-lity of the nucleotide substitution process*. Molecular Biology and Evolution 25(12), 2525-35

Messer P.W. and PF Arndt (2007): *The majority of recent short DNA insertions in the human genome are tandem duplications*. Molecular Biology and Evolution 24(5), 1190-7

Messer P.W., Bundschuh R., Vingron M. and Arndt P.F. (2006): *Alignment Statistics for Long-Range Correlated Genomic Sequences*. Lecture Notes in Computer Science 3909, 426-440

Selected invited talks

Arndt P.F. *Parity Rules and their Violation in Molecular Biology*, BMS Days 2009, Berlin, 16. – 17.2.2009

Arndt P.F. *The Evolutionary Processes that shape the human genome,* Kavli Institute for Theoretical Physics, Santa Barbara, 18.3. 2007

Arndt P.F. *The Evolutionary Processes that shape the human genome,* Center for Theoretical Biological Physics, San Diego, 9.3. 2007

Arndt P.F. *Substitution pattern of mammalian transposable elements*, 1st International Conference on the Genomic Impact of Eukaryotic Transposable Elements, Asilomar, 31.3. – 4.4.2006

Work as scientific editor

- Journal of Statistical Mechanics – Theory and Experiment

Work as scientific referee

Peter Arndt serves as scientific referee for the following journals and conference series: Nature, Proceedings of the National Academy of Sciences, Molecular Biology and Evolution, Journal of Molecular Evolution, Biophysical Journal, Nucleic Acids Research, Europhysics Letters, BioSystems, Physical Review Letters, Research in Computational Molecular Biology (RECOMB), Gene, Genetics.

In addition, Peter Arndt serves as scientific referee for the following institutions: National Science Foundation, National Institute of Health, German Israeli Foundation.

Appointments of former members of the Group

Philipp Messer: HFSP fellow at the Biology Department, Stanford University

Nicole de la Chaux: Doctoral Student, University of Zurich

Teaching activities

Winter 05/06; Summer 08: Seminar on Population Genetics

Winter 06/07: Lecture and Tutorials on Theoretical Genetics

Summer 07: Seminar on the Evolution of Sex

Winter 07/08; 08/09: Lecture and Tutorials on Population Genetics

Organization of scientific events

Otto Warburg International Summer School and Workshop on Regulatory (Epi-)Genom-ics, Harnack House Berlin, August 29 - September 6, 2009

Otto Warburg International Summer School and Workshop on Computational Systems Biology, Harnack House Berlin, August 27 - September 5, 2007

Symposium on the Evolution of Sex, XI Congress of The European Society for Evolutionary Biology, Uppsala, August 20-25, 2007

Otto Warburg International Summer School and Workshop on Evolutionary Genomics, Berlin, August 29 - September 8, 2006

Gene Structure & Array Design Group

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Anne-Katrin Emde (since 10/08, IMPRS)
Shen Lin (since 01/09)
Helge Roider (10/05 – 07/09)

Scientific programmers
Sean O'Keeffe (since 11/2006)
Ramu Chenna (12/2006 – 11/2009)

Scientific overview

The group focuses mainly on the analysis of genome-wide expression data to decipher mechanisms driving tissue-specific activation/repression of genes or distinct transcripts. As a complement we provide bioinformatics support related to the design of experiments and develop tools to facilitate processing/analysis of expression studies based on high-throuput technologies like microarrays or next-generation sequencing.

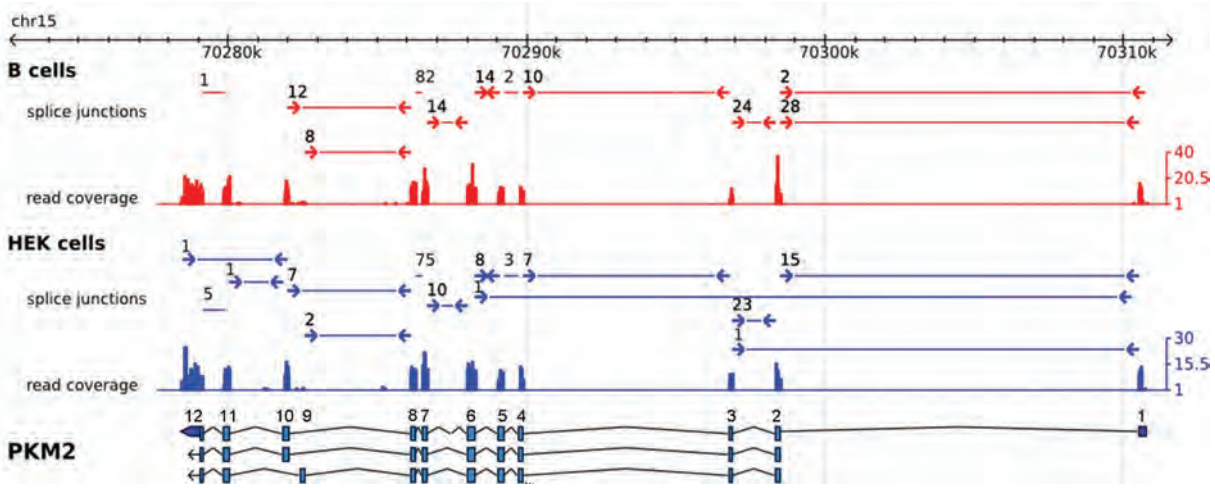
Prediction of alternative transcripts

[Gupta, Haas]

The huge variety of gene products expressed in an organism is to a major extend caused by differential usage of exons leading to alternative transcripts encoded by a single gene. In general, different mechanisms contribute to the formation of alternative transcript isoforms. While alternative splicing *via* the spliceosome causes variations in the usage of internal exons, alternative promoters and alternative polyadenylation will change the usage of alternative first or last exons, respectively.

By integrating public data sets targeted to determine transcriptional start sites (TSS) like CAGE and full-length clone data (DBTSS) together with Ensembl/RefSeq transcript annotations and our EST-based transcript predictions, we generated a comprehensive set of potential alternative transcriptional start sites. Our analysis revealed that most genes usually express a single, dominant transcript, whereas alternative transcripts are often expressed on lower levels. Although methods like

CAGE are tailored to detect TSSs, still the traditional data sets uniquely contribute a significant number of TSSs to the overall set. Such a set of TSSs can be used to select a representative transcript per gene based on expression rather than on location, which might be more appropriate when studying gene regulation. Furthermore, the integration of TSS predictions allows fine-tuning of TSS localization e.g. important in cases where only small proximal promoter regions have to be analyzed. The predicted TSSs are visualized in our Promotion Genome Browser (promotion.molgen.mpg.de) together with additional genomic features dedicated to facilitate the interpretation and integration of important aspects associated with gene regulation (e.g. sequence conservation, transcription factor binding affinities (TRAP), EST data (GeneNest)).



Alternative splicing of the PKM2 gene. Three transcripts annotated in ENSEMBL are shown next to the gene name, exons numbered. The read coverage is shown for each exon (blue for HEK and red for B cells). Splice-junction reads are shown as arrows; the numbers above the arrows represent the number of reads at junctions. Two different sequenced junctions connecting to either exon 9 or exon 10 reflect alternative transcripts with mutually exclusive exons in HEK and in B-cells.

Tissue-specific gene regulation

[Roider, O'Keeffe, Haas]

Regulation of gene expression is mainly controlled by chromatin modifications and the activity of specific transcription factors acting either on promoters close to the transcriptional start, or on distant enhancer elements. Since many genes are involved in a discrete biological context, the use of such contextual information is crucial to successfully unravel regulatory relationships between genes and transcription factors (TF). We therefore categorize genes according to their significance of expression in a certain tissue based on the statistical evaluation of EST data (T-STAG) or DNA-microarray/RNA-Seq expression measurements in a number of organisms (human, mouse, sheep etc.). Given the TSSs of such co-expressed genes we rank their potential proximal promoter regions by DNA-binding affinity of known transcription factors using our method TRAP. In order to detect candidate regulatory TFs we developed the method PASTAA, which iteratively tests for genes significantly ranked by tissue-specific expression as well as by high TF binding affinity. This way we were able to computationally predict a large number of functional TF-tissue associations well supported by literature. Intriguingly, we found that TFs predicted to regulate genes expressed in a certain tissue are frequently themselves significantly expressed in the respective tissue. In line with results revealed in another computational study performed in our department we observed potential auto-regulatory loops for many of the TFs involved in tissue-specific gene regulation.

In a subsequent study we could show that the success in predicting functional associations is strongly related to the CpG content of the promoters investigated. Functional predictions for transcription factors with respect to tissue-specificity are far more successful for promoters with low CpG content. However, even in high CpG promoters we could find functionally plausible TF-tissue association, e.g. NRSF-brain, which could be hardly detected when analyzing the full set of promoters. Thus, our computational analysis highlights the importance of categorizing promoters into high and low CpG groups since those promoters may be regulated by alternative biological mechanisms.

Next generation sequencing

[Richard, Emde, Schulz, O'Keeffe, Chenna, Haas]

The recent advances in next-generation sequencing technologies (NGS) provide the opportunity to tackle biological questions on genome scale in an unprecedented quality. However, the huge amount of data generated using these technologies requires the development of new algorithms to handle the data efficiently but also to analyse this new type of sequence information.

In this context, we were among the first studying the performance of next-generation sequencing in transcriptome sequencing (RNA-Seq) of two human cell lines. In collaboration with the group of Marie-Laure Yaspo we could show that NGS clearly outperforms state-of-the-art microarray technology in terms of sensitivity and noise thus revealing a more complete picture of the transcriptome. Although the transcriptomes in B- and HEK-cells are well studied we were able to discover extensions of exonic regions and a limited number of potential, so far unknown exons.

While the basic mapping of sequencing reads mainly provides information about genomic regions that are transcribed, details about differential splicing has to be determined by those sequencing reads mapping on exon junctions. We therefore generated an artificial set of all exon-junctions of a gene based on our comprehensive set of gene structures derived from EST data and the Ensembl database. The mapping of sequence reads to these junctions revealed a large number of alternative splicing events even with a relatively low sequencing depth.

In light of the steadily increasing sequence output of NGS we are currently implementing a generalised computational pipeline comprising statistical measures for quality control of sequencing runs as well as improved methods for efficient mapping of reads. In this context we are involved in the development of RazerS, a tool to perform read mapping allowing for short insertion/deletions, which is an important feature with respect to future applications aiming at the discovery of disease causing mutations by deep genomic sequencing.

Selected information

Selected publications

Roider,H.G., Lenhard,B., Kanhere,A., Haas,S.A., Vingron,M. (2009). *CpG-depleted promoters harbor tissue-specific transcription factor binding signals - implications for motif overrepresentation analyses.* Nucleic Acids Res., in press

Roider,H.G., Manke,T., O'Keeffe,S., Vingron,M., Haas,S.A. (2009). *PASTAA: identifying transcription factors associated with sets of co-regulated genes*. Bioinformatics 25(4):435-442

Sultan, M., Schulz, M.H., Richard, H., Magen, A., Klingenhoff, A., Scherf, M., Seifert, M., Borodina, T., Soldatov, A., Parkhomchuk, D., Schmidt, D., O'Keeffe, S., Haas,S., Vingron,M., Lehrach, H., Yaspo, M.L. (2008). *A Global View of Gene Activity and Alternative Splicing by Deep Sequencing of the Human Transcriptome*. Science 321 (5891): 956-960

Oberthuer, A., Berthold, F., Warnat, P., Hero, B., Kahlert, Y., Spitz, R., Ernestus, K., Koenig, R., Haas, S., Eils, R., Schwab, M., Brors, B., Westermann, F. and Fischer, M. (2006). *Gene-expression based classification of neuroblastoma patients using a customized oligonucleotide-microarray outperforms current clinical risk stratification*. J. Clin. Oncol. 24:5070-5078

Hecht, H., Kuhl, H., Haas, S.A., Bauer, S., Poustka, A.J., Lienau, J., Schell, H., Stiege, V., Seitz, V., Reinhardt, R., Duda, G.N., Mundlos, S. and Robinson, P.N. (2006). *Gene Identification and Analysis of Transcripts Differentially Regulated in Fracture Healing by EST Sequencing in the Domestic Sheep.* BMC Genomics, 7:172.

Selected invited talks

Haas S.A. *Lessons learned from 2nd Generation Sequencing of entire Transcriptomes,* Sixth Annual Meeting of the Italian Bioinformatics Society, Genoa, Italy, 18.-20.03.2009

Haas S.A. *Deep Sequencing of the Transcriptome of two Human Cell Lines,* ESF Symposium: Computational Challenges of the Next-Generation DNA Sequencing, Uppsala, Sweden, 15.-17.01.2009

Haas S.A. *Deep Sequencing of the Transcriptome of two Human Cell Lines,* Workshop: Parallel Sequencing, Basel, Switzerland, 02.10.2008

Richard H. *Estimating genetic richness in EST libraries,* JOBIM conference, Marseilles, France, 10.-12.07.2007

Haas S.A. *Integrating ESTs into a comprehensive Picture of Alternative Transcriptional Start Sites,* Symposium: Alternative Transcript Diversity, Heidelberg, Germany, 21.03.2006

Work as scientific referee

Stefan Haas serves as scientific referee for the following journals: BMC Genomics, BMC Bioinformatics, Bioinformatics, Genome Research, Genome Biology, Nucleic Acids Research.

Teaching activities

EMBO Course: *Next Generation Sequencing: ChIP-seq and RNA-seq*, 02/09

Public relations

Lange Nacht der Wissenschaft (06 – 09)

Tag der Talente, BMBF (09/08)

Presentations for High-School classes (07 – 09)

Mechanisms of Transcriptional Regulation Group

(Established: 09/2009)

Head:
Sebastiaan H. Meijsing (since 09/09)
Phone: +49 (0)30 8413-1176
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Email: meijsing@molgen.mpg.de

Technician
Edda Einfeldt (since 09/09)

Scientific overview

Recent technological advances facilitate the rapid genome-wide identification of transcription factor binding sites. *The long-term goal of our research is to understand the mechanisms by which these binding sites specify where and when genes are expressed.* Metazoan nuclear hormone receptors regulate the expression level of target genes to orchestrate development and to respond to changes in their environment. Target genes are not simply turned on or off, but instead their expression is fine-tuned to meet the needs of a cell. Hence mechanisms exist in metazoans that specify not only where and when genes are expressed but also at which level. To study gene regulation, we investigate transcriptional regulation by the glucocorticoid receptor (GR), a member of the steroid hormone receptor family. Upon binding endogenous hormones called glucocorticoids, GR translocates from the cytoplasm, where it is inactive, to the nucleus where it orchestrates the expression of a cell- or tissue-specific subset of target genes (Fig. 1). For example, in immune cells, GR-target genes mediate the anti-inflammatory effects of glucocorticoids, whereas in liver glucocorticoids affect metabolism *via* a different set of target genes to stimulate gluconeogenesis. An attractive feature of GR to study gene regulation is that its activity can simply be turned on or off by the addition or removal of hormone. This on/off switch facilitates the identification of genes with changed expression levels upon hormone treatment by a single experiment using gene ex-

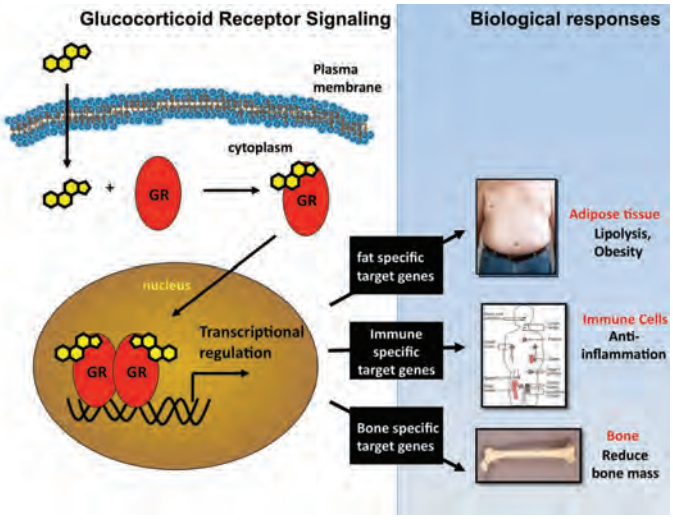


Figure 1: Transcriptional regulation by the Glucocorticoid receptor. Upon hormone binding, the receptor translocates into the nucleus where it activates a tissue specific set of target genes that in turn specify the biological response to hormone.

pression analysis such as microarrays or RNA-sequencing. In parallel experiments, Chromatin immunoprecipitation (ChIP) experiments reveal the genomic locations of GR DNA binding to identify primary target genes. Combining the expression and recruitment information in a single cell type aided by bioinformatical approaches yield leads for mechanisms that specify which genes are regulated and the magnitude of regulation. Similarly, by comparing cell types derived from different tissues, candidate mechanisms contributing to tissue specific regulation will be identified and studied.

Role of the DNA-sequence of binding site

The glucocorticoid receptor regulates target genes by associating with specific DNA binding sites, the sequences of which differ between genes. There is a paradox in how transcription factors, such as GR, bind DNA. On one hand binding sites can typically tolerate variation in its DNA-binding site, while still binding and activating genes (Fig. 2A). On the other, these sites are often conserved at individual genes, suggesting that the exact sequence is important for proper gene regulation (Fig. 2B). This paradox challenges the paradigm that transcription factor binding sites are simple docking sites. Using bioinformatical, structural, biochemical, and cell-based assays we found that GR binding sequences (GBSs), differing by as little as a single base pair, differentially affect GR conformation and regulatory activity. For example, we found GBS-specific requirements for cofactors and receptor domains, and GBS specific levels of transcriptional activation. Comparison of high resolution crystal structures we obtained, revealed GBS-specific GR conformations. Specifically, we observed alternative conformations of the lever arm, a domain connecting the DNA recognition helix and the dimer interface. In conclusion, we propose that DNA is a sequence-specific allosteric ligand of GR that tailors the activity of the receptor toward specific target genes.

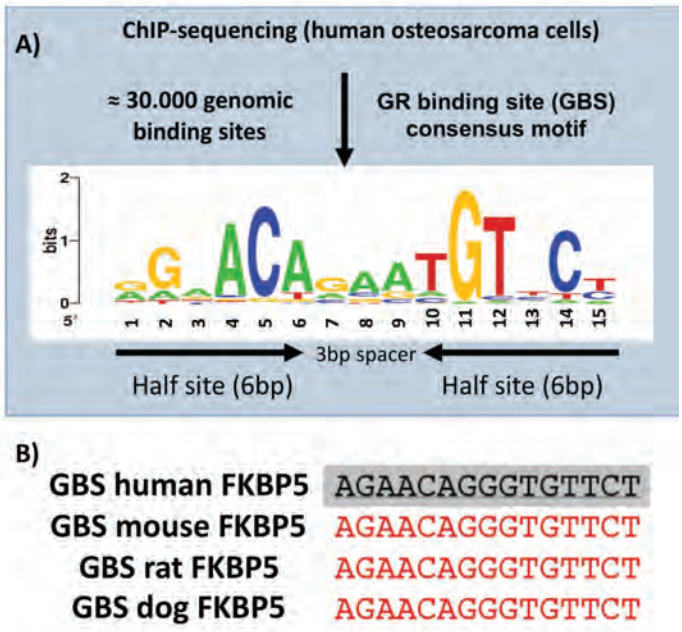


*Figure 2: **(A)** Binding site motif for the glucocorticoid receptor binding site based on binding sites identified by ChIP-sequencing. Height of nucleotide indicates sequence conservation at that position. **(B)** GR binding sequences are often highly conserved. Shown is an example for a target gene associated GBS from human, mouse, rat and dog.*

Outlook

One goal is to understand how GBS sequences direct distinct modes of transcriptional regulation and their role in spacio-temporal control of gene regulation. We will approach this problem using several strategies. One approach will be done in collaboration with Ulrich Stelzl from the Otto Warburg Laboratories using high throughput yeast two-hybrid screening to identify GBS specific cofactors. Furthermore, previous studies were focused on the role of the GBS in isolation, however, for GR-target genes the GBS typically collaborates with other *cis*-acting elements. To study this interplay we will flank GBS sequence variants with naturally occurring *cis*-elements to determine how several signals are integrated to specify the transcriptional output.

Alternative splicing generates a naturally occurring splice variant GRγ that inserts an arginine in the lever arm, the region that adopts alternative conformations depending on the sequence of the binding site. The inserted arginine does not affect DNA binding affinity and accordingly, its structure is superimposable on that of GRα the predominant splice isoform, except for the lever arm region. Hence, the extra amino acid specifically disrupts the lever arm while leaving the remainder of the receptor intact making it a great tool to understand the role of the lever arm in gene regulation. We assayed gene expression by microarray analysis and GR recruitment by ChIP-sequencing. Comparison of expression and recruitment data for GRα and GRγ indicates that insertion of the arginine influences transcriptional regulation at several steps and current efforts are aimed to identify the molecular mechanisms underlying the isoform specific regulation.
Furthermore, we plan to use the model organism *C. elegans* in unbiased approaches to identify novel factors and pathways that specify where and when target genes of the nuclear hormone receptor daf-12, are expressed and their role in physiology. The combination of these approaches allows us to determine how effectors of spacio-temporal gene regulation in whole animals act at individual genes and vice versa, how regulatory mechanisms acting at individual genes influence processes in whole animals.

Selected information

Selected publications

Meijsing SH, Pufall MA, So AY, Bates DL, Chen L, Yamamoto KR (2009). *DNA binding site sequence directs glucocorticoid receptor structure and activity*. Science 17:407-410.

Meijsing SH, Elbi C, Luecke HF, Hager GL, Yamamoto KR (2007). *The ligand binding domain controls glucocorticoid receptor dynamics independent of ligand release*. Mol Cell Biol. 27:2442-2451.

Wang JC, Shah N, Pantoja C, Meijsing SH, Ho JD, Scanlan TS, Yamamoto KR (2006). *Novel arylpyrazole compounds selectively modulate glucocorticoid receptor regulatory activity*. Genes & Dev. 15:689-699.

Selected invited talks

2008 Research in Progress series (UCSF).

2008 Keystone meeting: Nuclear Receptors: Steroid Sisters, Whistler, Canada.

2009 Otto Warburg International Summer School and Workshop on Regulatory (Epi)Genomics. Berlin, Germany.

2009 EMBO meeting: Nuclear receptors: From molecular mechanism to molecular medicine. Dubrovnik, Croatia.

Awards

2004 - 2007: Leukemia & Lymphoma Society Special Fellowship

2006 Keystone symposia Scholarship/ Travel award

2008 Keystone symposia Scholarship/ Travel award

Teaching activities

Teaching: Biochemistry/Pharmacology course for UCSF Medical students (2006).

External funding

2004 - 2007: Leukemia & Lymphoma Society Special Fellowship

Algorithmics

(Established: 05/2002 - 06/2009)

Head
Dr. Alexander Schliep (05/02-06/09)
Email: schliep@cs.rutgers.edu

PhD students
Wasinee Rungsarityotin (03-07)
Ivan G. Costa (04-08)
Benjamin Georgi (05-09)
Ruben B. Schilling (07-09)

Scientific overview

Our research focuses on methods from mathematics and statistics which are crucial for answering relevant biological questions. An emphasis is put on analyzing high-dimensional and heterogeneous data, such as time-courses or imaging data.

Clustering heterogeneous data

Detecting relevant groups of co-expressed genes as a foundation for building more detailed regulatory networks is still one of the central unsolved problems. The massive amounts of data created in molecular biology, the high error rates and the question of how to combine several heterogeneous data sets, pose challenges for research. We have contributed clustering methods based on mixture models, novel component models for specific data types. We also were the first to propose the framework of partially supervised learning or constrained clustering to simultaneously analyze two different sets of complimentary data (data fusion).
We studied gene regulation during early Drosophila development. Gene expression measurements during the development of the fly *Drosophila melanogaster* are routinely used to find functional modules of temporally co-expressed genes. Complimentary large data sets of in situ RNA hybridization images for different stages of the fly embryo elucidate the spatial expression patterns. Using a semi-supervised approach, constrained clustering with mixture models, we can find clusters of genes exhibiting spatio-temporal similarities in expression, or syn-expression. The temporal gene expression measurements are taken as primary data for which pairwise constraints are computed in an automated fashion from raw *in situ* images without the need for manual annotation. We investigate the influence of these pairwise constraints in the clustering and discuss the biological relevance of our results. Spatial information contributes to a detailed, biological meaningful

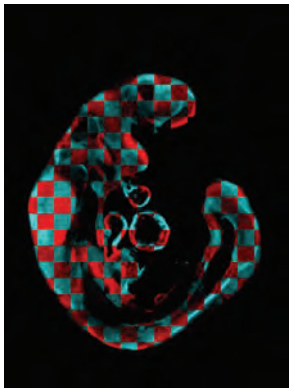


Figure 1: Two registered mouse embryos (cyan and red). Registration of in-situ stained mouse embryos facilitates the direct analysis of spatial gene expression patterns.

analysis of temporal gene expression data. In cooperation with the department of Developmental Genetics (B. Herrmann), we investigate the necessary image registration to deal with 3D *in situ* images of mouse embryos in a similar fashion (Figure 1).
Another model system we investigated is the lymphoid system. Gene expression measured in lymphoid cells in several distinguishable developmental stages helps in the elucidation of underlying molecular processes, which change gradually over time and lock cells in either the B cell, T cell or Natural Killer cell lineages. Large-scale analysis of these gene expression trees requires computational support for tasks ranging from visualization, querying, and finding clusters of similar genes, to answering detailed questions about the functional roles of individual genes.
We developed the first statistical framework designed to analyze gene expression data as it is collected in the course of lymphoid development through clusters of co-expressed genes and additional heterogeneous data. We introduce dependence trees for continuous variates, which model the inherent dependencies during the differentiation process naturally as gene expression trees. Such trees can have their structure estimated from the data or derived from expert knowledge. Several trees are combined in a mixture model to allow inference of potentially overlapping clusters of co-expressed genes. Computational results for several data sets from the lymphoid system demonstrate the relevance of this framework. We recover well-known biological facts and identify promising novel regulatory elements, including putative microRNAs, of genes and their functional assignments.
A complimentary technique for fusing heterogeneous datasets is based on a naive Bayes approach coupled with an innovative way of minimizing model complexity.
CSIMixture (Context-specific independence mixture) modeling for sequence motifs provides a more physically realistic description transcription factor binding site motifs. Previous studies showed that for transcription factors which bind to divergent binding sites, mixtures of multiple PWMs increase performance. However, estimating a conventional mixture distribution for each position will in many cases cause overfitting. We circumvent this problem by employing a context-specific independence (CSI) framework. In CSI mixtures model complexity is automatically adapted to match the variability found in a given data set.
Another application of the CSI mixture framework is clustering of protein families for simultaneous inference of subgroups and prediction of specificity determining residues based on multiple sequence alignments of protein families. Furthermore, CSI mixtures can be used for the joint analysis of genotype and phenotype data from ADHD-patients, or other complex diseases, to simultaneously identify disease subgroups and relevant genotypic features. Typically, in such applications, none of the variables will be fully informative with respect to distinguishing between clusters, which makes an a-priori feature selection infeasible.

Optimizing experimental designs

We developed the first linear time algorithms for designing DNA tiling arrays which find global optima, balancing uniformity of hybridization conditions, avoidance of cross-hybridization and probe placement. This has been used by several experimental groups interest in custom tiling arrays, primarily for bacteria. We also extended the generation of candidate probes, to include more realistic aspects

of hybrid formation in a computationally feasible manner. This is in stark contrast to the Blast-based approaches dominantly used in the field.
The same underlying hybridization reactions underlying gene expression analysis using DNA-Microarrays can also be used to infer presence and absence of biological agents, say viruses or bacteria, in a sample from the hybridization pattern of oligonucleotide probes to genomic DNA of the agents. This detection is crucial for epidemiological studies and vaccine design when used for detection of viral subtypes in Influenza or HIV, in food and safety control, in studies of microbial diversity or, particularly in the USA, in bio-threat reduction.
Due to close evolutionary relationships between agents oligonucleotide probes uniquely identifying agents cannot be found in many applications; consider identifying all Influenza virus subtypes. Nevertheless, even non-unique probes can be used for the detection with great success - indeed, in simulations, we can correctly identify previously unknown agents in biological samples with a success rate of up to 70% - as there is a connection to the mathematical field of statistical group testing. It bridges across combinatorial design theory, Bayesian statistics and Markov Chain Monte Carlo methods. The distilled computer science, non-unique probe sets and generalized group testing, which we first formulated directly based on the biological application at hand, has received a great deal of interest in the theoretical computer science literature.

Selected information

Selected publications

I.G. Costa, S. Roepcke, C. Hafemeister, A. Schliep (2008). *Inferring differentiation pathways from gene expression*. Bioinformatics (Proceedings of Intelligent Systems for Molecular Biology, ISMB 2008) 24(13):i156-64, 2008.

A. Schliep, R. Krause (2008). *Efficient algorithms for the computational design of optimal tiling arrays*. IEEE/ACM Trans Comput Biol Bioinform. 2008 Oct-Dec; 5(4):557-67 (Invited paper selected from WABI 2007)

I.G. Costa, R. Krause, L. Opitz, A. Schliep (2007). *Semi-supervised learning for the identification of syn-expressed genes from fused microarray and in situ image data*. BMC Bioinformatics 8(Suppl 10):S3, 2007

A. Schliep, S. Rahmann (2006). *Decoding non-unique oligonucleotide hybridization experiments of targets related by a phylogenetic tree*. Bioinformatics (Proceedings of Intelligent Systems in Molecular Biology, ISMB 2006), 22 (14): e424-e430, 2006

Selected invited talks

Group testing DNA Micro-arrays for detection of biological agents. DIMACS workshop on combinatorial group testing, 19.05.06

Treeprobes: Grouptesting biological agents related by phylogenetic trees. Los Alamos National Laboratory, 24.05.06.

Mixture models for heterogeneous biological data. Symposium on Bioinformatics and Biomathematics, Centrum voor Wiskunde en Informatica (CWI), Amsterdam, 06.04.07

Detecting functional modules from heterogenous mass data. College of Computing, Georgia Tech, 04.04.08

Detecting functional modules in heterogeneous biological data. Institute of Genetics and Molecular and Cellular Biology, Strasbourg, 02.07.08

Open access activities

Software packages implementing methods we develop are made available under open source licenses (GPL or LGPL), which guarantee open access and the freedom to extend the softwares: This includes GHMM, GQL, PyMix, Tileomatic, Gato and Mix DTrees (see http://algorithmics.molgen.mpg.de/Software/)

Work as scientific editor

- Associate Editor for Discrete Mathematics, Algorithms and Applications

Work as scientific referee

Alexander Schliep serves as scientific referee for the following journals and conference series: Bioinformatics, BMC Bioinformatics, Proteins, Functional and Integrative Genomics, Springer Verlag, IEEE/ACM Transactions on Computational Biology and Bioinformatics, Pattern Analysis and Applications, Journal of the American Statistical Association, Bulletin of Mathematical Biology, Journal of Bioinformatics and Computational Biology, Journal of Computational Statistics and Data Analysis, RECOMB, WABI, ISMB, GfKl, IFCS, ECCB.

In addition, Alexander Schliep serves as scientific referee for the following institutions: European Commission (IST - Future & Emerging Technologies), Netherlands Organization for Scientific Research (NWO, Horizon programme), US-Israel Binational Science Foundation.

Service to the scientific community

A. Schliep has been a member of the following program committees: NIPS workshop on machine learning in bioinformatics (2004-2009), Whistler BC, APBC Asia-Pacific Bioinformatics conference (2006), IAPR Workshop on Pattern Recognition in Bioinformatics (2006, 2007), German Conference on Bioinformatics (2007)

Appointments of former members

Alexander Schliep: Associate Professor, Dept. of Computer Science and BioMaPS Institute for Quantitative Biology, Rutgers University, New Jersey

Ivan G. Costa: Assistant Professor, Center of Informatics, Federal University of Pernambuco, Recife, Brasil

Benjamin Georgi: Postdoc with Maja Bucan, Department of Genetics, University of Pennsylvania

External funding

DAAD: *Meta-Learning for Selection and Combination of Clustering Algorithms Applied to Temporal Series*. With Universidade Federal de Pernambuco, Recife, Brasil.

Teaching activities

Winter 05/06: *Algorithmen der Bioinformatik*. FU Berlin (lecture course, one unit only); Algebraic statistics. FU Berlin (seminar course).

Winter term 05/06; 06/07: *Angewandtes Data Mining*. FU Berlin (course design, two week full-time lectures and labs).

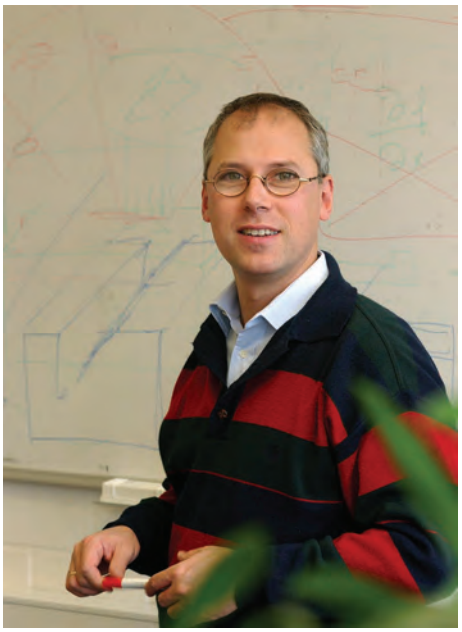
Organization of scientific events

Section chair Genome and DNA Analysis, GfKl meeting 2006 in Berlin.

Organizer of a Dagstuhl workshop on Group Testing in the life sciences, Juli 2008.

Transcriptional Regulation Group

(Established: October 2000)



Head
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Scientists
Thomas Manke (since 03/05)
Christine Steinhoff (since 08/05)
Ho-Ryun Chung (since 06/05)
Tomasz Zemojtel (since 02/04)
Szymon Kielbasa (since 03/05)
Julia Lasserre (since 04/08)
Anirban Banerjee (since 11/07)
Sarah Behrens (since 10/08)
Roman Brinzanik (since 11/07)
Andrew Hufton (since 11/06)
Morgane Thomas-Chollier (since 04/09)
Lloyd Demetrius (03-09)
Aditi Kanhere (09/05-05/08

PhD Students
Marcel Schulz (since 09/05)
Ewa Szczurek (since 10/06)
Marta Luksza (since 10/09)
Akdes Serin (since 08/06)
Rosa Karlic (since 10/07)
Jonathan Goeke* (since 09/2007)
Holger Klein (05/03-10/09)
Hannes Luz (04/0 -12/06)
Abha Singh Bais (06/03-05/07)
Utz Pape (11/0109/04)

Visitors
Prof. Dr. Ina Koch (since 06)
Dr. Pawel Gorecki (10/06-09/07)

Introduction

The theoretical study of transcriptional regulation has entered a new era with the availability of many fully sequenced genomes in conjunction with a number of new, high-throughput experimental techniques for the study of protein-DNA binding. The gene regulation group focuses on the delineation of regulatory motifs and interactions based on the integration and analysis of this variety of information sources.

Scientific overview

From CORG to TRAP: Transcription factor binding site prediction

(Thomas Manke, Helge Roider, Aditi Kanhere, Utz Pape)
A transcription factor tends to bind to particular DNA patterns which can be summarized by so-called Positional Weight Matrices (PWMs). After having explored the power and problems of matching PWMs to sequence in the CORG-database,

we have developed an alternative biophysics-inspired approach. This "TRAP" method (for Transcription Factor Affinity Prediction) transforms the match between a sequence and a pattern into a binding probability and integrates over the region of interest, say a promoter region. The TRAP method has been validated by comparison to large scale DNA binding experiments (ChIP-chip and ChIP seq experiments) and shown to be successful in predicting novel target genes of transcription factors. Based on a statistical normalization of the affinity scores, the most likely binding factors to a particular promoter can be inferred. Together with the group of Stefan Haas, TRAP has further been utilized to recognize transcription factor binding sites which are over-represented in co-expressed or tissue-specific groups of genes. Ongoing work applies TRAP for predicting possible effects of regulatory SNPs. It has also been applied in the context of deriving gene regulatory networks.
Estimating statistical significance of possible findings is crucial in the analysis of large data sets. To this end, we have derived probabilistic descriptions of the occurrence of hits to PWMs and of the distribution of TRAP scores. Significant efforts have gone into the development of measures of similarity among transcription factor binding sites, which in turn has proven instrumental in computing the probability of observing combinations of binding sites in a regulatory region, thus providing an instrument to study combinatorial regulation.

Epigenetic regulation

(Ho-Ryun Chung, Rosa Karlic, Julia Lasserre, Irit Gat-Viks)
Experimental progress over recent years has driven home the point, that in eukaryotes, and in particular in mammals, transcription factors are not the only regulators of gene expression. Chromatin structure, histone modifications, and DNA methylation also play vital roles or are at least correlated to expression status. Our own interest in this epigenetic level of regulation focuses on the question, in how far the DNA sequence can provide us with information not only about transcription factor binding sites, but also about, e.g., chromatin structure. While the sequence dependence of nucleosome positioning is still under debate, one does see a strong division of promoters into those with high vs. low contents of CpG dinucleotides. We have recently published that this distinction governs the localization and type of transcription factor binding sites, and are currently working on establishing that histone modification patterns also depend on these sequence features.

Evolution of regulation

(Tomasz Zemojtel, Szymon Kielbasa, Sarah Behrens, Andrew Hufton, Morgane Thomas-Chollier)
While protein evolution is nowadays generally described by a Markov process on the sequence positions with selection acting on the level of protein function, the evolution of regulatory DNA sequences is still badly understood. In collaboration with the Evolutionary Genomics Group of Peter Arndt, we have been studying the influence of the Cyotsine deamination on the appearance of binding sites. A mutation of a C in the context of a CpG dinucleotide due to deamination is much more likely than other mutations. A careful inspection of Alu repeats has shown that these transposable elements carry possible predecessors of binding sites, which are moved around the genome in the course of evolution, bearing the potential to become functional binding sites upon deamination. In this context we are now systematically investigating the role of transposable elements and numerous types of transcription factor binding sites as a possible source of novel binding sites in evolution. In contrast to this mechanism, studies in the time it would take for a binding site to evolve purely by point mutations indicate that this is not flexible

enough a process to explain regulatory evolution. This theoretical work is further complemented by studies in ancient conserved elements in collaboration with the Poustka/Panoupoulou group (Dept. Lehrach) and studies in the evolution of Hox genes.

Gene networks

(Ewa Szczurek, Thomas Manke, Anirban Banerjee, Lloyd Demetrius, Roman Brinzanik, Utz Pape)

Several projects, many of them in collaboration with experimentalist, try to delineate regulatory networks or study the general features of biological networks. In collaboration with C. Sers (Charité) we are studying the regulatory cascade downstream of the ras-triggered MAP kinase pathway. Gene regulatory networks in heart development were the topic of a collaboration S. Sperling (Dept. Lehrach). In a collaborative project with other Max Planck Institutes we are working on the delineation of regulatory networks integrating data from metabolomics and transcriptomics. The analysis of gene networks has also led us to propose an algorithmic framework to predict most informative experiments to elucidate regulatory dependencies.

Following their earlier work on the evolution of complex networks, Lloyd Demetrius, Thomas Manke and Anirban Banerjee have continued to develop a graph-theoretical framework for the characterisation of biological networks. Applying an entropic formalism to large-scale protein interaction data, they investigated the relationships between the essentiality of a protein and its overall position in the molecular networks of yeast and nematode worm. In numerical studies on model networks they found that network entropy correlates positively with many heuristic measures of structural and dynamical robustness of networks, such as the percolation threshold and mixing rates. This approach has been complemented by work on the graph spectrum as an alternative characterisation of biological networks. The normalized graph Laplacian spectrum does not only provide insights into the modular organisation of networks, but also helps to measure the distance between networks with different sizes (cooperation with J. Jost, MPI-MIS Leipzig). Lloyd Demetrius has continued his work on ageing models, proposing that differences among organisms in the rate of ageing and life span are due to differences in metabolic stability, rather than differences in metabolic rate. J. Adjaye (Dept. Lehrach) provided experimental evidence in support of this theory.

Collaborations

In addition to MPI-internal collaborations like the ones mentioned above, group members have collaborations within Berlin or Germany, as well as internationally. Frequently these collaborations will be in the context of a DFG-, BMBF- or EU-grant. While within Berlin we are closely cooperating with the FU bioinformatics group (K. Reinert) and with experimentalists at Charité (C. Sers), work together with A. Nordheim from Tübingen on SRF regulated genes has been very successful, too. On an international level, fruitful cooperation with B. Lenhard (Bergen, Norway), J. Tiuryn (Warsaw), E. Birney (Hinxton), and Fengzhu Sun (Los Angeles) need to be mentioned.

Selected information

Selected publications

Roider HG, Manke T, O'Keeffe S, Vingron M, Haas SA (2009). *PASTAA: identifying transcription factors associated with sets of co-regulated genes*. Bioinformatics 25(4): 435-442

Stritt C, Stern S, Harting K, Manke T, Sinske D, Schwarz H, Vingron M, Nordheim A, Knöll (2009). *Paracrine control of oligodendrocyte differentiation by SRF-directed neuronal gene expression.* Nat Neurosci. 12(4):418-27

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Zemojtel T, Kielbasa SZ, Arndt PF, Chung HR, Vingron M. (2008). *Methylation and deamination of CpGs generate p53-binding sites on a genomic scale.* Trends in Genetics 25(2):63-66

Roider HG, Kanhere A, Manke T, Vingron M (2007). *Predicting transcription factor affinities to DNA from a biophysical model.* Bioinformatics 23(2): 134–141

Manke T, Demetrius L, Vingron M (2006). *An entropic characterization of protein interaction networks and cellular robustness.* J R Soc Interface 3(11): 843-50

Demetrius L. (2006). *Aging in mouse and human systems: a comparative study*. Ann N Y Acad Sci. 1067:66-82 (review)

Selected invited talks (Martin Vingron)

- Heidelberg Spring Workshop on Cancer Biology, 04/09
- Center for Algorithmic and Systems Biology, CASB-20 meeting, San Diego, 03/09
- First RECOMB Satellite Conference on Bioinformatics Education, 03/09
- Asia Pacific Bioinformatics Conference, Beijing, 01/09
- NGFN Plus and NGFN Transfer, München, 12/08
- Mini EURO Conference on Computational Biology, Bioinformatics and Medicine, Rome, Italy, 09/08
- 6th Georgia Tech-Oak Ridge National Lab, International Conference on Bioinformatics, Atlanta, GA, 11/07
- European Conference on Computational Biology (ECCB) 2006, Eilat, Israel, 01/07
- Basel Computational Biology Basel Conference [BC]², 03/06

Membership in journal editorial boards

- J Comput Biol (associate editor)
- Bioinformatics
- Briefings in Bioinformatics
- BMC Bioinformatics and BMC Genomics
- Naturwissenschaften
- J Experimental Zoology Series B
- Interface - Journal of the Royal Society
- Int J Data Mining and Bioinformatics

Membership in professional societies (selected)

- ACM – Association for Computing Machinery
- RSS – Royal Statistical Society
- ISCB – International Society of Computational Biology

Service to Scientific Community (selected)

- Chair of the Steering Committee of the International Conference on Computational Molecular Biology RECOMB
- Member of Scientific Steering Committee of the Isaac Newton Institute (07-09)
- Member of Bioinformatics Advisory Council, European Bioinformatics Institute EMBL-EBI

Teaching activities

All courses given at Freie Universität Berlin.

Algorithmic Bioinformatics, 4hrs per week plus tutorials, held every other year during winter semester (alternatingly with Prof. Knut Reinert)

Probability and Statistics for Bioinformatics, winter 2007/08, 2 hrs per week

Algorithms for the Computation of Phylogenetic Trees, summer 2006, winter 2009/10, 2 hrs per week

Various seminars and practical courses

Organization of scientific events

Co-organizer of the German Conferences on Bioinformatics, GCB, Potsdam 2007

Chair of Program Committee for RECOMB 2008 in Singapore

Regular member of Program Committees of the European Conference on Computational Biology (ECCB), ISMB, and RECOMB.

Organization of Otto-Warburg Summer Schools in 2006, 2007, 2009

General information about the whole Department

Complete list of publications (2006-2009)

2009

Baek,Y.S., Haas,S., Hackstein,H., Bein,G., Santana,M.H., Lehrach,H., Sauer,S. and Seitz,H. (2009). *Identification of novel transcriptional regulators involved in macrophage differentiation and activation in U937 cells*. BMC Immunology 10:18

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Gat-Viks, I., Vingron, M. (2009). *Evidence for Gene-Specific Rather Than Transcription Rate–Dependent Histone H3 Exchange in Yeast Coding Regions*. PLoS Comput Biol 5(2): e1000282

Huang, X., Vingron, M. (2009). *Maximum Similarity: A New Formulation of Phylogenetic Reconstruction*. Journal of Computational Biology 16(7): 887-896

Kanhere, A., Vingron, M. (2009). *Horizontal Gene Transfers in prokaryotes show differential preferences for metabolic and translational genes*. BMC Evolutionary Biology 9:9

Ott CE, Bauer S, Manke T, Ahrens S, Rödelsperger C, Grünhagen J, Kornak U, Duda G, Mundlos S, Robinson PN (2009). *Promiscuous and Depolarization-Induced Immediate-Early Response Genes are Induced by Mechanical Strain of Osteoblasts Original Study.* J Bone Miner Res

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Steinhoff, C., Paulsen, M., Kielbasa, S., WalterJ., Vingron,M. (2009). *Expression profile and transcription factor binding site exploration of imprinted genes in human and mouse*. BMC Genomics 10:144

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Sultan, M.*, Schulz, M.H.*, Richard, H.*, Magen, A., Klingenhoff, A., Scherf, M., Seifert, M., Borodina, T., Soldatov, A., Parkhomchuk, D., Schmidt, D., O'Keeffe, S., Haas, S., Vingron, M., Lehrach, H., Yaspo, ML (2008). *A Global View of Gene Activity and Alternative Splicing by Deep Sequencing of the Human Transcriptome*. Science 321(5891), 956-960 // *shared first authorship

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de la Chaux, N., Messer, P.W., Arndt, P.F. (2007). *DNA indels in coding regions reveal selective constraints on protein evolution in the human lineage*. BMC Evol Biol. 7: 191

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PhD theses

2009

Benjamin Georgi: *Context-specific Independence Mixture Models for Cluster Analysis of Biological Data*. PhD Thesis, Bioinformatics, Freie Universität Berlin, 06/09 (supervisor: Alexander Schliep)

2008

Helge Roider: *Eukaryotic promoter analysis by means of a biophysical model for DNA transcription factor interactions*. PhD Theses, Freie Universität Berlin, 11/08 (supervisor: Stefan Haas)

Utz Pape: *Statistics for Transcription Factor Binding Sites*. PhD Thesis, Freie Universität Berlin, 10/08 (supervisor: Martin Vingron)

Ivan Gesteira Costa Filho: *Mixture Models for the Analysis of Gene Expression: Integration of Multiple Experiments and Cluster Validation*. PhD Thesis, Bioinformatics, Freie Universität Berlin, 06/08 (supervisor: Alexander Schliep)

Ho-Joon Lee: *Computational Genomic Analysis of Transcriptional Regulation*. PhD Thesis, Freie Universität Berlin, 04/08 (supervisor: Martin Vingron)

Philipp Messer: *Tandem Duplications in the Human Genome*, PhD Thesis, Freie Universität Berlin, 03/08 (supervisor: Peter Arndt)

2007

Wasinee Rungsarityotin: *Algorithms to identify protein complexes from high-throughput data*. PhD Thesis, Bioinformatics, Freie Universität Berlin, 11/07 (supervisor: Alexander Schliep)

Abha Singh Bais: *Annotated Alignments*. PhD Thesis, Freie Universität Berlin, 07/07 (supervisor: Martin Vingron)

2006

Hannes Luz: *Family Specific Rates of Protein Evolution*. PhD Thesis, Freie Universität Berlin, 12/06 (supervisor: Martin Vingron)

Dennis Kostka: *Methodology for exploring and communicating molecular characteristics of disease*. PhD Thesis, Freie Univer-sität Berlin, 12/06 (supervisor: Rainer Spang)

Stefanie Christina Scheid: *Novel Concepts for the Significance Analysis of Microarray Data.* PhD Thesis, Freie Universität Berlin, 10/06 (supervisor: Rainer Spang)

Jochen Jäger: *Deriving small diagnostic biomarker panels from genome wide, clinical microarray studies.* PhD Thesis, Freie Universität Berlin, 07/06 (supervisor: Rainer Spang)

Florian Markowetz: *Probabilistic Models for Gene Silencing Data.* PhD Thesis, Freie Universität Berlin, 04/06 (supervisor: Rainer Spang)

Student theses

2008

Christina Bianca Heitzer: *Prediction of Alternative Operons using an HMM approach with Transcription Factor Binding Sites and Intergenic Distances*. Diploma Thesis, Philipps-Universität Marburg, 2008 (supervisor: Roland Krause)

Sebastian Dominik Mackowiak: *Combined analysis of genome wide expression and copy number data of human tumors*. MSc Thesis, Freie Universität Berlin, 2008 (supervisor: Martin Vingron)

Marko Briesemann: *Evaluation and extension of a community detection approach using linear programming.* MSc Thesis, Freie Universität Berlin, 2008 (supervisor: Martin Vingron)

Christopher Hardt: *Evolutionary Rate Dynamics of Protein Families*. MSc Thesis, Freie Universität Berlin, 2008 (supervisor: Hannes Luz)

Christian Hoffmann: *Detection of Chimeric Small-Subunit rRNAs.* MSc Thesis, Freie Universität Berlin, 2008 (supervisor: Martin Vingron)

Christoph Hafemeister: *Efficient Computation of Probe Qualities*. MSc Thesis, Freie Universität Berlin Berlin, 2008.

Annekatrin Wiedenhoeft: *The regulatory power of 3'UTRs: The analysis of the 3'UTR sequences of human cytosolic RP genes*. BSc Thesis, Freie Universität Berlin, 2008 (supervisor: Martin Vingron)

Peter Hansen: *Kategorisierung von Aminosäuren und genomweite Zusammenfassung von Proteindomänen zur niederparametrischen Modellierung von Protein-Evolution*. BSc Thesis, Freie Universität Berlin, 2008 (supervisor: Hannes Luz)

Katharina Schmidt: *A Phylogenetic Parsimony Method Considering Neighbored Gaps*. BSc Thesis, Freie Universität Berlin, 2008 (supervisor: Hannes Luz)

Jevgeni Erehman: *Theoretische Untersuchungen von Proteinstrukturen alternativ gespleißter Proteine*. BSc Thesis, Freie Universität Berlin, 2008 (supervisor: Martin Vingron)

2007

Ruben Schilling: *Elastische Registrierung in 3D Volumen Daten.* Diploma Thesis, Universität Freiburg, 2007 (supervisor: Alexander Schliep)

M. Turewicz: *Lernen von CSI Mixturen mit MCMC Methoden*. Diploma Thesis, Martin Luther University, Halle, 2007 (supervisor: Alexander Schliep)

Nicole de la Chaux: The *Evolution of the Human Genome: Insertins and Deletions in Protein Coding Regions*. MSc Thesis, Freie Universität Berlin, 2007 (supervisor: Peter Arndt)

Christopher Hardt, MSc Thesis, Freie Universität Berlin, 2007 (supervisor: Hannes Luz)

Thomas Engleitner: *Insertions and Deletions in the Human Genome.* BSc Thesis, Freie Universität Berlin, 2007 (Supervisor: Peter Arndt)

Ricardo Raspe, BSc Thesis, Freie Universität Berlin, 2007 (supervisor: Hannes Luz)

J. Li: *Methoden für das Design von DNA Tiling arrays*. BSc Thesis, Freie Universität Berlin, 2007 (supervisor: Alexander Schliep)

M. Ruegen: *Optimale Probenauswahl fuer die Targeterkennung mit DNA microarrys*. BSc Thesis, Freie Universität Berlin, 2007 (supervisor: Alexander Schliep)

2006

Petko Fiziev, Diploma Thesis, Freie Universität Berlin, 2006 (supervisor: Martin Vingron)

Michael Seifert: *Analyzing microarray data using homogeneous and inhomogeneous Hidden Markov Models*; Diploma Thesis, Martin Luther University, Halle, 2006 (supervisor: Alexander Schliep)

Lennart Opitz: *Analyse von Bildern der mRNA- in Situ-Hybridisierung*. Diploma Thesis, Martin Luther University, Halle, 2006 (supervisor: Alexander Schliep, joint supervision with Dr. S. Posch, Halle)

Max Flöttmann, BSc Thesis, Freie Universität Berlin, 2006 (supervisor: Roland Krause)

Sebastian Mackowiak, BSc Thesis, Freie Universität Berlin, 2006 (supervisor: Roland Krause)

Christoph Hafemeister: *Learning topologies of conditional trees*. BSc Thesis, Freie Universität Berlin Berlin, 2006 (supervisor: Alexander Schliep)

Moritz Wade, BSc Thesis, Freie Universität Berlin Berlin, 2006 (supervisor: Aditi Kanhere)

Guest scientists since 2006

Shen Lin, Chinese Academy of Sciences, Wuhan, P.R. China, 11.01.2009 – 10.01.2010

Ina Koch, Technical University of Applied Sciences Berlin, since 2006

Pierre Nicodeme, Laboratoire LIX, Ecole Polytechnique, Palaiseau cedex, Frankreich, 24.08. – 05.09.2009; 10.09. – 01.10.2007; 25.08. – 05.10.2005

Peter Clote, Boston College, Chestnut Hill, MA, USA; 01.08. – 03.09.2009; 01.05. – 30.06.2007

Oliver Eulenstein, Iowa State University, Ames, Iowa, USA, 02.01. – 30.06.2009

Guillaume Bourque, Genome Institute of Singapore, Singapore, 10.06. – 12.06.2009

Martin Frith, AIST Tokyo, Japan, 22.04. – 13.05.2009

Roland Dosch, Universität Genf, Zürich, 07.01. – 10.01.2009

Lev Levitin, Boston University, Boston, MA, USA, 12.07. – 14.08.2008; 10.07. – 29.08.2007; 18.06. – 31.08.2006

Jerzy Tiuryn, Warsaw University; Polen; 06.-08.03.2008

Xiaoqiu Huang, Iowa State University, Iowa, USA; 15.08. – 14.11.2007

Ricardo Bringas, Centro de Ingenieria, La Habana, Kuba, 02.07. – 27.09.2007; 02.04. – 09.07.2005; 01.09. – 31.10.2004

Irit Gat-Viks, Tel-Aviv University, Israel; 29.05. – 01.06.2007

Marcilio Carlos Pereira de Souto, University of Tio Grande do Norte, Natal, Rn, Brasil, 01.10.2006 – 30.09.2007

Morgan Bishop, University SUNY Geneseo, New York, NY, USA, 30.4.-4.5.2007

Dmitri Petrov, Stanford University, 08/2007

Norbert Doyer, Warsaw University, Polen, 19.06. – 07.07.2006

Serdar Cakici, Sabanci University, Istanbul, Turkei, 28.07. – 28.08.2006

Pawel Gorecki, *Warsaw University,* Polen, 01.10.2006 – 30.09.2007

Abhinab Ray, Juni Line Faculty, India, 17.05. – 16.08.2006

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Dr. rer. nat. Eva Klopocki* (since 09/03)
Dr. rer. nat. Uwe Kornak* (since 03/04)
Dr. med. Pablo Villavicencio-Lorini* (since 02/05)
Dr. med. Claus Eric Ott* (since 03/05)
Dr. rer. nat. Jochen Hecht* (since 01/07)
Dr. rer. nat. Boris Thurisch* (since 08/07)
Dr. rer. nat. Johannes Egerer* (since 09/07)
Dr. med. Peter Krawitz* (since 01/09)
Dr. rer. nat. Pia Kuss* (since 07/09)
Dr. med. Katrin Hoffmann* (01/04-12/09)
Dr. rer. nat. Petra Seemann* (01/06-02/09)
Dr. med Alexander Jamsheer (06/08-12/08)
Dr. med. Katarina Lehmann* (08/00-03/08)
Dr. rer. nat. Volkhard Seitz (04/01-02/05)
Dr. med. Katrin Süring* (07/02-12/04)
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Florian Witte (01/05-06/09)
Julia Friedrich (06/08-03/09)
Haikuo Zhang (08/04-06/08)
Daniel Birker (11/05-12/07)
Jochen Hecht (10/01-12/06)
Nicole Verhey van Wijk (01/01-06/06)

Technicians

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Monika Osswald (since 05/05)
Carola Dietrich (since 10/09)
Maria Walther (02/07-12/09)
Kathrin Seidel (08/05-06/09)
Katja Schmidt (04/07-03/09)
Christine Zwingmann (08/00-12/08)

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PD Dr. Denise Horn*

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Dr. med. Seval Türkmen*
Dr rer. nat. Marc Trimborn*

Molecular Genetics

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Structure of the group

The research group Developmental & Disease focuses on the mechanisms by which genes control normal and abnormal development. The group is part of and works in close collaboration with the Institute for Medical Genetics (IMG), which is located at the Campus Virchow of the Charité - Universitätsmedizin Berlin. The IMG provides clinical and diagnostic genetic service locally, i.e. for the Berlin-Brandenburg area, as well as internationally. It consists of a unit for Clinical Genetics, Cytogenetics, and Molecular Genetics. Medical doctors in training rotate to work within the Development & Disease group and scientists from the MPIMG have the opportunity to specialize in Medical Genetics at the IMG. A shared infrastructure, exchange of technical achievements and expertise, as well as common research goals ensure a successful interdisciplinary approach to study the mechanisms of genetic disease. Thus, the research group Development & Disease and the IMG form a highly complementary unit that combines clinical expertise with a basic science approach to address genetic questions.
Development and regeneration are related and it is generally believed that developmental pathways get re-activated during healing processes. To synergistically use our expertise in the molecular control of cell differentiation and development with new advances in regenerative medicine we collaborate closely with the Berlin-Brandenburg Center for Regenerative Medicine (BCRT) which is funded by the BMBF. Two members of the lab, Jochen Hecht and Petra Seemann, were appointed as group leaders at the BCRT but remain affiliated with the group.

Research concept

The mechanisms by which DNA sequences influence human development, function and aging has moved into the center of medical research creating the basis for what is now called molecular medicine. Much of what we have learned over the past years is based on the knowledge about the human as well as other genomes and the use of model systems. However, understanding the pathomechanisms of human disease is frequently challenging due to complexity of the systems and limited transferability. We aim at combining basic biology, genetics and clinical medicine to generate in-depth knowledge of human diseases, especially those related to abnormal development (congenital malformations) and growth and homeostasis of the skeleton.
The research group at the MPI develops and analyzes genetically engineered *in vitro* and *in vivo* models to elucidate pathogenetic mechanisms for human disease. Through the IMG clinical and diagnostic services patient cohorts are generated that are analyzed for genetic defects. This involves patient recruitment, expert phenotyping, data management and analysis, as well as mutation detection, mapping, and disease gene identification. The latter has profited greatly from the recent technology developments at the MPIMG such as array-CGH and next generation sequencing. The identification of an ever increasing number of rare, potentially disease causing mutations necessitates in-depth functional analysis of the identified changes. At the Development & Disease group we are well equipped to test novel disease genes/mutations for their functional relevance in established *in vitro* and *in vivo* model systems. The major *in vivo* systems are genetically engineered mice, chicken embryos and, more recently, zebrafish. Thus, our approach synergistically combines basic science oriented research at the MPI with the more clinically oriented work at the IMG for research into human genetic disorders.

Scientific achievements / findings

Our research focuses on normal and abnormal mechanisms that influence development, growth, homeostasis, aging, and regeneration of the skeleton. The skeleton is a particularly informative model system for our phenotype driven approach, because of an almost unlimited number of distinct phenotypes. Over the past years our aim has been to learn about the origins of phenotypic variability, develop new definitions for disease entities in order to better predict the phenotype from the genotype. To achieve these goals the department is set up as an interactive unit with close cooperation between all members of the different research groups. Currently, our focus is on the following topics:

Molecular pathogenesis of the brachydactyly disease family

Brachydactyly (BD) refers to a limb malformation characterized by shortening of digits. We have been studying this group of conditions extensively over the last years to understand their molecular pathology (Fig. 1). Based on human phenotypes and families we identified a number of causes for various types of BDs. Our studies show that most of these conditions are due to mutations in genes of the bone morphogenetic protein (BMP) pathway, or in genes that interact with this pathway. These include mutations in the bone morphogenetic protein receptor BMPR1B, its ligand GDF5, a regulatory element in BMP2, the BMP antagonist NOGGIN, the transcription factors HOXD13 and SOX9, as well as the tyrosine kinase receptor ROR2. We have established mouse and *in vitro* models for most of these genes and investigated the developmental pathology of mutations and the normal gene action during mouse and chicken development. Our studies have shown that variability within this group of conditions is due to a general dysregulation of the pathway. Depending on whether the BMP signal is increased or decreased and on the ratio of receptor signaling different phenotypes arise. In addition, we identified basic mechanisms that control digit outgrowth such as the phalanx forming region which was shown to be controlled by Smad signaling. Based on these studies we have proposed the concept of molecular disease families defined as conditions that share certain phenotypes due to a common pathway dysregulation.

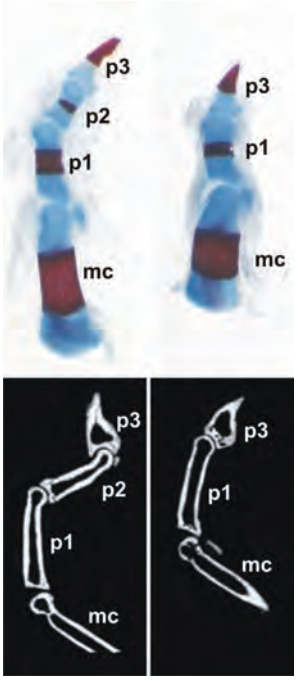

Figure 1: The Ror2-brachy mouse, a model for human brachydactyly. Skeletal preparations are shown on top, individual digits are labeled P1, P2, P3. Metacarpal is mc. µCT scan of digits (bottom panel). Note missing middle phalanx (P2) in mutant (right).

Sigmar Stricker has been studying the role of Ror2 in this process as well as basic mechanisms of digit development. Petra Seemann concentrated on the role of BMPs in digit and joint development.

Hox genes in limb development

Homeobox genes, and the proteins they encode, the homeodomain proteins, play important roles in the developmental processes of many multicellular organisms. Some of these genes have been shown to play important roles in limb development. However, it has remained a continuous challenge in the field to establish where Hox genes fit into the molecular genetic program of patterning and organogenesis of the limb elements.

In humans, mutations in HOXD13 results in synpolydactyly, a limb malformation characterized by an additional finger between digits 3 and 4 and a fusion of these three digits. The mutations that cause this condition are rather unusual as they comprise expansions of a polyalanine tract in the N-terminal region of the HOXD13 protein. We have studied the nature of this mutation in a mouse mutant that carries the exact same mutation found in humans (spdh) as well as in other Hox mutant mice. We showed that Hoxd13 regulates Raldh2, an enzyme critical for retinoid acid (RA) synthesis in the limb and that, consequently, RA is reduced in mutant limbs. RA is produced in the interdigital space where it suppresses chondrogenesis. The relevance of this finding was supported by the fact that treatment of pregnant mice with RA resulted in restoration of pentadactyly in spdh mice.
In a second set of experiments we showed that Hox genes control bone formation in the limbs by directly activating Runx2, the transcription factor essential for bone formation. Furthermore, we demonstrate that Hox genes determine the shape and identify limb bones and that their inactivation causes a homeotic transformation of long bones (metacarpals) into round bones (carpals). Together, our findings show that Hox genes are essential modifiers of shape and limb gestalt by controlling stem cell differentiation into chondrocytes or osteoblasts.
Pia Kuss and Pablo Villavicencio-Lorini have been the driving force in this project.

Mechanisms of bone regeneration

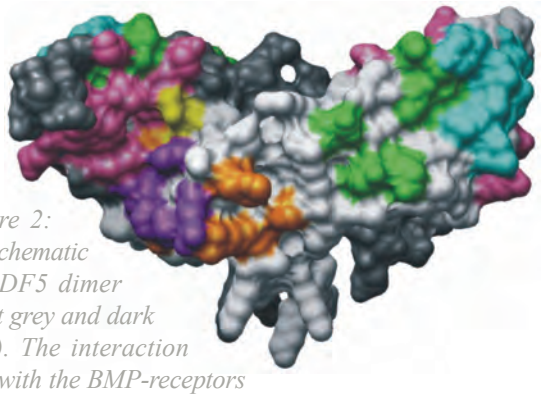

Figure 2: 3D-schematic of GDF5 dimer (light grey and dark grey). The interaction face with the BMP-receptors type I (orange/red) type II (green) and Noggin (pink) are color-coded. Alteration of amino acid N445 (yellow) disrupts the NOG interaction site and result in a GDF5 molecule that is no longer inhibited by NOG.

Bone has the unique capability to regenerate after injury. Considerable evidence indicates that the molecular control of this process uses similar pathways as during bone development. It is our aim to investigate these processes in order to understand regeneration and to develop new tools to improve bone healing in patients. We have been investigating gene regulation during fracture healing in the sheep using high throughput sequencing and have analyzed the data using bioinformatic tools. These data have given first insights into the genetic control of fracture healing. BMPs have been used for some time to improve bone formation. However, in some individuals their effect is limited and large amounts of the protein are needed. Based on our findings with the GDF5 mutations we have been developing new BMPs with improved biological activity, for example by manipulating the receptor specificity of by creating BMPs that are resistant to inhibition by e.g. Noggin (Fig. 2). These new BMPs are currently being tested in animal models.
Jochen Hecht and Peter Robinson have been working on the fracture model, Petra Seemann on the improvement of BMPs.

Osteoporosis and mechanisms of aging

In humans aging is invariably accompanied by changes in skin and bone. While age-related skin wrinkling is rather a cosmetic problem, age-related bone loss results in a increased susceptibility to fractures and thus a significant disease burden. To elucidate the molecular processes that govern aging in these tissues, we studied a group of recessively inherited diseases collectively characterized by the combination of wrinkly skin and osteoporotic bone. Over the last years we have

been able to identify disease causing mutations in three different genes, two of which are involved in the Golgi network. Mutations in the α2 subunit of the vacuolar-type proton pump (H(+)-ATPase or V-ATPase) ATP6V0A2 were shown to be associated with wrinkly skin syndrome. This pump is present in Golgi secretory vesicles and appears to be important for the proper protein modification, as patients with mutations in the gene show abnormal serum protein glycosylation pattern. In a similar condition, geroderma osteodysplastica, we identified mutations in GORAB, a novel Rab6 interacting golgin. Through further mapping analysis we identified a third group of patients with overlapping wrinkly skin phenotypes and identified mutations in PYCR1, the gene coding for the enzyme that catalyzes the NAD(P)H-dependent conversion of pyrroline-5-carboxylate to proline (pyrroline-5-carboxylate reductase). Further investigation *in vitro* and *in vivo* (zebrafish, xenopus) showed that the enzyme is located exclusively in mitochondria and that inactivation resulted in increased rates of cell death (Fig. 3). Intriguingly, similar observations were made in ATP6V0A2 mutant cells which show intracellular accumulation of secretory cargo and increased cell death.

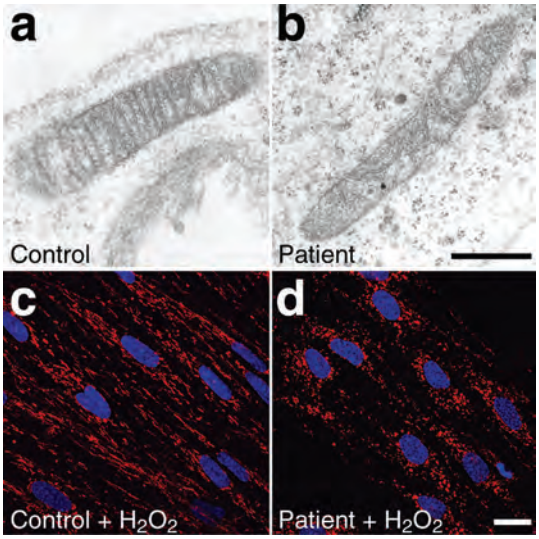

Figure 3: Electron microscopy of mitochondria from skin fibroblasts from a control ***(a)*** *and a patient* ***(b)*** *with a mutation in PYCR1. Note abnormal structure of mitochondria. Challenge of the cells with reactive oxygen species (H_2O_2) results in disintegration of mitochondria (red staining) in the patient's fibroblasts* ***(d)****, but not in control cells* ***(c)****.*

Our findings provide new insights into the molecular mechanisms of skin aging and osteoporosis. Proper function of the Golgi apparatus appears to be important for maintenance of healthy skin. Increased susceptibility to apoptosis may be an important trigger for age-related changes in skin and bone.
Uwe Kornak is in charge of this project.

Long range regulation

Most developmentally important genes have complex expression patterns that show distinct differences in temporal and spatial distribution. How this is achieved is largely unknown but cis-regulatory enhancer and suppressor elements are believed to play an important role. By screening large cohorts of patients with limb malformations *via* array-CGH we have identified a series of duplications involving conserved non-coding elements (CNEs) that are located in the vicinity of developmentally important genes. Sonic hedgehog (Shh) is a morphogen expressed asymmetrically in the posterior limb bud margin where it contributes to the overall bauplan of the autopod by determining the number and identity of digits. Shh is surrounded by a large gene desert containing numerous conserved elements that presumably act as enhancers. We identified duplications in one of these regions that are associated with duplicated and triphalangeal thumbs. In a large family with brachydactyly type A2 we identified a small (5 kb) duplication 3' of the BMP2 gene. A highly conserved sequence within this duplication was shown to drive expression in the distal digits suggesting that a dosage effect is causative for the phenotype (Fig. 4). Furthermore, in a limb malformation syndrome with absent nails and missing middle phalanges (Cooks syndrome) we identified a 1Mb duplication 5' of the SOX9 gene, a gene previously associated with a lethal skeletal dysplasia with sex reversal.

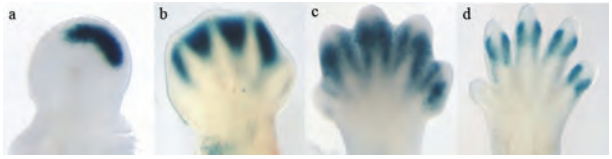

Figure 4: X-Gal staining of transgenic mice carrying the brachydactyly associated duplicated region of BMP2 at various stages of limb development. Staining is first visible in the progress zone of the limb bud ***(a)****, than between the digit anlagen* ***(b)****, and finally around the phalanges with highest staining around the middle phalanges* ***(c, d)****.*

Our findings identified duplications of CNEs as a novel mutation mechanism for human disease. In addition, they show that CNEs are important for fine tuning expression and that alterations in these regions can result in unexpected phenotypes. Alterations of regulatory CNEs are a likely mechanism for evolutionary change.
Eva Klopocki is in charge of this project.

Medical bioinformatics

Our group has developed an ontology to describe the phenotypic features seen in hereditary and other forms of human disease. This manually curated program can be used to study phenotypic features with bioinformatic tools and other forms of computational analysis. Following its publication in November 2008, the Human Phenotype Ontology (HPO) was featured as a research highlight in Nature Reviews Genetics and is already being adopted by international research groups for phenotyping, including most prominently the DECIPHER group at the European Bioinformatics Institute/Sanger Center. We have more recently used the HPO to develop a clinical diagnostics algorithm for human genetics that utilizes a novel statistical model of semantic similarities in ontologies to provide a ranking of the candidate differential diagnoses and have developed a novel graph algorithm that accelerates semantic searches in ontologies by many orders of magnitude. A patent application for a number of applications of these algorithms was recently submitted to the United States patent office. In addition, our bioinformatics group is active in a number of other areas including algorithms and support for ChIP-seq and other next generation sequencing applications as well as analysis of microRNA and mRNA microarray hybridizations and promoter analysis.
Peter Robinson leads the bioinformatics group.

Identification of disease genes

Our aim is to identify genetic factors that cause or modify monogenic diseases. Learning about the cause of a disease helps to understand, or to start to study, the subsequent disease processes and aims to develop more effective diagnostics and eventually preventive or therapeutic strategies. We have been using traditional linkage analyses, candidate gene sequencing, and array-CGH, and have now applied sequence capture and subsequent massive parallel sequencing to identify disease causing genes. With a sufficient supply of patient material this provides us with a continuous flow of novel genes and mutations. This project is interdisciplinary and involves clinicians for sampling, diagnosing, and phenotyping as well as bioinformatitions for the analysis of phenotypic and sequence data, and sequencing technology for mutation identification.
Denise Horn represents the clinical aspect of this project, Nick Robinson and Katrin Hoffmann the bioinformatic part, and Jochen Hecht is in charge of the sequencing. Peter Krawitz has established analysis routines for improving detection of indels in Illumina/Solexa data and for using the exon-enriched NGS sequencing for clinical diagnostics.

Cooperation within the institute

Cooperations over the past years have been with the Lehrach Dept. on a EU-funded large scale gene expression study using automated *in situ* hybridization technology (Maire-Laure Yaspo), the Evolution and Development group (Georgia

Panopoulou, Albert J. Poustka) on the evolution of Runx genes, and with Michal Schweiger (Cancer Genomics) on disease gene identification. We have been cooperating with the Herrman Dept. on novel technologies for 3D bone imaging. There is a long standing cooperation with the Ropers Dept. on array-CGH and the genetic causes of mental retardation. Close cooperations exist with the Vingron Dept. on computational analysis of ChIP-Seq data, analysis of sequencing data, ontologies and the role of a novel mitochondria localized gene. Together with Knud Nierhaus we are investigating the role of a novel ribosome-associated protein. Intense collaborations exist with the mouse and the sequencing facilities.

Special facilities / equipment

The research group as well as the IMG is equipped with the standard facilities for research into genetics, developmental biology, cell biology, and molecular biology. Special equipment includes the histology unit for the MPIMG and a sequencing facility for the Charité.

Planned developments

Future developments will aim at the integration of biological and phenotypic data. To achieve an in depth understanding of disease mechanisms we will aim at saturating disease groups by systematically analyzing phenotypes and disease genes/mutations. With an increasing number of conditions/genes we will be able to better understand the complexities of the dysregulated pathway(s) and their resulting phenotypes. To achieve this goal we will combine novel phenotyping methodology such as the HPO and screen patient samples using high throughput mutation analysis such as array-CGH, sequence capture, and whole exome/genome sequencing. Using this approach we will be able to combine phenotypic and molecular data in a systematic and comprehensive manner allowing us to better understand and ultimately predict the consequences of mutations.

General information about the whole group

Complete list of publications (2006-2009)

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Awards

Stefan Mundlos:

- Maroteaux Award, International Skeletal Dysplasia Society (2009)

Anja Brehm:

- 1. International BMP workshop 2009 Luzie Fabisch Award (2009)

Eva Klopocki:

- Young Scientist's Award, European Society of Human Genetics (2009)
- Vortragspreis 2009, Deutsche Gesellschaft für Humangenetik (2009)
- Finalistin Trainee Award, American Society of Human Genetics (2009)

Uwe Kornak:

- Ian T. Boyle Award of the European Calcified Tissue Society (ECTS) (2007)

Petra Seemann:

- Otto Hahn Medaille (2007) of the Max Planck Society

Work as scientific referee

S. Mundlos work as scientific referee for the following journals: Nature Genetics, American Journal of Human Genetics, American Journal of Medical Genetics, European Journal of Genetics, Journal of Medical Genetics, Human Genetics, Clinical Genetics, Development, Developmental Dynamics,

Mechanisms of Development, Journal of Clinical Investigation, Human Molecular Genetics, Histochemistry, Bone, Experimental Cell Research.

Membership in journal editorial boards

- American Journal of Medical Genetics
- Clinical Genetics
- Histochemistry

Service to scientific community

S. Mundlos serves as a referee for the following science organizations and institutions: Deutsche Forschungsgemeinschaft (DFG), Bundesministerium für Bildung und Forschung (BMBF), Wellcome Trust, European Union, Boltzmann Institute, Swiss National Science Foundation.

Postdoctoral lecture qualification (Habilitation)

2009

Katrin Hoffmann (2009) *Kopplungsanalysen zur Aufklärung monogener und komplexer Krankheiten*

Peter N. Robinson (2009) *Molekulare und klinische Untersuchungen beim Marfan Syndrom und verwandten Erkrankungen*

2008

Georg Schwabe (2009) *Molekulare Grundlagen von Organmalformationen am Beispiel kongenitaler Extremitätenfehlbildungen und eines Lateralisierungsdefekts*

2007

Denise Horn (2009) *Syndromale Erkrankungsbilder mit mentaler Retardierung*

PhD theses

2009

Florian Witte (2009) *Analyse der Ror2-Funktion in vivo und in vitro - Die Ror2 W749X-Maus als Modell für humane Brachydaktyliy Typ B*. PhD Thesis

Uli Wilkening (2009) *Funktionelle Analyse von in der Skelettentwicklung differentiell regulierten Genen*. PhD Thesis

Chayarop Supanchart (2009) *Characterization of an Osteopetrosis mouse model*. PhD Thesis

Friederike Kremer (2009) *Nonsense-mediated mRNA decay in collagen X*. PhD Thesis

Pia Kuss (2009) *Molekulare Pathologie und Embryologie von Hoxd13-assoziierten Fehlbildungen der Extremitäten*. PhD Thesis

Charlotte Wilhelmina Ockeloen (2009) *Split hand/split foot malformation: determing the frequency of genomic aberrations with molecular-genetic methods.* PhD Thesis

2008

Seval Türkmen (2008) *Molekulare Analyse genetisch bedingter Entwicklungsstörungen.* PhD Thesis

Haikuo Zhang (2008) *Investigating the molecular mechanism underlying Geroderma osteodysplastica (GO) Syndrome.* PhD Thesis

2007

Andreas Ney (2007) *Zur Pathogenese des Marfan Syndroms: Untersuchung der Matrix-Metalloproteinase-Regulierung nach Stimulierung mit rekombinanten Fibrillin-1-Konstrukten und Untersuchung der Selbstassoziation eines rekombinanten Versikan-Konstruktes*. PhD Thesis

2006

Petra Seemann (2006) *Zur Bedeutung des Wachstumfaktors GDF5*. PhD Thesis

Nicole Verhey van Wijk (2006) *Identifizierung intrazelllulärer Bindungspartner von Ror2*. PhD Thesis

Jochen Hecht (2006) *Genexpressionsanalysen zum besseren Verständnis von Knochenheilung und -entwicklung*. PhD Thesis

Student theses

2009

Julia Meier (2009) *Die Etablierung eines siRNA-Systems zur funktionellen Analyse der Odd-skipped-related-Gene Osr1 und Osr2 am Beispiel des Hühnerembryos*. Diploma Thesis

Annika Mahl (2009) *Charakterisierung der Interaktion der Rezeptortyrosinkinase Ror2 mit dem Liganden Noggin.* Diploma Thesis

Nadine Gladow (2009) *Molekulargenetische Untersuchungen des NSD1-Promotors bei Patienten mit Sotos Syndrom*. Diploma Thesis

Dajana Lichtenstein (2009) *Deletionsanalyse im NSD1- und FOXL2-Gen bei Patienten mit Sotos- und BPES Syndrom*. Bachelor Thesis

2008

Denise Pankalla (2008) *Histologische und Expressionsanalyse der Mausmutante ank/ank.* Diploma Thesis

Jolike Van Oosterwijk (2008) *Investigation and Characterization of Genomic Aberrations in Patients with Limb Malformations, with Focus on Brachydactylies*. Master Thesis

K. Guse (2008) *Untersuchung des Neurofibromatose Typ1 – Gen Promoters*. Diploma Thesis

Ines Dabow (2008) *Untersuchungen zur Rolle des Transkriptionsfaktors Mef2c in der Skelettentwicklung.* Diploma Thesis

Mareike Trams (2008) *Regulation des Sonic hedgehog durch cisregulatorische Ion range enhancer.* Diploma Thesis

Janine Dokas (2008) *Analyse des Wnt-Signalweges in der Knorpelentwicklung der Mausextremitäten.* Diploma Thesis

2007

B. Mehmedi (2007) *Erzeugung und funktionelle Untersuchungen von Phosphorylierungsstellen im ANK-Protein.* Diploma Thesis

Cindy Ast (2007) *Funktionelle Analysen zur molekularten Pathogenese der Fibrodysplasia ossificans progressiva (FOP)*. Diploma Thesis

Franziska Neuendorf (2007) *Interaktionen von Ror2 mit dem Wnt – Signalweg. 1. Expressionsanalyse der Wnt-Proteine und Antagonisten im Verlauf der frühen Handentwicklung. 2. Interaktionsstudien mit Hilfe vom Coimmunopräzipitationen*. Diploma Thesis

Fabian Grammes (2007) *Charakterisierung der PKA Regulation im NF1 Signalweg*. Diploma Thesis

Sören Zeidler (2007) *Charakterisierung von atNOA1*. Diploma Thesis

Anne Baude (2007) *Untersuchungen zur in vitro-Strukturbildung der Pro-Form des humanen Wachstumsfaktors GDF5: Vergleich des Wildtyps mit einer krankheitsassoziierten Variante*. Diploma Thesis

N. Beck (2007) *Kandidatengenanalyse zum Perrault Syndrom*. Diploma Thesis

S. Köhler (2007) *Support Vector Machines for Disease Gene Prediction from Protein-Protein-Interaction Data,* Master Thesis

2006

Manuela Magarin (2006) *Das Down Syndrom – Symptomatik, Expressionsanalyse der Chromosom 21 Gene und funktionelle Charakterisierung knorpelrelevanter Kandidaten*. Diploma Thesis

Julia Haupt (2006) *Analysis of expression and function of the transcription factor odd-skipped related (Osr2) during limb development*. Diploma Thesis

External funding

DFG: SFB 665, Projekt C04: *Klinisch-genetische Untersuchungseinheit*, 07/09-06/13

DFG: *HOXD assoziierte Fehlbildungen,* 07/09-06/12

EU: *Fighting Aneurismal Disease (FAD),* 06/08-05/12

BMBF: *Netzwerk Neurofibromatosis: TP4: Analyse von PD98059 als potenzielles Therapeutikum,* 02/09-01/12

DFG: *Computational phenotypic analysis,* 10/08-09/11

Fritz Thyssen Stiftung: *ATPase subunit a2,* 07/09-06/11

DFG: *Array CGH-Analyse bei Extremitätenfehlbildungen,* 04/08-04/11

BMBF: *Pathogenese der autosomal dominanten Osteopetrose,* 04/08-03/11

DFG: SFB 760, TP A1: *The molecular biology of fracture healing,* 01/07-12/10

DFG: SFB 760, TP A2: *Improving bone regeneration by modification of BMP-inhibition,* 01/07-12/10

EU: *Berlin Bedrest Study BBR2-2,* 09/07-11/10

BMBF: BCRT (Berlin Center für Regenerative Therapien): TP A: *Basis Research,* 10/06-09/10

DFG: *Cohenlike-Syndrom,* 06/09-05/10

BMBF/Koop. Biopharm: *BiochancePlus: GDF5 für therapeutische Anwendungen,* 10/06-9/09

Children´s Tumor Foundation/USA – Young Investigator Award Applicatiom: *Role of FGF signaling in the genesis of multiple bone phenotypes in NF1Prx1 mouse,* 07/07-06/09

BMBF: *Skelnet*, 10/06-12/11

EU: *EuroGrow: Pathophysiology of chondrodysplasia resulting from Prx1-Cre mediated knockout of NF1 (Neurofibromatosis 1) gene*, 04/07-03/10

DFG: SFB 577 TP A4: *Craniometaphyseal dysplasia (CMD) - Clinical Variability and Pathogenic Pathways,* 07/01-06/09

DFG: SFB 577 TP A6: *Molecular Pathology and Embryology of HOXD-related Limb Malformations*, 07/01-06/09

DFG: SFB 577 TP A8: *Analysis of the receptor tyrosine kinase ROR2: a central regulator in the brachdactyly disease family*, 07/04-06/09

DFG: SFB 577 TP Z 05: *Central Facility for Animal Model Generation*, 07/04-06/09

DFG, SFB 665: Projekt A5: *Sonic hedgehog regulation during development and in disorders of the nervous system*, 07/05-06/09

Otto Warburg Laboratory
Neurodegenerative Disorders

(Established: 02/02 as part of the Dept. of Vertebrate Genomics, independent since 09/08)

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Franziska Welzel (since 07/06)
Anja Nowka (since 02/08)
Christian Linke (since 04/08)
Christian Kähler (since 05/09)
Karolin Huckauf (09/02-05/08)
Ute Nonhoff (05/02-08/07)
Markus Ralser (06/03-06/06)

Technician
Silke Wehrmeyer (since 11/04)

Scientific overview

Human life expectancy is steadily rising in industrialized western countries and as a result fatal late-onset neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, or the polyglutamine related diseases, are among the leading causes of disability and death representing one of the major challenges of today's modern medicine. Millions of people worldwide suffer from these devastating disorders or are at risk, and a marked rise in the economic and social burden caused by these disorders will be noticed over the upcoming decades. Even though these diseases are quite common, the mechanisms responsible for their pathologies are in most cases still poorly understood and effective preventative therapies are currently not at hand. For the heritable forms of these neurodegenerative disorders linkage studies have led to the discovery of the causative genes. Current knowledge of the underlying molecular mechanisms accountable for the observed neurodegenerative processes was gained mainly from studying inherited disease variants, and these resulted in the identification of genetic and metabolic factors modulating disease onset and progression. Of note, similarities in the clinical and neuropathologic features have suggested that neurodegenerative diseases may share similar mechanisms of pathogenesis related to abnormal protein folding, aggrega-

tion, cellular dysfunction and cell death. In consequence, a comprehensive characterization of the molecular mechanisms implicated in the clinical heterogeneity of specific neurodegenerative disorders should help in defining the complete picture of potential pathomechanisms.

Projects

The main research interest of our group is to elucidate molecular mechanisms contributing to neurodegenerative disorders, in particular Alzheimer´s disease and the polyglutamine disorder Spinocerebellar Ataxia type 2, by combining yeast genetics/models, functional genomics and systems biology approaches.

Molecular mechanisms in neurodegenerative disorders

The current understanding in this research field is that a combination of multiple pathways is causative for neuronal dysfunction and finally neuronal death. In this respective, our studies revealed several pathways in which the disease-causing proteins are implicated and we are further exploring whether and how these pathways can be correlated to pathogenesis.

mRNA metabolism

Aberrant expression or functions of proteins implicated in several aspects of the cellular mRNA metabolism have been linked to human diseases, amongst others neurodegenerative disorders. We have discovered that the *SCA2* gene product ataxin-2, causing Spinocerebellar Ataxia type 2 (SCA2), is found in association with components central for regulating and controlling mRNA degradation and translation. We have demonstrated that ataxin-2 is a component of "stress granules", cellular compartments that represent sites of mRNA triage assembling in mammalian cells as response to specific cellular stresses. Moreover, we discovered that ataxin-2 plays a role in their assembly/composition. In regard to SCA2 pathogenesis, we revealed that an elevated ataxin-2 concentration – a condition detected in affected Purkinje neurons of SCA2 patients - interferes with the occurrence of so-called "processing-bodies", cellular compartments regulating mRNA degradation. Additionally, we showed that ataxin-2 levels regulate the intracellular concentration of the poly(A)-binding protein, a key factor for translational control. Currently, we are focusing on the association of ataxin-2 with further factors involved in the cellular mRNA metabolism, particularly splicing factors. How this is subject to regulation by cell signalling pathways and response to stress is here of central interest. These studies also comprise the ataxin-2-like protein and the SCA1-causing protein, ataxin-1, that has been found in association with ataxin-2.

Transcriptional regulatory networks

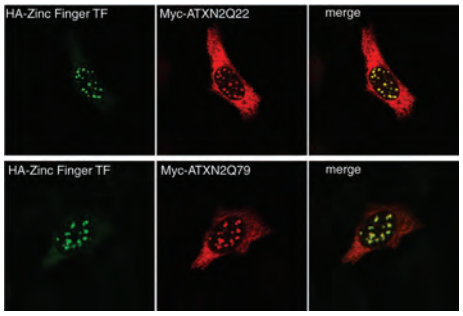

Figure 1: Overexpression of an interacting transcription factor (HA-Zinc Finger TF, green) causes recruitment of over-expressed ataxin-2 with a normal (Myc-ATXN2Q22, red) or an expanded (Myc-ATXN2Q79, red) polyglutamine stretch into nuclear bodies.

Aberrant interactions between polyglutamine proteins and transcriptional regulators have been found in respective cell culture, animal models and in the brains of patients indicating that perturbation of transcription frequently results in neuronal dysfunction in polyglutamine disorders. In order to gain insight into the cellular function of ataxin-2, we have performed comprehensive yeast-2-hybrid analyses with different

regions of the ataxin-2 protein. Interestingly, these screens resulted in the isolation of ataxin-2 interaction partners known to act as transcription factor or transcriptional regulator. Most exciting, one of these interactions seems to be affected by the length of the intrinsic glutamine-stretch within ataxin-2. To verify and comprehend the significance of the observed interactions, we are currently utilizing functional approaches in mammalian cells, such as localization studies, promoter analyses and RNAi knock down experiments. One further tool we are exploiting in this regard is the application of intracellularily expressed antibodies (intrabodies) with inhibitory activity. For this, we have developed in collaboration with Zoltán Konthur a screening procedure that is based on a combination of *in vitro/in vivo* ligand screening methods using the phage display technology and the yeast-2-hybrid system. In general, this technique is applicable for all protein-protein interactions happening in the yeast-2-hybrid system. In addition, we exploited the RNAi technology in combination with microarray analysis to gain insight into the nature and complexity of the ataxin-2 network on the transcriptional level. This work is complemented by studies of the ataxin-2 yeast homolog Pbp1p in order to discover novel/conserved target proteins as well as pathways that could contribute to SCA2 pathogenesis.

Involvement of glycolytic pathways

Over the last years it has been discussed that a reduced cellular energy production might contribute to the pathogenesis of several neurodegenerative diseases. This assumption is mainly based on the facts that a reduced activity of the glycolytic enzyme glycerinaldehyd-3-phosphat-dehydrogenase (GAPDH) as well as alterations of its cellular localization and physical properties was observed in cell culture, transgenic animal models and patient materials of several neurodegenerative disorders. Moreover, several polyglutamine proteins have been found in complex with GAPDH; however, the significance of these interactions is not understood. In this perspective, we have discovered an interaction between ataxin-2 and the glycolytic enzyme triosephosphate isomerase (TPI) by means of yeast-2 hybrid analyses. Functional characterization revealed that the isolated variant has no glycolytic activity. Interestingly, glycolytic inactivation of TPI, caused by particular mutations, has been linked to the pathogenesis of the human disorder triosephosphate isomerase deficiency. In this cellular context we were able to demonstrate that the disease-causing TPI mutations *per se* are not accountable for the observed reduced TPI activity measured in patient extracts; instead we discovered that alterations in the dimer formation of this enzyme as well as regulation defects contribute to the pathogenesis of triosephosphate isomerase deficiency.
A systems biology approach further revealed that reduced TPI activities lead to an increased resistance against a particular oxidant, which is also conserved in the worm *C.elegans*. Exploiting yeast genetics combined with mass spectrometry analyses, we discovered that inactivation of TPI led to an increased activity of the pentose phosphate pathway. Moreover, we demonstrated that inactivation of GAPDH increases the concentration of pentose phosphate pathway metabolites as well. To this end, we discovered that the pentose phosphate pathway activity is a regulator of normal lifespan in yeast and *C.elegans*, and that the observed metabolic switch provoked increased cellular NADPH concentrations which are accountable for protection against oxidative stress. Since chronic oxidative stress has been clearly linked to neurodegenerative disorders and first evidence was provided demonstrating that increased cellular NADPH concentrations possess a stimulating effect on the aggregation properties of the prion protein, it will be interesting to investigate whether and how alterations in the cellular redox state influence survival of neuronal cells and the aggregation properties of the disease proteins.

Identification of genes causing early onset Alzheimer´s disease

In this project, we aim in collaboration with Lars Bertram to identify novel early-onset Alzheimer´s disease (AD) genes and functionally characterize their respective gene products. The comprehensive mutational screening of AD patient DNA material is performed in Bertram's group with subsequent functional characterization of potential AD genes carried out in our group. Interestingly, first comparative studies revealed that proteins of the ataxin-2 network seem to be implicated in AD as well. Amongst others, these candidates have been analyzed genetically in the Bertram group and three candidates showed significant evidence of association with AD risk.

Links between neurodegenerative processes and cancer

Biochemical, genetic and epidemiologic evidence was provided over the last years linking a number of genes to both oncogenesis and neuronal dysfunction. Although tumorigenesis and neurodegeneration are different pathologies, common factors and overlapping molecular pathways have been identified in the generation and progression of these human disorders but often with complementary relationships. Of particular interest is that the dysregulation of genes that are implicated in the control of cell cycle progression and DNA repair is a hallmark of tumorigenesis and the same defects seem to contribute to the degeneration of post-mitotic neurons under certain conditions. Strong evidence has been provided that deregulation of cell cycle control is associated with neurodegeneration and that re-entry into the cell cycle occurs before substantial AD brain pathology can be observed. We are addressing this issue by performing a systems biology approach in which we are studying particular cell cycle aspects in the yeast *S.cerevisiae*. The outcome will be correlated to neurodegeneration by investigating whether expression levels/localization/protein-protein interactions of particular proteins involved in cell cycle regulation and DNA replication are affected by the expression of polyglutamine proteins or the APP protein in yeast with subsequent transfer to the human system. In this context, recent data obtained in budding yeast about a novel role of specific regulators of cell cycle progression in the regulation of the kinase activity will help to focus on the targets that could be potential key regulators in neurodegenerative disorders (in cooperation with Prof. E. Klipp). Besides, the administration of the commonly used chemotherapeutic imatinib has been shown to improve the survival of Purkinje cells in a Niemann-Pick disease mouse model. Since the underlying mechanisms are not fully understood and Purkinje cells are the primary target in SCA2 as well, we have recently exploited yeast genetics to identify genes implicated and future work will concentrate on their validation in mammalian cell culture.

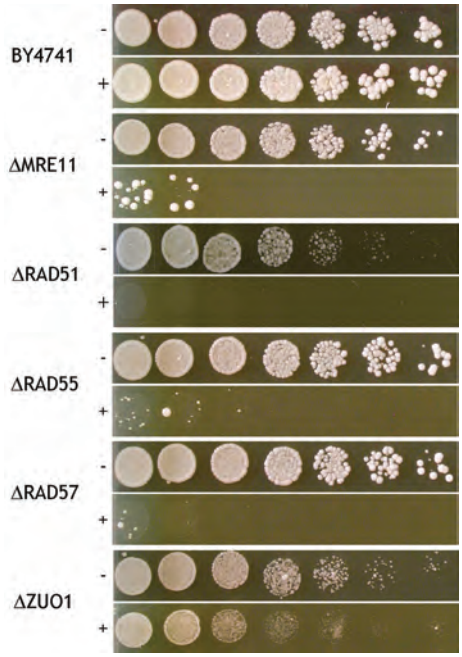

Figure 2: Selected results from a global drug screen in S. cerevisiae. Growth of wild type (BY4741) and deletion strains in the presence of solvent control (-) and chemotherapeutic drug (+).

Perspectives

In the future perspective, we will continue our efforts in understanding disease protein functions and mechanisms contributing to neurodegenerative processes. Since recent evidence exists linking several disease proteins in neurodegenerative disorders to cancer as well, we will broaden our efforts in this perspective. In collaboration with Michal Schweiger we started lately a project aiming at the identification of proteins conferring resistance to chemotherapeutics by means of yeast genetics. This project is likely to result also in the identification of pathways involved in neurodegeneration taken into consideration that overlapping common factors and molecular pathways have been observed in both pathologies.

Internal cooperations

- Zoltán Konthur, Dept. of Vertebrate Genomics
- Bernd Timmermann, Service group Next Generation Sequencing
- Michal Schweiger, Dept. of Vertebrate Genomics
- Lars Bertram, Dept. of Vertebrate Genomics
- Holger Klein / Martin Vingron, Dept. of Computational Molecular Biology
- Vera Kalscheuer, Dept. of Human Molecular Genetics
- Wilfried Nietfeld, Dept. of Vertebrate Genomics
- Lars Wittler / Bernhard Herrmann, Dept. Developmental Genetics
- Alexey Soldatov, Dept. of Vertebrate Genomics
- Ralf Herwig / Christoph Wierling, Dept. of Vertebrate Genomics
- Jörn Glökler, Dept. of Vertebrate Genomics

External cooperations

- Prof. Dr. Dr. Christian Betzel, University of Hamburg, Germany
- Prof. Michael Breitenbach, University of Salzburg, Austria
- Prof. Dr. Thomas Lengauer/Dr. Mario Albrecht, Max Planck Institute for Informatics, Saarbruecken, Germany
- Prof. Dr. Stefan Schreiber, Institute for Clinical Molecular Biology, University Hospital Schleswig-Holstein, Kiel, Germany
- Prof. Erich Wanker, Max Delbrueck Center for Molecular Medicine, Berlin, Germany
- PD Dr. Stefan Kindler, University of Hamburg, Germany
- Prof. Dr. Joachim Klose, Charité, Berlin, Germany
- Dr. Karl Skriner, Charité, Dept. of Rheumatology and Clinical Immunology, Berlin, Germany
- Prof. Dr. Edda Klipp, Humboldt-University, Berlin, Germany
- Johanna Gostner/ PD Dr. Gilbert Spizzo, Tyrolean Cancer Research Institute, Innsbruck, Austria

General information

Complete list of publications (2006 – 2009)

2009

Parkhomchuk D, Borodina T, Amstislavskiy V, Banaru M, Hallen L, Krobitsch S, Lehrach H, Soldatov A. (2009) *Transcriptome analysis by strand-specific sequencing of complementary DNA.* NAR 37(18):e123

2008

Ralser M, Wamelink MM, Struys EA, Joppich C, Krobitsch S, Jakobs C, Lehrach H. (2008) *A catabolic block does not sufficiently explain how 2-deoxy-D-glucose inhibits cell growth*. PNAS 105(46):17807-11.

Ralser M, Nebel A, Kleindorp R, Krobitsch S, Lehrach H, Schreiber S, Reinhardt R, Timmermann, B (2008). *Sequencing and genotypic analysis of the triosephosphate isomerase (TPI1) locus in a large sample of long-lived Germans*. BMC Genet. 29(9):38.

2007

Ralser M, Wamelink MM, Kowald A, Gerisch B, Heeren G, Struys EA, Klipp E, Jakobs C, Breitenbach M, Lehrach H, Krobitsch S (2007). *Dynamic re-routing of the carbohydrate flux is key to counteracting oxidative stress*. Journal of Biology 6(4):10.

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Taussig, M.J., Stoevesandt, O., Borrebaeck, C.A.K, Bradbury, A.R., Cahill, D., Cambillau, C., de Daruvar, A., Dübel, S., Eichler, J., Frank, R., Gibson, T.J., Gloriam, D., Gold, L., Herberg, F.W., Hermjakob, H., Hoheisel, J.D., Joos, T.O., Kallioniemi, O., Koegl, M., Konthur, Z., Korn, B., Kremmer, E., Krobitsch, S., Landegren, U., van der Maarel, S., McCafferty, J., Muyldermans, S., Nygren, P.A., Palcy, S., Plückthun, A., Polic, B., Przybylski, M., Saviranta, P., Sawyer, A., Sherman, D.J., Skerra, A., Templin, M., Ueffing, M., and Uhlén, M. (2007). *ProteomeBinders: Planning a European Resource of Affinity Reagents for Analysis of the Human Proteome*. Nature Methods 4 (1): 13-17.

2006

Ralser, M., Heeren, G., Breitenbach, M., Lehrach, H., and Krobitsch, S. (2006). *Triose Phosphate Isomerase Deficiency is caused by altered dimerization – not catalytic inactivity – of the mutant enzymes*. PloS ONE 1 (1): e30.

Ralser, M., Querfurth, R., Warnatz, HJ., Lehrach, H., Yaspo, ML., and Krobitsch, S. (2006). *An efficient and economic enhancer mix for PCR*. Biochem. Biophys. Res. Commun. 347(3): 747-751.

Invited lectures

FASI Conference, Dublin, Ireland, 2008

University of Hamburg, Department of Human Genetics, Hamburg, Germany, 2008

University of Leicester, Genetics seminar series, Leicester, UK, 2007

University of Salzburg, Salzburg, Austria, 2007

University of Hamburg, Biochemical and Molecular Biology seminar, Hamburg, Germany, 2006

Center of Molecular Medicine of the University of Cologne, Seminar series, Cologne, Germany, 2006

Awards

Sylvia Krobitsch - W 2 Special Program for the Advancement of Outstanding Female Scientists at the Max Planck Society (Minerva program), 2008

Markus Ralser - BioMed Central Research Award, Biology, 2007

Work as scientific referee

Sylvia Krobitsch serves as scientific referee for the following journals: Biotechniques, BBA - Molecular Cell Research, Experimental Neurology, Human Molecular Genetics, Journal of Cell Science, Journal of Cellular Biology, Journal of Neurochemistry.

PhD Theses

Ute Nonhoff (2007) *Untersuchungen zur Rolle von Ataxin-2 im zellulären mRNA-Metabolismus*, PhD Thesis, Freie Universität Berlin (supervisor: Sylvia Krobitsch)

Markus Ralser (2006) *Exploring molecular pathways contributing to spinocerebellar ataxia type 2*, PhD Thesis, University of Salzburg (supervisor: Sylvia Krobitsch)

Student theses

Lina Milbrand (2009) *Untersuchung zur zellulären Funktion von Ataxin-2*, Diploma thesis, Universität Greifswald (supervisor: Sylvia Krobitsch)

Melanie Isau (2009) *Untersuchungen zur zellulären Funktion des Proteins Ataxin-2*, Diploma thesis, Freie Universität Berlin (supervisor: Sylvia Krobitsch)

Christian Kähler (2009) *Funktionelle Charakterisierung von Ataxin-2-like: Untersuchungen zur Rolle des Ataxin-2 -like Proteins im zellulären mRNA-Metabolismus*, Diploma thesis, Freie Universität Berlin (supervisor: Sylvia Krobitsch)

Susanne Weber (2009) *Identifizierung funktioneller Intrabodies für die Untersuchung von Protein-Protein-Wechselwirkungen in SCA2*, Diploma thesis, Technische Universität Braunschweig (supervisor: Sylvia Krobitsch)

Mareike Schnaars (2007) *Funktionelle Charakterisierung der Proteininteraktionen zwischen Ataxin-2, LSm12 und Chromogranin B*, Diploma thesis, Universität Bremen (supervisor: Sylvia Krobitsch)

Franziska Welzel (2006) *Funktionelle Charakterisierung der Interaktion von Ataxin-2 und DDX6*, Diploma thesis, Freie Universität Berlin (supervisor: Sylvia Krobitsch)

Desagani N. Kumar (2006) *Investigations into molecular pathways involved in spinocerebellar ataxia type 2*, Diploma thesis, University of Skövde, (supervisor: Sylvia Krobitsch)

External funding

EU-FP7: *Eukaryotic unicellular organism biology – systems biology of the control of cell growth and proliferation, 04/08-03/13*

NGFN: *Alzheimer Krankheit - Integriertes Genom Forschungsnetzwerk (AD-IG), 06/08-05/11*

EU-FP6: *A European Infrastructure of Ligand Binding Molecules against the human proteome, 03/06-02/10*

Ataxia UK: *Investigations into the cellular function of ataxin-2 by the use of ssRNA aptamers,09/05-08/08*

NGFN: *Analysis of transcription regulatory networks by RNA interference expression analysis, 05/05-04/08*

Guest scientists

Dr. Matteo Barberis, Humboldt University Berlin, 01-12/09

Johanna Gostner, Tiroler Krebsforschungsinstitut Innsbruck, 09-11/09

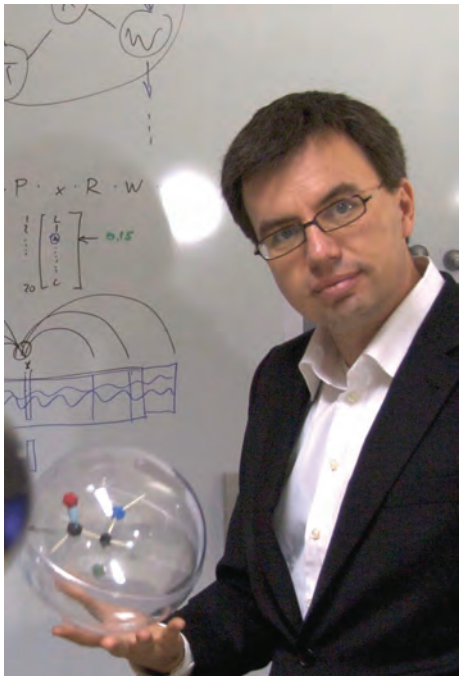
Teaching

Practical course: Physikalische Übungen für Pharmazeuten, Universität Hamburg, each winter term since 2005

Participitation in Lecture Biophysikalische Chemie, Universität Hamburg, summer terms 06/07/08

Otto Warburg Laboratory
Bioinformatics / Structural Proteomics

(Established: 10/04)



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Elisabeth Sahler (since 10/08)

Scientists
Sathyapriya Rajagopal (since 03/08)
Dan Bolser (08/06-07/08)
Ganesh Bagler (10/08-09/09)
Patrick Slama*
Brijnesh Johannes Jain

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Henning Stehr (since 10/05, IMPRS)
Ioannis Filippis (01/05-08/09)
Hyeong Jun An

Scientific programmers
Jose Duarte (12/05-11/09)
Lars Petzold

Scientific overview

The Independent Junior Research Group Bioinformatics / Structural Proteomics has been established in October 2004. Our research efforts aim at a system-wide understanding of cellular processes by predicting the structure and interactions of proteins at high resolution. Investigating the molecular basis of folding and flexible docking builds the foundations for rational design of novel therapeutic agents. Based on available partial datasets we developed a robust estimate of the number of interactions in human at about 650,000. This means that current data covers less than 1% of the entire interaction network and we are still a long way from achieving a comprehensive picture. Thanks to experimental high-throughput screening this gap can be expected to close in the years to come in a similar fashion as structural genomics projects have contributed ever more structural information in the PDB. Ultimately, we want to know how these interactions work on the molecular level by bridging between networks at different levels of resolution, rang-

* externally funded

ing from the atomic and residue level *via* protein structures and complexes up to phenotype. In order to meet this challenge, the problem of flexible docking needs to be addressed. Our research rationale is based on the idea that folding and binding follow the same biophysical principles. By finding network-based solutions to the problems of structural flexibility and co-operativity, we aim at major contributions to protein structure prediction and docking. At the same time, the results will advance our understanding of higher level networks. The PDB (ProteinDataBank) represents one of the "hardest", oldest and best curated databases in bioinformatics. Using this as a reliable source we have performed extensive work on the topic of converting protein structures into networks and back to 3D structures. In general, we aim at using the architectural principles and intrinsic properties of biomolecular networks to crack problems which, in their general form, are NP-complete. In this context the Protein Folding problem, which has often been called the "holy grail" of bioinformatics, presents itself in a new and exciting form.

An example: The structural basis of the epigenetic code

The need for flexible docking of proteins in order to understand the assembly of large molecular complexes is highlighted by docking histone modifying enzymes (HMEs). The post-translational modifications of histone complexes have received a lot of attention due to their intimate link with gene regulation, development, stem cell reprogramming, aging and cancer. Especially modifications of the flexible tails are well studied, however the detailed molecular mechanisms of the epigenetic code are unclear to date. Recently modifications of the histone core domains have been described. This leads to the 'lateral surface' hypothesis, stating that modifications at the histone-DNA interface determine nucleosome mobility and positioning. A structural analysis of several HMEs reveals a possible common mode of action. We performed docking of HMEs of known structure to the nucleosome which provides novel insights as to how tail modifications could be translated into core modifications in a condition dependent manner, hence providing a structural basis to the epigenetic code. However, such a practical application

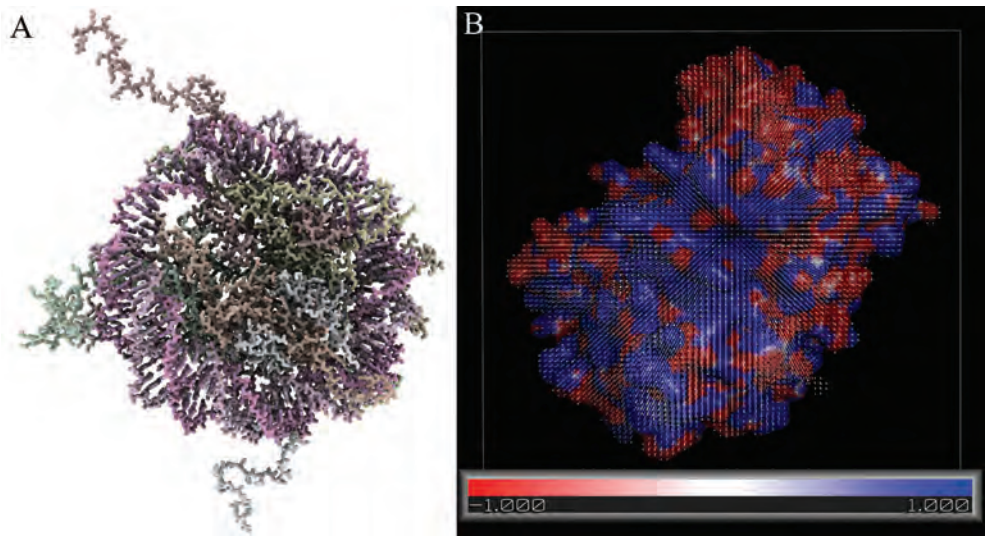

Figure 1: The structural basis of the epigenetic code ***(A)*** *Overview of the nucleosome shows the histone octamer with the double-stranded DNA wrapped around it. The interface between DNA and the histone-core is called the lateral surface of the "wheel-like" core structure. Several Lysine and Arginine positions on the lateral surface are known to be target sites for histone modifying enzymes.* ***(B)*** *Electrostatic surface representation of Rtt109 (PDB 3d35), a fungal-specific histone acetyltransferase (HAT) that modifies histone H3 lysine 56 on the lateral surface to promote genome stability. Rtt109 does not show sequence conservation with other known HATs and depends on association with either of two histone chaperones, Asf1 or Vps75, for HAT activity. The electrostatic surface of the protein is shown with the electro-positive surface in blue and the electro-negative surface in red. A long (blue) stretch of the electro-positive surface on Rtt109 reveals a potential binding site for nucleosomal DNA. The binding of the Rtt109 onto the nucleosomal DNA is proposed as a prerequisite of its ability to modify the histone core residue H3K56. (Figure rendered using A) QuteMol, B) PyMol.)*

highlights the shortcomings of existing approaches: To-date, docking is generally treated as a "rigid-body" problem. Even when the structures of all individual components are known it is remains impossible to satisfy all known experimental restraints simultaneously when the interacting proteins undergo conformational changes upon binding. Since most complexes are far to big for molecular dynamics (MD) simulations, fast flexible docking methods based on contact maps is the most promising way forward. Treating proteins as networks of non-covalent interactions has several advantages, as such a coarse-grained description codes for an entire ensemble of similar structures and, like NMR ensembles, captures aspects of protein dynamics. Since contact maps are binary adjacency matrices, this view offers new lines of investigation within a graph-theoretic framework. Constraint-based methods are several orders of magnitude faster than classical MD-simulations, hence our development of novel multi-body potentials as energy functions within such a probabilistic framework are described below.

3D Reconstruction from contact maps

Figure 2: 3D Reconstruction Ensemble. Reconstruction into three dimensions from a contact map produces a typical ensemble of conformations consistent with the given spatial constraints. All conformations are within 2Å C_α rmsd of the native crystal structure (shown in blue). Shown here is an ensemble for PDB 1bxyA, calculated using an implementation of the EMBED algorithm (distance geometry from the TINKER molecular dynamics package).

The conversion of contact maps back into a three-dimensional structure is central to our research. Using distance geometry we implemented a reconstruction pipeline based on the TINKER package. In principle this is similar to structure resolution using NMR spectroscopy. The structures obtained in this way are better than most NMR based structures, as the quality of the input constraints provided by crystal structures is better than the NOEs used in NMR. Like in NMR, there is usually not a unique solution in terms of 3D co-ordinates: a contact map encodes an entire ensemble of structures consistent with the given distance constraints.

Given the contact map information derived from a crystal structure, we obtain reconstructions usually better than 2 Å C_α rmsd (root-mean-square deviation), approaching the experimental limit. Given a set of biophysically realisable (native) contacts, we are now able to recover the protein structure at near experimental accuracy. We also observe that up to 80% of contact information is redundant and can be removed (at random) without significantly affecting reconstruction quality.

Protein structure prediction

In order to evaluate the performance of our newly developed structure prediction pipeline, we participated in the 8th edition of CASP (Critical Assessment of protein Structure Prediction). The prediction season lasted from May to July 2008, where a total of 57 targets were released. Our methods integrate information from several related templates by "graph averaging" which is generating consensus contact maps. Our contributions in the human expert and the fully automated category performed relatively well in comparison with state-of-the-art methods. In particular the fully automated predictions, in the meta-server category, did especially well in the residue-residue contacts category beating most of the groups in many targets. With the advanced concepts of essential subnetworks and multi-

body scoring functions we are preparing for CASP 9 in 2010 to put our contact prediction and embedding methods to the test in a rigorous international competition.

Mutations and cancer

The understanding of protein structures in terms of residue interaction networks is ideally suited to the analysis of nsSNPs. Current high-throughput sequencing technologies in combination with targeted enrichment methods enable genome-wide studies of cancer-causing mutations with large sample sizes. In the context of the Mutanom project (www.mutanom.org) we investigate several known oncogenes in order to find patterns of mutations in the underlying structure networks. The explicit goal is to make sense of the massive influx of next generation sequencing data. The results are used to select target mutations for further functional analysis and to obtain statistical trends of cancer-causing mechanisms at the structural level. In an initial study we have analysed 211 mutations from four different tumor types (breast, colon, stomach, prostate) in 19 well-known cancer genes. By comparing cancer mutations to a control set of mutations in healthy populations, we obtain statistical trends and assess the significance of the findings. Preliminary results indicate that in oncogenes frequently observed mutations tend to occur and cluster in mutation hotspots at or near the surface of the protein, putatively involved in binding. Probably such nsSNPs are non-disruptive to the structure of oncogenes but tend to affect functional sites with more subtle consequences to binding affinities and specificity.

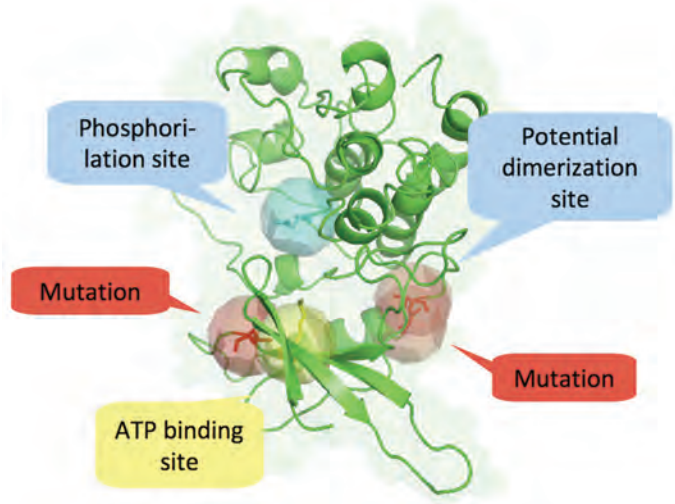

Figure 3: Mapping cancer mutations to protein structures. The epidermal growth factor receptor 2 (ERBB2) is implicated in aggravating tumor growth in breast cancer. The figure shows mutations found in cancer patients (red spheres) and important sites for the function of this protein. Proximity between mutations and functional sites is often not visible in the sequence, but becomes apparent in the context of the three dimensional structure. The effects of mutations on functional sites provide insights into possible mechanisms of cancer initiation and help to select subsequent experiments.

The essence of protein structures

With the reconstruction pipeline established, we addressed the questions of "What are the important interactions in a protein?" and "Is there an 'essence' of a structure?". The goal is to determine a set of key residue contacts that are sufficient to dene the structure: a minimal reconstructable subset. We developed several algorithms capable of deriving extremely sparse subsets with approx. 4,8 Å $C_\alpha$rmsd reconstruction accuracy with less than 10% of the native contacts. At the same time, a randomly chosen subset of native contacts needs about twice as many contacts to reach the same level of accuracy. This is the first algorithm ever described that significantly outperforms random selection and presents a big step towards identifying essential contacts of protein structures. This "structural essence" opens new avenues in the fields of structure prediction, empirical potentials and docking. Furthermore such a minimal set obviously is entropically favorable. This establishes a theoretical link to the Transition State Ensemble - hence providing new insights into one of the most central notions in protein folding.

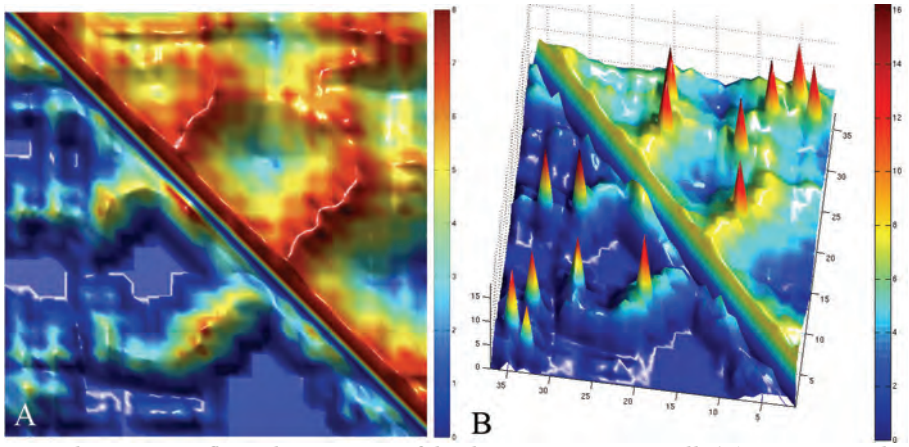

Figure 4: Essential residue contacts in protein structure reconstruction. ***(A)*** *Protein structures can be represented as networks of non-covalent contacts, but not all contact make the same contribution towards structural integrity. The upper right displays the distance matrix of a protein (PDB 1e0l) from which the contact map is derived. The lower left shows the common neighborhood size computed from the contact map. Obviously, such a network property reflects the structure of the distance map very well.* ***(B)*** *Using a combination of sequence and network descriptors a small number of essential contacts is identified. This "structural essence" constitutes less than 20% of the native contacts. Shown are the matrices from A) with essential contacts highlighted as cones. These cones have the property of covering the underlying distance landscape.*

Contact-map prediction

Of course, the next logical step is to predict contact maps accurately in the absence of any related structure - the equivalent of *de novo* structure prediction. In order to infer contacts from sequence alone, we are working on efficient algorithmic solutions to multi-body scoring functions. Existing approaches for structure prediction or scoring native conformations from decoys are usually based on pairwise interaction potentials. Essentially, the log-likelihood of observed vs. expected frequencies of contacts between pairs of amino-acids is calculated. However, the expected frequencies are estimated from a reference state that assumes the amino-acid residues are statistically independent like in the gas-phase. It has been shown that pairwise potentials cannot distinguish properly folded from unfolded structures accurately, but scoring based on essential subnets could provide an avenue for novel scoring functions and structure prediction methods. Our current line of research represents a quite radical shift in paradigm: In order to capture the multitude of co-operative effects that characterize the folding problem we turned to devise statistical multi-body potentials.

Multi-body scoring functions

Put briefly, the representation of a proteins conformation as a contact map implies a discretisation of the available conformational search-space. We actually use the dynamic nature of proteins to compare the current to all neighboring states. This can be expressed quite simply by insert- and delete-operations in contact map space. The resulting dynamic programming matrix is used as a proxy for the conformational entropy accessible to each residue. The important difference to existing methods is the choice of reference state (i.e. the quasi-chemical approximation). In contrast, our multi-body potential makes minimal assumptions about the reference state such that only minimal and biophysically realistic changes from the current environment have to be considered. Initial tests indicate that such a scoring function is indeed capable of distinguishing native from perturbed residue environments. Advancing multi-body scoring functions beyond the limits of existing approaches is of crucial importance. In this context, an information theoretic treatment leads to an optimised definition of the initial contact model. We observe a distinct peak of information entropy with varying cut-off. In addition, the discretisation also decreases the search-space for folding simulations by several orders of magnitude. A prototype of such a potential already exhibits two crucial characteristics: First of all, the native state is stabilised compared to decoy

structures. And secondly, even when crucial long-range interactions are removed, the potential is able to re-predict some of the missing contact information. While completing the work on statistical multi-body potentials for *de novo* structure prediction, we are currently building a "hybrid engine" by combining statistical inference in contact-graphs with geometric reconstruction to ensure 3D-embedability of all predicted contacts.

Summary & outlook

We advanced and developed novel approaches to the problems of protein folding and exible docking, based on the view that they follow the same principles as being the "two sides of the same coin". This work is motivated by insights into biological networks and their emergent properties. At the same time, the practical applications of this work are in protein design, mutational analysis, design of evolvable libraries for directed evolution, novel interaction screening technologies and structure-based drug design. As indicated before, such work opens new avenues for *de novo* inference of contact maps and consequently, structure prediction. The subsequent extension to flexible docking is relatively straightforward: Rather than finding the set of intra-chain contacts of the structure, the task becomes to find the inter-chain contacts that describe the protein-protein interface. Starting with a small number of biophysical realistic contacts between surface residues will avoid a combinatorial explosion of the search space. From such a "seed" the entire interface could be grown, using the multi-body potentials as described above. In summary, two key components (essential contact networks and multi-body scoring) to tackle the problems of protein folding and flexible docking are now at our disposal. With some optimisation remaining, we are now in a position to apply them successfully to some of the most pressing biological questions of our time.

General information

Complete list of publications (2006-2009)

2009

Sathyapriya R, Duarte JM, Stehr H, Filippis I, Lappe M. *Defining an Essence of Structure Determining Residue Contacts in Protein Structures*. PLOS Comput Biol 2009; 5(12):e1000584. doi:10.1371/journal.pcbi.1000584

Lappe M, Bagler G, Filippis I, Stehr H, Duarte JM, Sathyapriya R. *Designing evolvable libraries using multi-body potentials*. Curr Opin Biotech, doi:10.1016/j.copbio.2009.07.008

Daujat S, Weiss T, Mohn F, Lange UC, Ziegler-Birling C, Zeissler U, Lappe M, Schubeler D, Torres-Padilla M-E, Schneider R. *H3K64 trimethylation marks heterochromatin and is dynamically remodeled during developmental reprogramming*. Nature Struct Mol Biol, doi:10.1038/nsmb.1629

Milenkovic T, Filippis I, Lappe M, Przulj N. *Optimized Null Model for Protein Structure Networks*. PLoS ONE 4(6): e5967. doi:10.1371/journal.pone.0005967

2008

Bolser DM, Filippis I, Stehr H, Duarte J, Lappe M. *Residue contact-count potentials are as effective as residue-residue contact-type potentials for ranking protein decoys*. BMC Structural Biology 2008, 8:53 doi:10.1186/1472-6807-8-53

Slama P, Filippis I, Lappe M. *Detection of protein catalytic residues at high precision using local network properties*. BMC Bioinformatics 2008, 9:517 doi:10.1186/1471-2105-9-517

Stumpf MP, Thorne T, de Silva E, Stewart R, An HJ, Lappe M, Wiuf C. *Estimating the size of the human interactome*. Proc Natl Acad Sci USA 2008 May13; 105(19):6959-64. doi:10.1073/pnas.0708078105

2007

Jain BJ, Lappe M. *Joining Softassign and Dynamic Programming for the Contact Map Overlap Problem*. Springer Lecture Notes in Computer Science, S. Hochreiter and R. Wagner (Eds.): BIRD 2007, LNBI 4414, pp. 410-423.

2006

Paszkiewicz KH, Sternberg MJ, Lappe M. *Prediction of viable circular permutants using a graph theoretic approach*. Bioinformatics 22, 1353-8.

Open access activities

We continued to be active in the open-access front mainly through the creation and support of PDBWiki, a community based resource for the annotation of biological macromolecular structures. We created PDBWiki in August 2007 and were awarded the second place in the 3rd International BioWiki Contest.

Wiki-databases are becoming a significant contribution to the emerging field of biological community annotation. Our system features a single structured page for each PDB entry to which users can attach categorized comments. The resource has been successful in gathering part of the PDB community as demonstrated by over 40,000 visits to the home page and the numerous contributions to the PDB FAQ, cross reference tools and entry annotations. All software related to PDBWiki is accessible through an open subversion repository hosted at bioinformatics.org.

In line with this, some of our software has also been made publicly available using the open source paradigm. An example of it is CMView, a tool for contact map visualization and analysis. CMView is freely available from at http://www.molgen.mpg.de/~lappe/cmview. The source code is distributed under the GPL v2 license.

Finally, we published in Open Access journals whenever appropriate and possible, as most of our recent publications show.

Selected invited talks

Molecular Interaction Workshop 2009, Berlin, 16.-18.09.2009

Seminar at the Institute for Condensed Matter Physics (Festkörperphysik), TU Darmstadt, 02.-03.04.2009

Seminar at the Center for Bioinformatics, Hamburg, 26.06.2008

Seminar on Computational Proteomics, Dagstuhl, 02.03.-07.03.08

eScience-Seminar der GWDG, Göttingen, 22.03.2007

Work as scientific referee

Michael Lappe serves as scientific referee for the following journals: Advances in Data Analysis and Classification, Bioinformatics, BMC Bioinformatics, Genome Biology, IEEE/ACM Transactions on Computational Biology and Bioinformatics, IEEE Proc. Systems Biology, PLoS Computational Biology, Proteins: Structure Function and Bioinformatics, PROTEOMICS, Theoretical Chemistry Accounts.

In addition, Michael Lappe serves as scientific referee for the Deutsche Forschungsgemeinschaft (DFG).

Appointments of former members of group

Ioannis Filippis - Bioinformatician at the Centre for Bioinformatics, Division of Molecular Biosciences, Imperial College London, UK.

Dan Bolser - PostDoc at University of Dundee, UK.

Student thesis

Joachim von Eichborn (2006) *Network Analysis of Protein Sequence Conservation*, Bachelor thesis, Freie Universität Berlin (supervisor: Michael Lappe, together with Prof. Dr. Knuth Reinert, FU Berlin)

External funding

EU MRTN "ProSA": Selection and Analysis of Protein-Protein Interactions

International Max Planck Research School for Computational Biology and Scientific Computing

Teaching activities

Lecture *Algorithmic Bioinformatics* at Free University Berlin, section on Structural Bioinformatics

EU MRTN ProSA network teaching coordinator, organizing workshops on *Bioinformatics tools for experimental partners*

Workshop on *Molecular Modelling* at the internal PhD programm of the MPIMG

Organization of scientific events

PC member of the German Conference on Bioinformatics GCB2008

Chairman of the Dahlem Colloquium committee (Seminar Series in Molecular Genetics)

Training co-ordinator on EU Marie-Curie Research Training Network "ProSA".

Otto Warburg Laboratory
Nutrigenomics and Gene Regulation

(Established: 2003 as part of the Dept. of Vertebrate Genomics, independent since 01/08)

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Magdalena Kliem*
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Technician
Beata Lukaschewska-McGreal*

Research Concept

Many processes during embryonic and postnatal development as well as response to environmental factors such as (mal-) nutrition are controlled by complex molecular mechanisms. In order to prevent health decline and prolong the quality of life and as epidemiological studies in humans do not substantially prove causal connections between diet and disease, the acceptance of nutrition for the prevention of disease processes requires further molecular, cellular and whole-organism studies. Functional food and nutraceuticals, i.e. extracts or compounds of edible biomaterials with a medicinal effect on human health are attracting more and more scientific and public interest due to their high potential in health promotion and disease prophylactics. Moreover, highly potent natural products may be useful to develop pharmaceutical products.

Figure 1: Logo of the group

The Nutrigenomics and Gene regulation Group has been established in January 2008 as an independent, BMBF-funded Junior Research Group. Prior to his appointment as an independent group leader, Dr. Sascha Sauer was a scientist in the department of Prof. Hans Lehrach during 2003-2007. As a newly founded research group in 2008 at the Max Planck Institute for Molecular Genetics, the research group has been exploring health implications of the interaction between nutrition and genomics or the so-called "nutrigenomics". The regulation of genes plays an important role in various molecular processes of metabolic disorders such as insulin resistance. One emphasis

* externally funded

of our research lies in analysing the modulation of gene expression in cellular processes, for example cell differentiation. These processes can be significantly influenced through the interaction between genes and naturally occurring compounds. Consequently, as the second emphasis of our research group, we study the capability and mechanisms of natural products to interact with genes and gene products.

In order to identify active natural products, we screen and systematically characterise compounds including their mechanistic mode of action that are derived from small molecule libraries with a large structural variability. Our multidisciplinary approach consists of fundamental as well as applied research. The data obtained by systematic studies will be useful for modeling effects of natural products on, for instance, fat cell differentiation. Clearly, natural products with a beneficially active profile can be further exploited, for the development of nutraceuticals and/or for the development of novel chemical structures for treating insulin resistance and obesity. Furthermore, we work on the development of precise diagnostics for bacteria and complex diseases such as inflammatory barrier diseases like Crohn's disease, which include genetic susceptibility, anomalies of the bacterial gut flora, and the influence of nutrition.

Red grapes contain the natural product resveratrol.

Scientific achievements

In the past the group has developed a battery of methods for nucleic acids analysis including genotyping, molecular haplotyping, small molecular screening and protein analysis for basic research and diagnostic purposes. These techniques have been used in a number of collaborative national and international projects and are continuously being improved. Workhorses are several complementary mass spectrometers and a second generation sequencing platform. For the precise diagnosis of bacteria we have set up new comprehensive tools based on MALDI mass spectrometry that provide combined information on the nucleic acids and protein level. For example, we have published standardised methods for the efficient analysis of bacteria using this methodology.

Furthermore, the group has set up the facilities and protocols required for studying systematically gene-regulation processes and the interaction of small molecules such as natural products with cellular key regulators such as nuclear receptors and histone-modifying enzymes. For example, we have developed a novel functional high-throughput mass spectrometry assay to screen and characterise small molecules interacting with protein-modifying enzymes such as deacetylases or acetyl transferases. Moreover, we have - amongst others - identified novel structural classes of highly efficient natural products that specifically modulate transcriptional regulators such as PPARgamma, PPARalpha and LXRalpha or the human deacetylase Sirtuin 1. These compounds are currently being exploited in collaboration with the Lead Discovery Centre in Dortmund, Germany. Moreover, in collaboration with Sirtris Pharmaceuticals, a Glaxo Smith Kline company, we have discovered the mode of action of highly potent antidiabetic drugs such as SRT1720.

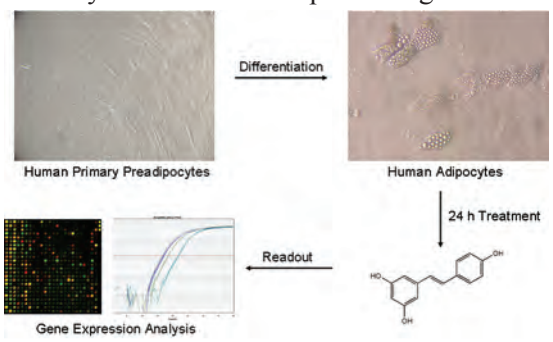

Figure 3: Investigation of PPARγ ligands in mature adipocytes.

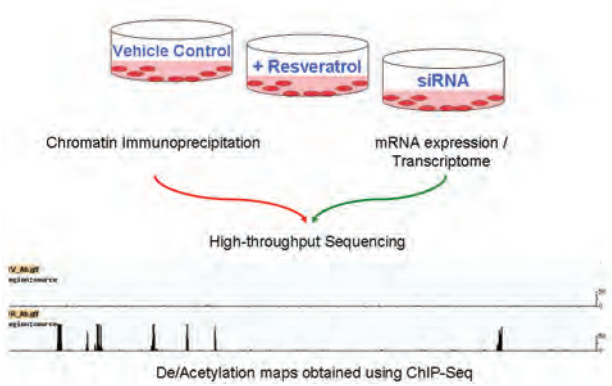

Figure 4: Genome-wide detection of histon modification caused by resveratrol treatment of cells.

In more basic research approaches, we discovered the molecular mechanisms of calorie-restriction mimetics such as the compound resveratrol that contributes to longevity in model organisms. Furthermore, we have set up the methodology to study genome-wide transient structural variation caused by transcription during cell differentiation processes. Thereby we could discover the snap-shots of transient genome-wide structural variation in the human genome caused among others by gene transcription.

All these recent results of the Nutrigenomics and Gene Regulation group are currently being patented and are in manuscript preparation.

Cooperation within the institute

- Martin Vingron: Bioinformatics analyses of 2nd generation sequencing data
- Richard Reinhardt, Hans Lehrach: Methods development

Special facilities/equipment of the group

- Nano-HPLC LTQ Orbitrap XL (EDT) ESI Mass Spectrometer (Thermo)
- Cap-LC HCT ultra mass spectrometer (Bruker)
- Genome Analyser II (Illumina)

General information

Complete list of publications (2006-2009)

2009

Baek YS, Haas S, Hackstein H, Bein G, Santana MH, Lehrach H, Sauer S, Seitz H. *Identification of novel transcriptional regulators involved in macrophage differentiation and activation in U937 cells*. BMC Immunol. 2009 Apr 2;10(1):18.

Darii E, Lebeau D, Papin N, Rubina AY, Stomakhin A, Tost J, Sauer S, Savva-teeva E, Dementieva E, Zasedatelev A, Makarov AA, Gut IG. *Quantification of target proteins using hydrogel antibody arrays and MALDI time-of-flight mass spectrometry (A2M2S)*. N Biotechnol. 2009 Mar 14.

Freiwald A, Sauer S. *Phylogenetic classification and identification of bacteria by mass spectrometry*. Nature Protoc. 2009;4(5):732-42.

Han CT, Holzhauser S, Sauer S. *Nutrigenomics and Gene Regulation*. Journal of Biotechnology in Berlin-Brandenburg: BioTOPics 36_Molecular Nutrition Research and Food Technology. 2009;36: 14-15.

Konrad K, Dempfle A, Friedel S, Heiser P, Holtkamp K, Walitza S, Sauer S, Warnke A, Remschmidt H, Gilsbach S, Schäfer H, Hinney A, Hebebrand J, Herpertz-Dahlmann B. *Familiality and molecular genetics of attention networks in ADHD*. Am J Med Genet B Neuropsychiatr Genet. 2009 May 5.

Sauer S, Kliem M. *Mass spectrometry tools for classification and identification of bacteria*. Nature Rev. Microbiology, accepted

Sauer S. "Tools for Detection of DNA Polymorphisms", ed. T.P. Begley, Wiley Encyclopedia of Chemical Biology, Wiley& Sons, 2009

Schimmelmann BG, Friedel S, Nguyen TT, Sauer S, et al. *Exploring the genetic link between RLS and ADHD*. J Psychiatr Res. 2009 Feb 14.

Stracke S, Haseneyer G, Veyrieras JB, Geiger HH, Sauer S, Graner A, Piepho HP. *Association mapping reveals gene action and interactions in the determination of flowering time in barley*. Theor Appl Genet. 2009 Jan;118(2):259-73.

2008

Haseneyer G, Ravel C, Dardevet M, Balfourier F, Sourdille P, Charmet G, Brunel D, Sauer S, Geiger HH, Graner A, Stracke S. *High level of conservation between genes coding for the GAMYB transcription factor in barley (Hordeum vulgare L.) and bread wheat (Triticum aestivum L.) collections*. Theor Appl Genet. 2008;117(3):321-31.

Sauer S, Freiwald A, Maier T, Kube M, Reinhardt R, Kostrzewa M, Geider K. *Classification and identification of bacteria by mass spectrometry and computational analysis*. PLoS ONE. 2008;3(7): e2843.

Sauer S. "Chapter 20: *Matrix-assisted Laser Desorption/Ionization Mass Spectrometry: Principles and Applications in Life Sciences*", Lasers in Chemistry, Volume 1, 593-616, Wiley-VCH, 2008

2007

Friedel S, Saar K, Sauer S, et al. *Association and linkage of allelic variants of the dopamine transporter gene in ADHD*. Mol Psychiatry. 2007;12(10):923-33. Epub 2007 Apr 10. (Abstract)

Sauer S. *The essence of DNA sample preparation for MALDI mass spectrometry*. J Biochem Biophys Methods. 2007;70(2):311-8. Epub 2006 Oct 21.

Sauer S. *MALDI mass spectrometry detection of oligonucleotides*. Bio Tech International. 2007;19(1):11-13.

2006

Kepper P, Reinhardt R, Dahl A, Lehrach H, Sauer S. *Matrix-assisted laser desorption/ionization mass spectrometric analysis of DNA on microarrays*. Clin Chem. 2006;52(7):1303-10.

Sauer S, Reinhardt R, Lehrach H, Gut IG. *Single Nucleotide Polymorphisms: Analysis by Mass Spectrometry*. Nature Protoc. 2006;1(4):1761-1771.

Sauer S. *Typing of single nucleotide polymorphisms by MALDI mass spectrometry: principles and diagnostic applications*. Clin Chim Acta. 2006;363(1-2):95-105.

Sauer S. "*SNP Detection and Mass Spectrometry*", Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine, Springer, 2006

Sauer S. "*Analysis of DNA variation by MALDI mass spectrometry: Recent developments and perspectives*", Recent Developments in Nucleic Acids Research, 1-14, Transworld Research Network, 2006

Selected invited talks

Next Generation Tools for the Analysis of Nucleic Acids, World Genome and DNA DAY Conference, Chair of Session "SNPs", Dalian/China, 28.4.2005

Analysing nucleic acids by mass spectrometry: applications to SNP geno-typing in microbial and human genetics, Institut Pasteur, Paris, France, 10.3.2005

Memberships

- Network Nutrigenomics Berlin-Brandenburg (www.nutrigenomik.de)
- National Network of Genome Research (NGFN) (www.ngfn.de)
- MolTools Consortium (www.moltools.org)
- READNA consortium (www.cng.fr/READNA)

Work as scientific referee

Sascha Sauer serves as scientific referee for the following journals: Nucleic Acids Research, BMC Genomics, Pharmacogenomics, BioTechniques, Expert Review of Proteomics, Journal of Biotechnology, Journal of Mass Spectrometry.

Student theses

Viola Nicolaysen (2008), Bachelor thesis, University of Potsdam (supervisor: Sascha Sauer)

Christopher Weidner (2007), Diploma Thesis, Freie Universität Berlin (supervisor: Sascha Sauer)

Patents

Method of analyzing nucleic acids with mass spectrometry, Sascha Sauer, Pamela Kepper, Richard Reinhardt, Hans Lehrach, EP 1775348 A1

Method for high-throughput screening of test molecules binding to target molecules (II), Sascha Sauer, Konrad Büssow, Hans Lehrach, WO 2006/015797

Method for high-throughput screening of test molecules binding to target molecules (I), Sascha Sauer, Konrad Büssow, Hans Lehrach, WO 2006/015796

External funding

BMBF (Junior Research Group/Molecular Nutrition Research)

NGFNplus

READNA (EU-FP7)

GABI-Génoplante

BioProfile

NGFN2

Moltools (EU-FP6)

Guest scientist

Dr. Lei Mao, Charité Berlin, 04/08 - 04/10

Teaching activities

Lecture *Analyse von kleinen Molekülen und Nukleinsäuren* (original title), each term since summer 07, FU Berlin

Lecture *Moderne Methoden der Bioanalytik* (original title), SS 06, WS 06/07, FU Berlin

Practical course *Freie Mitarbeiten in der Nukleinsäureanalytik*, SS 06, FU Berlin

Lecture *Moderne Methoden der Proteinanalytik* (original title), WS 05/06, FU Berlin

Lecture *Von der Suche nach vasoaktiven Hormonen zum Blutdruck senkenden Therapeutikum* (original title), SS 05, WS 05/06 FU Berlin

Seminar and practical course *Massenspektrometrische Analyse von Nukleinsäuren und kleinen Molekülen* (original title), SS 05, WS 05/06 FU Berlin

Organisation of scientific events

READNA SYMPOSIUM and Project Meeting ON ADVANCED NUCLEIC ACID ANALYSIS METHODS, Harnack-Haus, Berlin, 6th-7th of July 2009

READNA Workshop on DNA Enrichment Methods, Harnack-Haus, Berlin, 15th-16th of January 2009

Public relations

Berlin Long Night of Sciences, open house visit and presentation of the laboratory to the public (13/06/2009)

Otto Warburg Laboratory
Molecular Interaction Networks

(Established: 06/2007)

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Scientists
Nouhad Benlasfer (since 05/08, guest)
Petra Birth (since 06/08)
Helman Reynaldo López-Mirabal* (since 07/08)

PhD students
Arndt Grossmann (since 07/07)
Josphine Worseck (since 09/07)
Mareike Weimann (since 12/07)

Engineer
Thomas Przewieslik (05/08-03/09, part time)

Technician
Anna Hegele* (since 06/07)

Introduction

Protein-protein interaction (PPI) networks are very useful for the prediction of the function of proteins, their involvement in cellular processes and their ability to form protein complexes. Moreover, PPI networks provide a framework for a systems understanding of the molecular biology of the cell. Recent global PPI studies e.g. suggest that cellular processes are orchestrated by much larger protein networks than previously thought. Comprehensive understanding of how the human protein machinery interacts is important as large molecular assemblies, both static and dynamic, transmit and respond to intra and extra cellular alterations determining the phenotypic outcome of an organism.
Concomitant to offering a more comprehensive understanding of cellular biology, molecular network studies can improve the practice of medicine. This involves the study of network properties of human disease genes, the use of PPI information to implicate disease modulating genes, the identification of disease related subnetworks or the network based classification of patient samples.
The group is focusing on the analysis of molecular interaction networks with the aim to understand the dynamics of molecular networks underlying cellular processes related to human disease. Experimental functional genomics techniques, e.g. high-throughput yeast two-hybrid (Y2H) screening, are utilized in combination with biochemical, cell biological and computational methods.

* externally funded

Systematic generation of protein-protein interaction networks

Despite successful attempts to systematically map protein-protein interaction networks and their usefulness in further studies, it remains difficult to assess the quality of existing data sets. To provide thorough experimental and theoretical assessment of current PPI screening techniques and data we teamed up in an international collaboration (led by the Vidal Lab, CCSB, Boston MA). First, a conceptual framework for binary interactome mapping was introduced. It allows empirical assessment of the quality of interaction datasets by combining positive and negative training sets with independent interaction assays. Second, we experimentally assessed our data in comparison to large literature PPI collections demonstrating that protein interaction mapping can be achieved with high sensitivity and specificity using high-throughput Y2H setups. Furthermore we estimate that the human interactome contains ~ 130,000 binary interactions, most of which remain to be mapped (Venkatesan et al. Nature Meth. 2009).

This work provides a reference point for large scale PPI mapping and revealed many clues relevant to develop improved screening strategies. At the institute we set up robotic systems for Y2H interaction matrix screening. The prey matrix was extended now harboring ~ 12000 full length human proteins (with E.E. Wanker, MDC Berlin) and we applied novel repeat screening protocols yielding PPI data with significantly improved specificity and sensitivity. The setup is extensively used for interaction screens in the lab and in collaborative projects *e.g.* with the Dept. Herrmann (Schrewe, Bauer, Grote). In addition we are developing protocols applying 2nd generation Illumina sequencing. Because of the large number of pairwise protein combinations, the primary screening step in the current automated mating protocol is the bottle neck in terms of time and cost. To overcome this step, interaction pairs are pooled and interacting proteins are fully sequenced using parallel sequencing technology followed by deconvolution and pairwise retest of interacting proteins. This approach has the potential to increase the throughput of high quality protein interaction mapping up to ten fold.

Dynamic alterations in protein-protein interaction networks

Activation of cellular networks often occurs in response to extracelluar stimuli. In signaling networks the information dynamically propagates from the plasma membrane through a network of coupled signaling protein interactions to the nucleus regulating components necessary to express a response phenotype. However, PPI information obtained from Y2H analysis as such is a qualitative, static representation of protein-protein relationships that do not directly relate to any biological situation in the cell. Although the PPI network includes information about components potentially involved in cellular signaling pathways it does not provide immediate information about the signal flow that propagates from membrane receptors leading to transcription factor activation.

To obtain a dynamic view of a cellular signaling network and to predict potential signaling modulators, we created a network for proteins implicated in EGF/Erk signaling connecting 1126 proteins *via* 2626 PPIs using automated Y2H interaction mating. From this interaction map, a network model of activated signaling was generated using a naïve Bayesian classifier. Information on shortest PPI paths from membrane receptors to transcription factors was exploited to predict input/output relationships between interacting proteins (Fig. 1). The input/output relationships mimic the signal transmission in the cell. Analysis of the model revealed that activation of EGF/Erk signaling modulates and can be modulated by many proteins which are distant to the core EGF/Erk pathway members. Integration of time resolved phosphoproteomics data gave a first dynamic view on how the EGF stimulus transmits and spreads in a cellular interaction network. Moreover, signal

flow analysis in our directed network allowed predictions of potential modulators of EGF/Erk signaling. 19 of 50 candidate proteins were validated successfully in mammalian cell-based assays demonstrating that these proteins can change Erk phosphorylation quantitatively in the presence or absence of EGF. Proteins identified here may contribute e.g. to up regulation of MAPK pathway activity in human cancers (cooperation with the lab of E. E. Wanker, MDC Berlin). This experimental and computational approach is generic and provides a framework for elucidating causal connections between proteins facilitating the identification of factors modulating the flow of information in signaling networks.

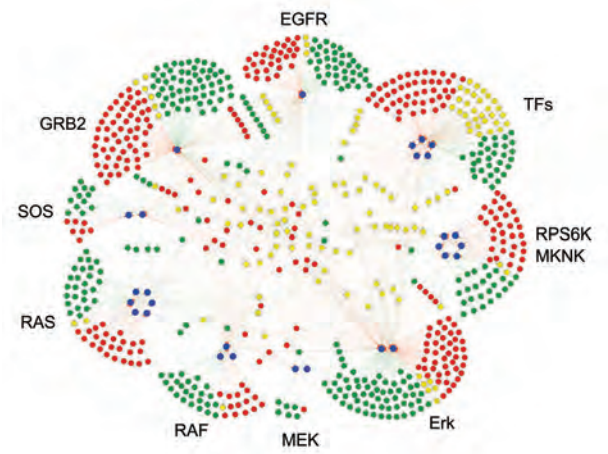

Figure 1: Model of a directed PPI signaling network. The EGF/Erk pathway and 733 direct interacting partners are shown. Blue nodes represent the 28 members of the EGF/Erk core pathway in a counterclockwise arrangement. Red and green nodes correspond to input and output nodes linked to the core pathway, respectively. Proteins that are both input and output nodes are shown in yellow.

Modification-dependent protein interactions

The cellular response to changing conditions is frequently mediated by the reversible covalent modification of proteins. In eukaryotic cells, phosphorylation of the amino acids serine, threonine, and tyrosine of proteins is very common and of great importance for cellular function. Phosphorylation (P)-dependent interactions are particularly relevant for the dynamics of PPI networks, as these interactions are clue to the fast activation/deactivation of cellular signaling cascades. Current methods for the identification of kinase substrates and P-dependent interactions are mainly based on *in silico* homology studies, *in vivo* knockout strategies or *in vitro* techniques. However, the majority of P-dependent interactions remains to be discovered. This task is particularly important, as several kinases as well as proteins with P-binding domains have been shown to be major players in oncogenic signaling networks determining growth control pathways and thus contributing e.g. to human malignancies.

We have established a modified Y2H setup employing kinases to detect P-dependent protein-protein interactions. Combinations of bait and kinase proteins are used to search for interacting human prey proteins. Interactions that do show up in the presence of active kinases but not in their absence indicate that phosphorylation of the prey or bait protein is necessary for interaction (Fig. 2). In a genome wide approach, we have identified more than 150 novel P-dependent PPIs that show high specificity with respect to human kinases. P-dependent interactions are further analyzed in mammalian cell culture systems using e.g. co-immunoprecipitation

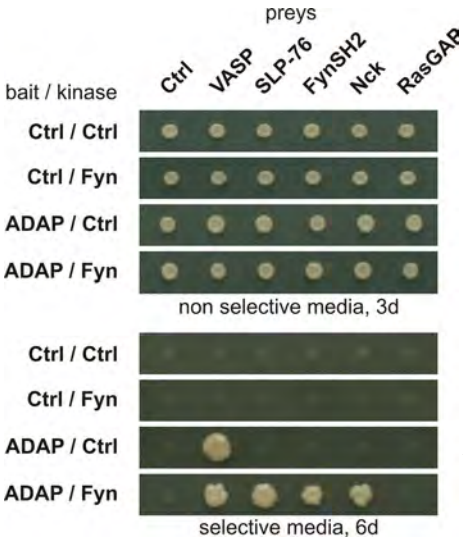

Figure 2: Analysis of P-dependent interactions involved in T-cell activation in a modified Y2H system. Diploid yeast colonies expressing indicated bait, prey and kinase constructs were assayed for growth on non selective and selective media, respectively. Y2H interaction between ADAP and VASP is independent of the Fyn kinase. Growth on selective media of ADAP in combination with SLP-76, Fyn-SH2 and Nck preys is strictly dependent on the presence of active Fyn, indicating direct, P-dependent protein interactions.

strategies. Candidate modified amino acid residues are identified using an immunoprecipitation / mass spectrometry approach, *via* integration of large scale phosphoproteomics data or motif search algorithms.
Our screening approach will be extended to other postranslational protein modification such as acetylation, methylation, ubiquitylation or ADP-ribosylation. It is complementary to existing strategies e.g. peptide arrays or AP-MS techniques as we are analyzing binary P-dependent interactions between full length proteins in a cellular environment. The results indicate that the context of interacting proteins strongly determines specificity of P-dependent interactions. Integration with time resolved proteomics data that e.g. record phosphorylation patterns of proteins in response to cellular stimuli results comprehensive probabilistic models of distinct network states that correlate with cellular phenotypes. This will promote a systems understanding of cellular processes and their alteration in human disease revealing novel starting points for therapeutic intervention.

General information

List of publications (06/2007-2009, with MPIMG affiliation)

2009

Palidwor GA, Shcherbinin S, Huska MR, Rasko T, Stelzl U, Arumughan A, Foulle R, Porras P, Sanchez-Pulido L, Wanker EE, Andrade-Navarro MA. *Detection of alpha-rod protein repeats using a neural network and application to huntingtin*. PLoS Comput Biol. 2009 Mar;5(3):e1000304.

Venkatesan K*, Rual JF*, Vazquez A*, Stelzl U*, Lemmens I*, Hirozane-Kishikawa T, Hao T, Zenkner M, Xin X, Goh KI, Yildirim MA, Simonis N, Heinzmann K, Gebreab F, Sahalie JM, Cevik S, Simon C, de Smet AS, Dann E, Smolyar A, Vinayagam A, Yu H, Szeto D, Borick H, Dricot A, Klitgord N, Murray RR, Lin C, Lalowski M, Timm J, Rau K, Boone C, Braun P, Cusick ME, Roth FP, Hill DE, Tavernier J, Wanker EE, Barabási AL, Vidal M. *An empirical framework for binary interactome mapping*. Nature Methods. 2009 Jan;6(1):83-90. (*These authors contributed equally to this work.)

Vinayagam A, Stelzl U, Wanker EE. *Repeated two-hybrid screening detects transient protein–protein interactions*. Theor Chem Acc 2009; DOI 10.1007/s00214-009-0651-8

2008

Stelzl U, Nierhaus KH. *In vitro selection of random RNA fragments to identify protein-binding sites within large RNAs*. Methods Mol Biol. 2008;488:247-55.

Ozlem Tastan Bishop A, Stelzl U, Pech M, Nierhaus KH. *Characterization of RNA-protein interactions by phosphorothioate footprinting and its applications to the ribosome*. Methods Mol Biol. 2008;488:129-51.

Selected invited talks

Constructing directed protein interaction networks for activated EGF/Erk signaling, EBI Seminars in Systems Biology: European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, UK, 02.06.2009

Constructing causal protein interaction networks for activated EGF/Erk signaling, CSHL Meeting Systems Biology: Networks, Cold Spring Harbor Laboratory, New York, USA, 18.-22.03.2009

Systematic Analysis of Human Protein-Protein Interactions, First Status Seminar of the Helmholtz Alliance on Systems Biology, Potsdam, Germany, 22.-24.06.2008

Future developments of proteomics for cancer risk assessment, invited talk at the International Workshop: „Omics for Assessing Unclear Risks", Berlin, Germany, 26.-28.05.2008

Improving Y2H protein-protein interaction networks, HUPO PSI Spring Meeting 2007, Lyon, France, 23.-25.04. 2007

Awards

Erwin Schrödinger Prize 2008 (http://www.helmholtz.de/en/research/research_awards/)

Scientific referee

Ulrich Stelzl serves as scientific referee for the following journals: Molecular Systems Biology, PLoS Computational Biology, Trends in Biotechnology, Trends in Biochemical Sciences, BMC Pharmacology, Briefings in Functional Genomics and Proteomics, Cell Research (NPG, China), Protein Science, Journal of Proteome Research, BBA - Proteins and Proteomics, Bioinformatics.

In addition, Ulrich Stelzl serves as referee for the following institutions: Leibniz Gemeinschaft (SAW-Verfahren), Dutch National Science Foundation (NWO).

External funding

BMBF, NGFNplus, *NeuroNet, TP3*, 06/08 - 05/11

HGF, *Alliance on Systems Biology*, 01/08 - 12/11 (jointly with E. Wanker, MDC, Berlin-Buch)

Guest scientists

Monserat Soler-López, Institute for Research in Biomedicine and Barcelona Supercomputing Center, Barcelona, Spain, 01.-31.07.2008

Public relations

Participation in the Lange Nacht der Wissenschaften 2009

Erwin Schrödinger Price 2008:

- Portrait by the Austrian Television ORF, broadcast 09/2008 at ORF1.
- Reports in newspapers / magazines, e.g. Hamburger Abendblatt, Salzburger Nachrichten, Der Standard, Max-Planck Intern

Ribosome Group

Translation, Structure & Function of Ribosomes

(Established: 1969-03/2010)

Head
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Scientists
Dr. Markus Pech* (since 07)
Dr. Hiroshi Yamamoto* (since 07)

PhD students
Romi Gupta (since 06)
Jarek Kijek* (since 08)
Zhala Karim (since 06)

Technicians
Renate Albrecht (since 07)
Edda Einfeldt (since 89)

Research concept

We study assembly, structure and function of the ribosome, mainly that of the bacteria *E. coli*. The structure of functional complexes is preferentially analyzed *via* cryo-electron microscopy (cryo-EM) in cooperation with the group of Prof. Dr. Christian Spahn, Charité Berlin.

Scientific achievements /findings

Antibiotics

The inhibition mechanism of *fusidic acid*, an inhibitor of the translocation factor EF-G was kinetically unraveled [Seo et al., 2006, together with B. Cooperman, Philadelphia]. The binding site of *kasugamycin* and its specificity of blocking the 70S initiation rather then the 30S initiation was unraveled [Schluenzen et al., 2006, together with P. Fucini, MPIMG Berlin].

Evolution of the translational machinery

A new ribosome-type 61S was found in *E. coli* upon administering the drug kasugamycin. The 61S ribosome has a small subunit lacking 11 out of 21 proteins, the 50S is normal. This particle translates exclusively leaderless mRNA, a molecular fossil present in all domains, and it allows a view on a translational machine existing about 3 billion years ago [Kaberdina et al, 2009, together with I. Moll, Vienna]. We published two short reviews with some ideas of the ancient translation machinery and some ways to save energy during protein synthesis [Nierhaus 2007; Szaflarski & Nierhaus, 2007].

* externally funded

Principles of ribosomal functions

We detected a new elongation factor practically present in all bacteria, mitochondria and chloroplasts, namely LepA which we re-named EF4. This factor is one of the highest conserved proteins known and has a new function in that it back-translocates stalled ribosomes, i.e. the opposite reaction of the translocase EF-G. In that way it mobilizes stalled ribosomes and increases the active fraction of the synthesized protein [Qin et al., 2006]. We also identified its binding site on the ribosome *via* cryo-EM, which illustrated the mechanism of the back-translocation [Connell et al., 2008, together with C.M.T. Spahn, Charité Berlin]. We analyzed in detail functional features of ribosomes from higher eukaryotes (rabbit liver) and compared them with features of the bacterial ribosome [Szaflarski et al., 2008; together with A. El'skaya, Kiev]. We identified an essential importance of the E-site for the accuracy during the elongation process, and we also could show that the Shine-Dalgarno interaction functionally replaces the lack of codon-anticodon interaction of the E-tRNA just after initiation [Di Giacco et al., 2008; together with F. Triana-Alonso, Maracay, Venezuela]. Small iRNAs regulate initiation of bacterial protein synthesis; we could demonstrate the first case that an iRNA interferes with the decoding region of an mRNA also blocking initiation [Bouvier et al., 2008; together with J. Vogel, Charité Berlin]. Bacterial EF-G factors have two different functions, namely promoting (i) translocation and (ii) the termination process. The details of the latter involvement are unknown. Mitochondria have two EF-G's, mEF-G1 and mEFG2. We could demonstrate mEF-G1 promotes exclusively translocates, whereas EF-G2 is exclusively involved in the termination process. This opens the possibility to analyze in detail the latter process [Tsuboi et al., 2009; together with N. Takeuchi, Tokyo].

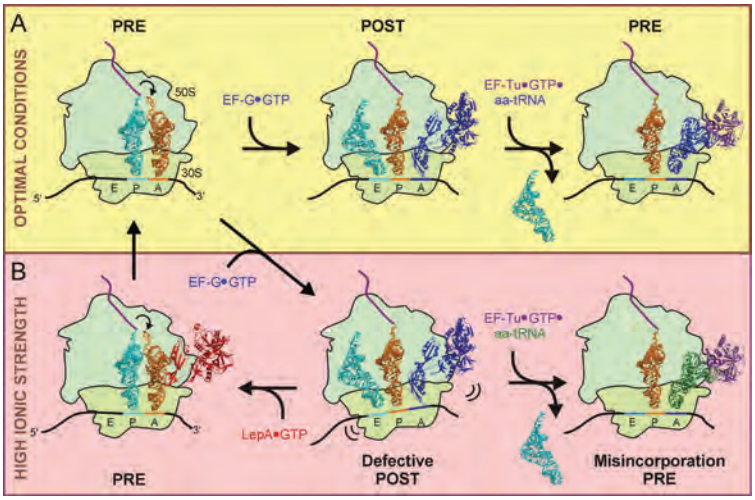


Figure 1: Model for LepA (EF4) Function. ***(A)*** *Under optimal growth conditions the translocation has a very low rate of error, and therefore, EF4 is not so important under such conditions. Translocation involves the movement of tRNAs at the A and P sites (PRE state) to the P and E sites (POST state). This reaction is catalyzed by elongation factor G (EF-G, blue) and GTP. After dissociation of EF-G, the A site is now free for binding of the next ternary complex aa-tRNA EF-Tu GTP (blue tRNA to blue A site codon), which leads to release of the E-tRNA (cyan).* ***(B)*** *In the rare case that EF-G malfunctions, a defective translocation complex may result. This is likely to occur more frequently under conditions of high ionic strength. The consequences of the defective translocation complex are 2-fold: (1) ribosomes may incorrectly display the A site codon, allowing binding of near-cognate ternary complexes and therefore misincorporation, as illustrated by the right-hand pathway (binding of green tRNA to blue codon); (2) under extreme conditions, the ribosome may even become stuck, thus precluding continued translation. The defective translocation state is recognized by EF4 GTP (red), which induces a back-translocation, allowing EF-G a second chance to catalyze a correct POST state. In this way EF4 reduces translational errors and relieves stuck ribosomes.*

General information

Complete list of publications (2006-2009)

2009

A. Katranidis, D. Atta, R. Schlesinger, K. H. Nierhaus, T. Choli-Papadopoulou, I. Gregor, M. Gerrits, G. Büldt and J. Fitter: *Fast Biosynthesis of GFP Molecules: A Single-Molecule Fluorescence Study*. Angew. Chem. Int. Ed. Engl. 48:1758-1761 (2009)

A. C. Kaberdina, W. Szaflarski, K. H. Nierhaus and I. Moll: *An unexpected type of ribosomes induced by kasuga-mycin: a look into ancestral times of protein synthesis*? Mol. Cell. 33:227-236 (2009)

D. N. Wilson, R. Gupta, A. Mikolajka, and K. H. Nierhaus: *Ribosomal Proteins: Role in Ribosomal Functions.* In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0000687.pub3 (March 2009)

D. N. Wilson, A. L. Starosta, H. Yamamoto and K. H. Nierhaus: *Inhibitors of the elongation cycle of protein synthesis*. In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0000550.pub2 (June 2009)

M. Tsuboi, H. Morita, Y. Nozaki, K. Akama, T. Ueda, K. Ito, K. H. Nierhaus and N. Takeuchi: *EF-G2mt is an exclusive recycling factor in mammalian mitochondrial protein synthesis*. Mol. Cell 35:502–510 (2009)

2008

M. Pech and K. H. Nierhaus: *Ribosomal peptide-bond formation.* Chem Biol. 15:417-419 (2008)

W. Szaflarski, O. Vesper, Y. Teraoka, B. Plitta, D. N. Wilson and K. H. Nierhaus: *New features of the ribosome and ribosomal Inhibitors: Non-enzymatic recycling, misreading and back-translocation.* J. Mol. Biol. doi:10.1016/j.jmb.2008.04.060 380:193-205 (2008)

T. V. Budkevich, A. V. El'skaya and K. H. Nierhaus: *Features of 80S mammalian ribosome and its subunits.* Nucl. Acids Res. doi:10.1093/nar/gkn424, 36:4736-4744 (2008)

V. Di Giacco, V. Márquez, Y. Qin, M. Pech, F. J. Triana-Alonso, D. N. Wilson and K. H. Nierhaus: *Shine–Dalgarno interaction prevents incorporation of noncognate amino acids at the codon following the AUG.* Proc. Natl. Acad. Sci. 105: 10715–10720 (2008)

S. R. Connell, M. Topf, Y. Qin, D. N. Wilson, T. Mielke, P. Fucini, K. H. Nierhaus and C. M. T. Spahn: *A new tRNA intermediate revealed on the ribosome during EF4-mediated back-translocation.* Nature Structure Molecular Biology 15: 910-915 (2008)

A. Özlem Tastan Bishop, U. Stelzl, M. Pech, and K. H. Nierhaus: *Characterization of RNA-protein interactions by phosphorothioate footprinting and its applications to the ribosome.* Methods Mol. Biol. 488:129-151 (2008)

U. Stelzl and K. H. Nierhaus: *In Vitro Selection of Random RNA Fragments to Identify Protein-Binding Sites Within Large RNAs*. Methods Mol. Biol. 488:247-255 (2008)

M. Bouvier, C. M. Sharma, F. Mika, K. H. Nierhaus, and J. Vogel: *Small RNA binding to 50 mRNA coding region inhibits translational initiation.* Mol. Cell 32:827-837 (2008)

2007

D. N. Wilson and K. H. Nierhaus: *The oxazolidinone class of drugs find their orientation on the ribosome*. Mol. Cell 26: 460-462 (2007)

D. N. Wilson and K. H. Nierhaus: *The weird and wonderful world of bacterial ribosome regulation.* Crit. Rev. Biochem Mol. Biol. 42:187-219 (2007)

K. H. Nierhaus: *Early Steps of Evolution and Some Ideas About a Simplified Translational Machinery.* Orig. Life Evol. Biosph. 37:391-398 (2007)

W. Szaflarski and K. H. Nierhaus: *Optimized Energy Consumption for Protein Synthesis*. Orig. Life Evol. Biosph. 37: 423-428 (2007)

2006

H. S. Seo, S. Abedin, D. Kamp, D. N. Wilson, K. H. Nierhaus, B. S. Cooperman: *EF-G-dependent GTPase on the ribosome: Conformational change and fusidic acid inhibition.* Biochemistry 45:2504-2514 (2006).

K. H. Nierhaus: *Decoding errors and the involvement of the E-site.* Biochimie 88:1013-1019 (2006)

F. Schluenzen, C. Takemoto , D. N. Wilson, T. Kaminishi, J. Harms, K. Hanawa-Suetsugu, W. Szaflarski, M. Kawazoe, K. H. Nierhaus, S. Yokoyama and P. Fucini: *The antibiotic kasugamycin mimics mRNA nucleotides to destabilize tRNA binding and inhibit canonical translation initiation.* Nat. Struct. Mol. Biol. 13:871-878 (2006)

D. N. Wilson and K. H. Nierhaus: *The E-site story: The importance of maintaining two tRNAs on the ribosome during protein synthesis*. Cell. Mol. Life Sci. 63: 2725–2737 (2006)

M. B. Iskakova, W. Szaflarski, M. Dreyfus, J. Remme and K. H. Nierhaus: *Troubleshooting coupled in in vitro transcription–translation system derived from Escherichia coli cells: synthesis of high-yield fully active proteins.* Nucl. Acids Res. 34:e135 (2006)

Y. Qin, N. Polacek, O. Vesper, E. Staub, E. Einfeldt, D. N. Wilson and K. H. Nierhaus: *The Highly Conserved LepA Is a Ribosomal Elongation Factor that Back-Translocates the Ribosome*. Cell 127: 721-733 (2006)

D. Wittek and K. H. Nierhaus: *Wer "A" sagt, muß auch "E" sagen: Die ribosomale E Stelle*. BIOspektrum 12: 723-725 (2006)

Selected invited talks

Function and ribosomal binding site of EF4 (LepA), an old factor with a new function, Autrans, France, 27.01.2009

Principles of Translation: Maintenance of Accuracy at the Elongation and Initiation Phase, Munich, 04.05.2009

Early Cells and Protein-Synthesis Machineries, San Sebastian, Spain, 21.05.2009

4.5 Billion Years in 45 min: Evolutionary Relics in the Current Translational Apparatus, Patras, Greece, 05.06.2009

Principles of Translation: Maintenance of Accuracy at the Elongation and Initiation Phase, Arkhangelsk, Russia, 19.06.2009

Principles of Translation: The tricks of the ribosome to be fast and accurate, Regensburg, 26.08.2009

5 Billion Years in 50 min: A Short Story of Evolution of Life on Earth, Berlin, 06.09.2009

The ribosome as the preferential target of antibiotics, Poznan, 22.01.2008

News from the ribosomal elongation cycle: The importance of the third tRNA-binding site (E site) and the initiation problem, Zürich, 06.05.2008, and Basle, 07.05.2008

The importance of the third tRNA-binding site (E site) on the ribosome, Beijing, China, 15.05.2008

LepA (EF4) is an old factor with a new function, Shanghai, 27.05.2008

LepA (EF4) is an old factor with a new function, Konstanz, 14.07.2008

Principles of Proteinsynthesis: The importance of the third tRNA-binding site (E site) and the initiation problem, Pretoria, South Africa, 05.09.2008

Principles of protein synthesis: Maintaining the reading frame, Johannesburg, South Africa,1109.2008

The tricks of the ribosome to maintain an accurate protein synthesis, Valencia, Venezuela, 10.10.2008

4.5 Billion Years in 45 min: Evolutionary Relics in the Current Translational Apparatus, Philadelphia, USA, 22.10.2008

The third elongation factor EF3 and its relation to conserved features of the ribosome, Philadelphia, 23.10.2008[r]

Principles of protein synthesis: Maintaining the reading frame, Philadelphia, 27.10.2008

Principles of Proteinsynthesis: Preventing mis-incorporation of non-cognate amino acids and the initiation problem, Philadelphia, 29.10.2008

Why ribosomes have three tRNA binding sites? Philadelphia, 30.10.2008

LepA (EF4) is an old ribosomal factor with a new function, Philadelphia, 31.10.2008

Why ribosomes have three tRNA binding sites? Maintaining the reading frame, Washington, 04.11.2008

Principles of Proteinsynthesis: Preventing mis-incorporation of non-cognate amino acids and the initiation problem, Baltimore, 06.11.2008

4.5 Billion Years in 45 min: Evolutionary Relics in the Current Translational Apparatus, New York, Memorial Sloan-Kettering Cancer Center, 10.11.2008

LepA (EF4) is an old ribosomal factor with a new function, Chapel Hill, USA, 13.11.2008

The highly conserved LepA is a ribosomal elongation factor in all bacteria, Poznan, Poland, 15.01.2007

The highly conserved LepA is a ribosomal elongation factor in all bacteria, Geneva, Switzerland, 07.05.2007

The highly conserved LepA is a ribosomal elongation factor in all bacteria, Cambridge, 14.05.2007

LepA (EF4), one of the most conserved proteins and present in all bacteria and mitochondria, is a ribosomal elongation factor with a new function, Cape Cod, USA, 05.06.2007

LepA (EF4), one of the most conserved proteins and present in all bacteria and mitochondria, is a ribosomal elongation factor with a new function, Biocatalysis Conference Moscow, 18.06.2007

Deacylated tRNA at the ribosomal A site: Dissection of the stringent response regulation, Lübeck, 01.07.2007

Recent excitements in ribosome research: The universal LepA (EF4) is a ribosomal elongation factor with a new function, Dortmund, 04.07.2007

LepA is a ribosomal elongation factor with a new function, Heidelberg, 13.09.2007

The E-site Story: The Importance of the Third tRNA Binding Site on Ribosomes, Fukuoka, Japan, 15.10.2007

Unravelling the hidden life of IF1: A novel hypothesis on IF1 function, Niigata, Japan, 17.10.2007

Parameters for highly efficient and accurate in vitro protein synthesis: Energy, ionic conditions, factors, Tokyo, 19.10.2007

The role of the E site and a new elongation factor with a new function, Tokyo, 22.10.2007

Three billion years of perfection: The tricks of the nanomachine ribosome, Hongkong, 02.11.2007

News from the ribosomal elongation cycle: The importance of the third tRNA-binding site (E site) and an elongation factor with a new function, Regensburg, 06.11.2007

The E-site story: New aspects of protein-synthesis, Madrid, 20.01.2006

The highly conserved LepA is a ribosomal elongation factor in all bacteria and mitochondria, Weimar, 17.03.2006

The E-site story: New aspects of protein-synthesis. Athens, 30.03.2006

The E-site story: Fundamental aspects of protein-synthesis, La-Londes-les-Maures, France, 15.06.2006

The E-site Story: The Importance of the Third tRNA Binding Site on Ribosomes, Gif-sur-Yvette, France, 13.07.2006

The highly conserved LepA is a ribosomal elongation factor in all bacteria and mitochondria, Philadelphia, New York and Chicago, 09/2006

Minimal Set of Components for Translation of an mRNA, Erice, Italy, 03.10.2006

The highly conserved LepA is a ribosomal elongation factor in all bacteria, Camerino, Italy, 04.11.2006

The E-site story: Fundamental aspects of protein-synthesis, Camerino, Italy, 05.11.2006

The highly conserved LepA is a ribosomal elongation factor in all bacteria, Homburg, 14.12.2006

Selected memberships /scientific honours

- Dr. *honoris causa* of the University of Patras, Greece, 2009
- Distinguished International Scholar of the University of Pennsylvania, Philadelphia, USA, 2008
- "Adjunct Professor of Molecular Biology" of the Moscow State University, 1999
- Elected Member of the European Molecular Biology Organisation (EMBO), 1984

Work as scientific referee

Knud Nierhaus reviews about 40 manuscripts per year for more than 35 journals.

PhD Theses

2007

Witold Szaflarski (2007) *Antibiotics as translation inhibitors: new methods-new insights,* PhD thesis, Freie Universität Berlin (supervisor: Knud Nierhaus)

Oliver Vesper (2007) *Analyse funktionelle Komplexe des Ribosoms in Regulations- und Terminationsprozessen,* PhD thesis, Freie Universität Berlin (supervisor: Knud Nierhaus)

2006

Yan Qin (2006) *The highly conserved LepA is a ribosomal elongation factor that back-translocates the ribosome and is essential for viability at high ionic strength,* PhD thesis, Freie Universität Berlin (supervisor: Knud Nierhaus)

Student theses

2009

Jaroslav Kijek (2009) *Functional analysis of EF-P, a non-canonical factor of the translational apparatus*, Diploma thesis, University MC Sklodowska (supervisor: Knud Nierhaus)

David Ramrath (2009), Diploma thesis, Universität Lübeck (supervisor: Knud Nierhaus)

2006

Marianne Collier (2006) *Funktionelle Analyse der ribosomalen E-Stelle in E. coli: Die Position C2394 der 23S rRNA und ihre Bedeutung,* Diploma thesis, Freie Universität Berlin (supervisor: Knud Nierhaus)

Daniela Kaul (2006) *Analyse einer E-Stellen Mutante von* E. coli *Ribosomen,* Diploma thesis, Freie Universität Berlin (supervisor: Knud Nierhaus)

Daniela Wittek (2006) *Bindung von tmRNA an programmierte Ribosomen als experimentelle Voraussetzung für die Darstellung von tmRNA•70S Komplexen für Cryo-EM,* Diploma thesis, Freie Universität Berlin (supervisor: Knud Nierhaus)

Patents

Europäische Patentanmeldung Nr. 06 754 661.4 basierend auf PCT/EP2006/006503 vom 4. July 2006 *"Use of LepA for improving the accuracy of protein synthesis in vitro"*

External funding

DFG, Alexander-von-Humboldt Stiftung, Fonds der Chemischen Industrie, Bundesministerium für Bildung und Forschung.

Guest scientists

2009

Ana Cristina Gomez, RNA Biology Lab, CESAM – Department of Biology, University of Aveiro, Portugal, 08.-18.07.2009

2008

Jarek Kijek, Marie-Curie Sklodowska University Lublin, Poland, 01.07.-30.09.2008

2007

Tsagkalia Aikaterini, Aristotle University of Thessaloniki, Greece, 06.02.-31.05.2007; 01.11.-20.12.2007

Alexandros Katranidis, Aristotle University of Thessaloniki, Greece, 12.02.-23.03.2007; 14.-28.05.2007

2006

Alexandros Petropoulos, University of Patras, School of Medicine, Greece, 01.04.-31.08.2006

Dimity Lesnyak, Moscow State University Russia, 18.08.-13.10.2006

Organization of scientific events

10th International Workshop *Experimental Strategies for Ribosome Research* at the Schloss Ringberg of the Max-Planck-Society, 19.-22.04.2009

Symposium on Exciting Advances in Ribosome Research, 04.08.2008

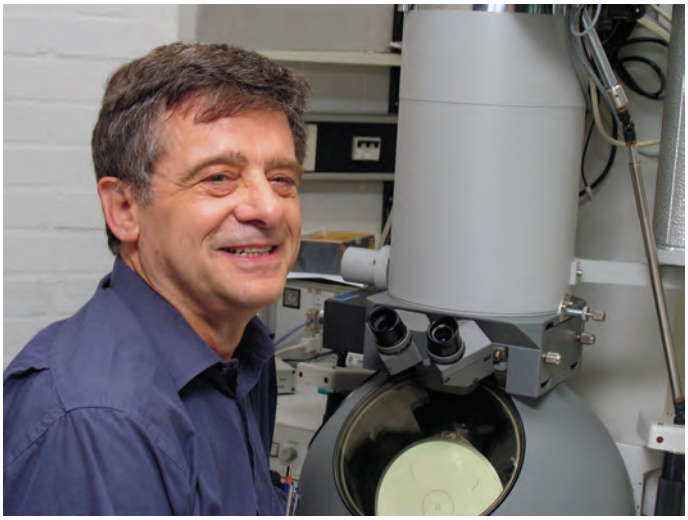
Protein Structure and Function as Revealed by Work on Ribosomes, Harnack House Berlin, 18.-19.05.2007

Berlin Workshop on Experiments of the Dynamics of Single Molecules, 07.-09.12.2007

9th Workshop on *Experimental Strategies for Ribosomal Research* in Patras-Psathopirgos, Greece, 20.-25.05.2006

CSH Translational Control, Chairman and Organizer of the Session "*Elongation and Termination*", 06.-10.09.2006

Miscellaneous Research Groups
Microscopy



Head
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Technician
Beatrix Fauler

Scientific overview

The lab is running two transmission electron microscopes: Philips CM100 and Tecnai Spirit. In addition, a high-resolution Tecnai Polara cryo-EM is available through the cooperation with the cryo-EM group in the institute (Thorsten Mielke, USN, Anwenderzentrum). All EMs are equipped with CCD cameras and optional cryo or tomography holder.

A broad range of preparation methods is established. Our main focus is on:
- ultra-thin sections of embedded samples;
- specific labelling of sections or isolated structures;
- visualization of nucleic acids and nucleic acid - protein interactions;
- cryo preparation and image acquisition of samples in vitreous ice.
- fine structural analysis of protein complexes or viruses after negative staining.

Within the institute we perform projects with all departments having wet labs.

Research Group Development & Disease (Stefan Mundlos)
- M. Kolancyk: *Immuno-gold localzation of a nitric oxide synthase.*
- U. Kornak: *Structural changes in mitochondria after expression of PYCR1.*

Dept. of Human Molecular Genetics (H.-Hilger Ropers)
- A. Kuss: *Structure of the nuclear membrane in Bod1 transfected MatCat cells*
- R. Ullmann: *Protein localization by immunolabelling of IMR-90 in fetal fibroblasts.*
- C. Scharff /S. Scotto: *Neurogenesis in bird brain.*

Dept. of Vertebrate Genomics (Hans Lehrach)

- G. Panopoulou: *Expression patterns in sections of in situ labelled sea urchin and amphioxus embryos.*
- J. Adjaye: *Morphological changes characterization of iPSC and ESC line during cultivation*.
- B. Lange: *The function of two cancer-related proteins for maintaining centriole integrity was confirmed in U2OS osteosarcoma cells after protein depletion by siRNA.*

Dept. of Developmental Genetics (Bernhard Herrmann)

- N. Véron: *Localization of the t-complex responder locus (Tcr).*
- M. Mayer: *Mice kidney podocytes in Slit12 knockouts.*

Collaborations outside the institute are often in continuation of projects started during the stay of the people in this institute.

- J.C. Alonso/S. Ayora, Centro Nacional de Biotecnologia, Departamento de Biotecnologia Microbiana, Madrid: *Replication and recombination in B. subtilis and phage SPP1.*
- M. Espinosa / A. Bravo, Centro de Investigaciones Biológicas (CSIC), Madrid: *Replication and mobilization of the promiscuous plasmid pMV158***.**
- K. Geider, JKI Dossenheim, *Analysis of morphology and DNA of Erwinia phages.*
- S. Hertwig, Bundesinstitut für Risikobewertung (BfR), Berlin: *Phage PY54 from Yersinia enterocolitica replicates as a linear chromosome. EM analysis of the complexes of the C1 repressor with the operator region.*
- M. J. Loessner, Swiss Federal Institute of Technology (ETH), Zürich: *EM characterisation of phages (Listeria) and their DNA.*
- G. Multhaup, FU Berlin, Institut für Chemie-Biochemie, *Assembly of β-amyloid fibrils monitored by EM.*
- E. Orlova, Birkbeck College, Dept. of Crystallography, University of London: Image processing of data sets from components of phage SPP1.
- A. Pingoud / V. Pingoud, Institut für Biochemie, Justus-Liebig-Universität Giessen: *Interaction of nucleases (CAD, EndoG, DNaseII) and regulatory proteins with DNA. Specific interaction of restriction enzymes and their recognition sequence on the DNA*
- C. Speck, DNA Replication Group MRC, Clinical Sciences CentreFaculty of Medicine, Imperial College, LondonHammersmith, *Yeast replication: visualization of the MCM2-7 helicase loaded onto origin DNA.*
- P. Tavares, Laboratoire de Virology Moléculaire et Structurale, CNRS, Gif-sur-Yvette: *Life cycle of B. subtilis bacteriophage SPP1 (morphogenesis, DNA packaging, infection); fine structural analysis of the phage components.*
- J.N. Reeve, Ohio State University, Columbus: *Mapping of the archaeal transcription repressor (TrpY) from Methanothermobacter*.
- E. Wanker, Max-Delbrück-Centrum Berlin-Buch: *Effect of selected drugs on the formation of huntingtin, α-synuclein or β-amyloid fibrils monitored by EM.*
- E. Weinhold, Institut für organische Chemie, RWTH Aachen: *Sequence specific labeling of DNA by covalent binding of modified cofactors of restriction enzymes*.
- J. Zakrzewska-Czerwinska, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw: *Architecture of the partitioning complexes (segregosomes) in Streptomyces coelicolor*.

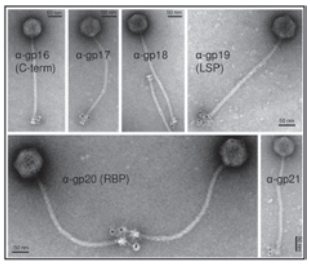

Figure1: Immunolabelling of tail tip proteins of Listeria phage A118. LSP=lytic structural protein, RBP receptor binding protein. (collaboration with group Loessner, Zürich).

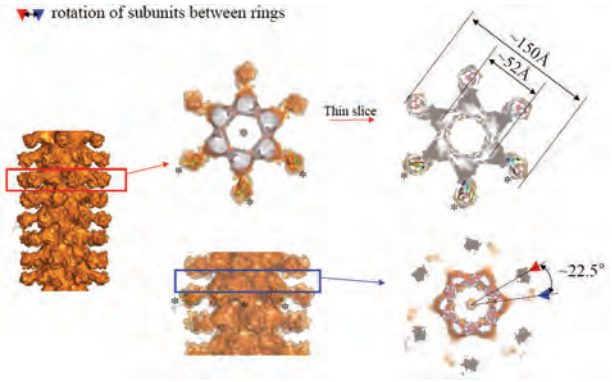

Figure 2: 3D Image reconstruction of the phage tail of the translational frameshift mutant pIA8 of phage SPP1 showing an additional IG like domain. (collaboration with the goups of P. Tavares and E. Orlova)

General information

Complete list of publications (2006-2009)

2009

Evrin C, P Clarke, J Zech, R Lurz, J Sun, S Uhle, H Li, B Stillman, C Speck (2009) *A double-hexameric MCM2-7 complex is loaded onto origin DNA during licensing of eukaryotic DNA replication*. Proc Natl Acad Sci U S A 106(48): 20240-45.

Garcia P, B Martinez, J M Obeso, R Lavigne, R Lurz, *A Rodriguez (2009) Functional genomic analysis of two Staphylococcus aureus phages isolated from the dairy environment*. Appl Environ Microbiol 75 (24): 7663-73.

2008

Aranda-Orgilles B, J Aigner, M Kunath, R Lurz, R Schneider, S Schweiger (2008) *Active transport of the ubiquitin ligase MID1 along the microtubules is regulated by protein phosphatase 2A*. PLoS One 3(10): 3507.

Auzat I., A Dröge, F Weise, R Lurz, P Tavares (2008) *Origin and function of the two major tail proteins of bacteriophage SPP1*. Mol Microbiol 70(3): 557-69.

Ehrnhoefer DE, J Bieschke, A Boeddrich, M Herbst, L Masino, R Lurz, S Engemann, A Pastore, EE Wanker (2008) *EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers*. Nat Struct Mol Biol 15(6): 558-66.

Gasset-Rosa F, T Diaz-Lopez, R Lurz, A Prieto, M E Fernandez-Tresguerres, R Giraldo (2008) *Negative regulation of pPS10 plasmid replication: origin pairing by zipping-up DNA-bound RepA monomers*. Mol Microbiol 68(3): 560-72.

Graeber I, I Kaesler, M S Borchert, R Dieckmann, T Pape, R Lurz, P Nielsen, von Dohren, W Michaelis, U Szewzyk (2008) *Spongiibacter marinus gen. nov., sp. nov., a halophilic marine bacterium isolated from the boreal sponge Haliclona sp. 1*. Int J Syst Evol Microbiol 58 (Pt 3): 585-90.

Kaesler I, I Graeber, M S Borchert, T Pape, . Dieckmann, H von Dohren, P Nielsen, R Lurz, W Michaelis, U Szewzyk (2008) *Spongiispira norvegica gen. nov., sp. nov., a marine bacterium isolated from the boreal sponge Isops phlegraei.* Int J Syst Evol Microbiol 58 (Pt 8): 1815-20.

Karr EA, K Sandman, R Lurz, JN Reeve (2008) *TrpY regulation of trpB2 transcription in Methanothermobacter thermautotrophicus*. J Bacteriol 190 (7): 2637-41.

Klumpp J, J Dorscht, R Lurz, R Bielmann, M Wieland, M Zimmer, R Calendar and M J Loessner (2008) *The terminally redundant, nonpermuted genome of Listeria bacteriophage A511: a model for the SPO1-like myoviruses of gram-positive bacteria*. J Bacteriol 190 (17): 5753-65.

Lange M, B Kaynak, U B Forster, M Tonjes, J J Fischer, C Grimm, J Schlesinger, S Just, I Dunkel, T Krueger, S Mebus, H Lehrach, R Lurz, J Gobom, W Rottbauer, S Abdelilah-Seyfried, S Sperling (2008) *Regulation of muscle development by DPF3, a novel histone acetylation and methylation reader of the BAF chromatin remodeling complex*. Genes Dev 22 (17): 2370-84.

Poh S L, F El Khadali, C Berrier, R Lurz, R Melki, P Tavares (2008) *Oligomerization of the SPP1 Scaffolding Protein*. J Mol Biol 378 (3): 551-64.

Pratto F, A Cicek, W A Weihofen, R Lurz, W Saenger, JC Alonso (2008) *Streptococcus pyogenes pSM19035 requires dynamic assembly of ATP-bound ParA and ParB on parS DNA during plasmid segregation*. Nucleic Acids Res 36(11): 3676-89.

2007

Pljevaljcic G, F Schmidt, A J Scheidig, R Lurz, E Weinhold (2007) *Quantitative labeling of long plasmid DNA with nanometer precision*. Chembiochem 8(13): 1516-9.

Ruiz-Maso J A, R Lurz, M Espinosa, G Del Solar (2007) *Interactions between the RepB initiator protein of plasmid pMV158 and two distant DNA regions within the origin of replication*. Nucleic Acids Res 35(4):1230-44.

Zawilak-Pawlik A, A.Kois, K.Stingl, I.G.Boneca, P Skrobuk, J Piotr, R Lurz, J Zakrzewska-Czerwinska, A Labigne (2007) *HobA—a novel protein involved in initiation of chromosomal replication in Helicobacter pylori*. Mol Microbiol 65(4): 979-94.

2006

Alonso J C, P Tavares, R Lurz, TA Trautner (2006) *Bacteriophage SPP1 in R Calendar* (ed) The Bacteriophages. Oxford University Press

Boeddrich A, S Gaumer, A Haacke, N Tzvetkov, M Albrecht, B O Evert, E C Muller, R Lurz, P Breuer, N Schugardt, S Plassmann, K Xu, J M Warrick, J Suopanki, U Wullner, R Frank, U F Hartl, N M Bonini, EE Wanker (2006) *An arginine/lysine-rich motif is crucial for VCP/p97-mediated modulation of ataxin-3 fibrillogenesis*. Embo J 25(7): 1547-58.

Sao-Jose C, S Lhuillier, R Lurz, R Melki, J Lepault., MA Santos, P Tavares (2006) *The ectodomain of the viral receptor YueB forms a fiber that triggers ejection of bacteriophage SPP1 DNA*. J Biol Chem. 281(17):11464-70.

Zemojtel T, M Kolanczyk, N Kossler, S Stricker, R Lurz, I Mikula, M Duchniewicz, M Schuelke, P Ghafourifar, P Martasek, M Vingron, S Mundlos (2006) *Mammalian mitochondrial nitric oxide synthase: characterization of a novel candidate*. FEBS Lett 580(2): 455-62.

Vinga I, A Dröge, A C Stiege, R Lurz, M A Santos, R Daugelavièius, P Tavares (2006) *The minor capsid protein gp7 of bacteriophage SPP1 is required for efficient infection of Bacillus subtilis*. Mol Microbiol. 61(6):1609-21.

Lioy V S, M T Martin, A G Camacho, R Lurz, H Antelmann, M Hecker, E Hitchin, Y Ridge, J M Wells, J C Alonso. (2006). *pSM19035-encoded {zeta} toxin induces stasis followed by death in a subpopulation of cells*. Microbiology 152(Pt 8): 2365-79.

Guest scientists

J. Klumpp, Swiss Federal Institute of Technology (ETH), Zürich, 07.-13.05. 2006

F. Pratto, Centro Nacional de Biotecnologia and C. Manfredi, Universidad Autonoma de Madrid, Madrid, 13.-26.05. 2006

A. Bravo, Centro de Investigaciones Biológicas (CSIC), Madrid, 28.05.-12.06. 2006

Fátima Gaaset Rosa, Centro de Investigaciones Biológicas (CSIC), Madrid, 25.06.-25.07. 2007

R.Bielmann; Swiss Federal Institute of Technology (ETH), Zürich, 20.08.-14.09. 2007

P. Tavares, Unité de Virologie Moléculaire et Structurale, Gif-sur-Yvette, 17.-24.09. 2007

Juan López Villarejo/ Ramon Diaz Orejas, Centro de Investigaciones Biologicas (CSIC), 18.11.-07.12.2007

Ambra Lo Piano/Sylvia, Centro Nacional de Biotecnologia, Madrid, 01.-28.09. 2008

J. Klumpp, Swiss Federal Institute of Technology (ETH), Zürich, 20.-30.04.2009

Public relations

Demonstration of the EM facility to the public within *Die lange Nacht der Wissenschaften*. Practical demonstrations to classes schools in and outside Berlin. Several school apprentices (Schülerpraktikanten) for 2 to 3 weeks each.

Miscellaneous Research Groups

Cryo-electron Microscopy: Structure Determination of Macromolecular Complexes & Protein Modules

(Established: 2004)

Head
Dr. Thorsten Mielke* (since 04)
Phone: +49 (0)30 8413-1644
Fax: +49 (0)30 8413-1385
Email: mielke@molgen.mpg.de

Scientist
Justus Loerke* (04/05-11/08)

Technicians
Jörg Bürger* (since 08/06)
Matthias Brünner* (since 09/07)

Introduction

Sequencing the genomes of multiple organisms including *Drosophila*, zebra fish and human has revealed the total number of genes and the sequence of the encoded proteins. The next steps towards understanding biological processes including diseases require the characterization of the protein composition in a given cell, the protein function and protein protein-interactions. Consequently, understanding protein function at a molecular level requires profound knowledge of the structure of a protein and its different functional states.

However, key processes in biology involve large protein complexes such as multi-enzyme-complexes, the ribosome and RNA polymerases. Many of these complexes act as highly organized molecular machines, but often show a variable assembly and are subject to dynamical regulation. Furthermore, many signalling complexes or protein modules form only temporarily, making structure determination even more difficult.

Our group aims for structure determination of macromolecular protein complexes and protein modules using cryo-electron microscopy (cryo-EM) in combination with the single-particle approach. This technique has emerged as the key technology to gain structural information on macromolecular protein complexes without the need to crystallize these complex and dynamic systems. The molecules of interest are embedded in a thin layer of vitreous ice under near physiological conditions and imaged by transmission cryo-electron microscopy. Image processing then allows the reconstruction of the three-dimensional structure at sub-nanometer resolution.

* externally funded

Results

Starting in 2004, we have established a state-of-the-art cryo-EM facility within the Berlin-Brandenburg-wide research consortium UltraStructure Network (USN). The USN aims for a systematic analysis of macromolecular protein complexes combining methodology for complex isolation, analysis of the protein composition and function and, finally, cryo-EM structure determination. Our facility provides the latest techniques for cryo-EM structure determination including screening, semi-automated sample vitrification, data acquisition and intense computing resources for image processing. The Core instrument is a helium-cooled 300 kV Tecnai G2 Polara electron microscope (FEI) equipped with a 4k CCD camera (TVIPS). Additionally, a 100 kV Philips CM100 and a 120 kV Tecnai Spirit electron microscope are available through the close collaboration with the microscopy group of our institute (AG Rudi Lurz).

Within the framework of the USN, we support the isolation of protein complexes of various groups e.g. the "Protein Complexes and Cell Organelle Assembly Group" (AG Bodo Lange, MPIMG) and the "ribosome group" (Knud Nierhaus, MPIMG) with EM-screening techniques. We further solved several mainly ribosomal complexes at 1 nm resolution or below, which revealed important new insides into different functional states of the cellular protein biosynthesis machinery. One example is the structure of the active ribosome in complex with the signal recognition particle (SRP) and the SRP receptor at 8 Å resolution. This structure unveiled how the interaction of the SRP receptor with both, the ribosome and the SRP, displaces parts of the SRP molecule, leading to the exposition of ribosomal translocon binding sites (Halic et al. (2006) Science 312, 745-747). The cryo-EM structure of the yeast 80S ribosome in complex with the cricket paralysis virus IRES element at 7.3 Å resolution allowed for the very first time the de-novo modelling of the complete viral IRES RNA (Schüler et al. (2006) Nature Structural & Molecular Biology 13, 1092-1096).

The project "Anwenderzentrum" focused on new methods to further exploit the potential of cryo-EM structure determination in the sub-nanometer range, where conformational sub-states as well as different ligand binding states become increasingly crucial. New strategies for particle classification and sorting based on 3D variance analysis and multi-reference alignment allowed us in cooperation with Knud Nierhaus, Paola Fucini (MPIMG) and Christian Spahn (Charité) to identify a new tRNA intermediate of the 70S *E. coli* ribosome during EF4-mediated back-translocation (Connell et al. (2008) Nature Structural & Molecular Biology 15, 910-915). In cooperation with Roland Beckmann (LMU Munich) and Thomas A. Steitz (Yale University, USA) we could trace the ordered TnaC nascent chain within the exit tunnel of the ribosome (Seidelt et al. (2009) Science, in press). Visualizing eukaryotic ribosome-Sec61 complexes, we could further trace the nascent into the protein conducting channel. These structures provide a new structural basis for co-translational protein translocation, proving that the nascent chain interacts only with a single copy of the Sec61 complex (Becker et al. (2009) Science, in press). In Cooperation with Venkatraman Ramakrishnan (MRC-LMB, UK), we could for the very first visualize an antibiotic, kirromycin, in the ternary complex of the T. thermophilus 70S ribosome and elongation factor Tu (Schuette et al. (2009) EMBO J. 28, 755-765), proving the potential of cryo-EM to address pharmaceutical and medical relevant questions, which so far were out of reach of this technique.

Current activities and future perspectives

Technical developments in the field of cryo-EM including modern electron microscopes such as the G2 Polara allow structure determination of macromolecular

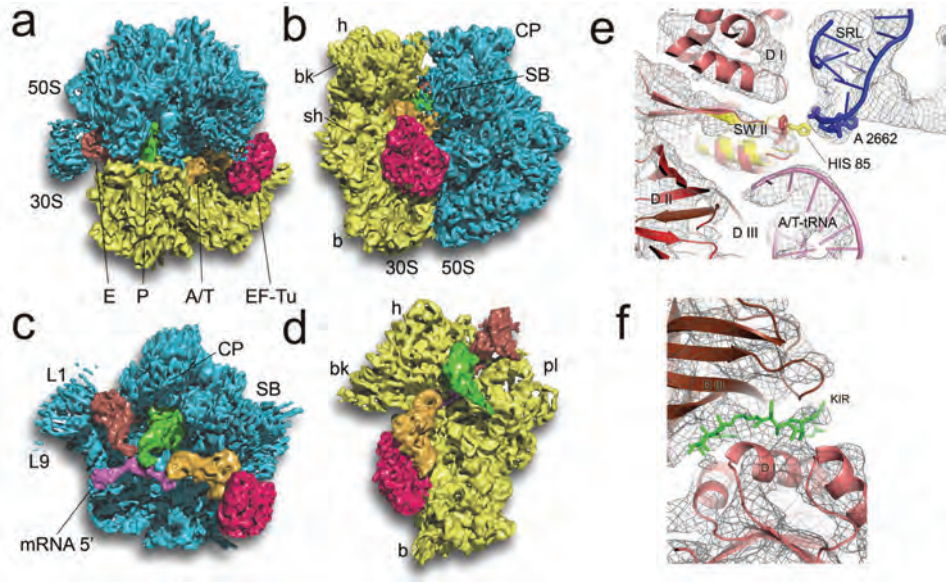

Figure 1: Cryo-EM structure of the 70S ribosome in complex with elongation factor EF-Tu, KPhe-tRNA, GDP and kirromycin shown from the top ***(a)****, from the L7/L12 side* ***(b)****, from the 30S side, with the 30S subunit removed* ***(c)*** *and from the 50S side, with 50S subunit removed* ***(d)****. The colors distinct the different components: 30S subunit, yellow; 50S subunit, blue; EF-Tu, red; A/T-tRNA, orange; P-tRNA, green; E-tRNA, brown; mRNA, pink. Details of the switch II region* ***(e)*** *and the kirromycin binding site* ***(f)*** *are highlighted (modified from Schuette et al. (2009) EMBO J. 28, 755-765).*

complexes at sub-nanometer resolution. However, in the sub-nanometer range, intense image processing techniques such as particle classification and sorting are mandatory to overcome sample heterogeneity due to conformational flexibility. This requires not only more computing power, but also a high degree of automation for both, image acquisition and image processing, to process not only a few hundred thousands but also millions of particles. We currently focus on automated data acquisition using the Leginon system (AMI group, The Scripps Research Institute, La Jolla, USA) or Emtools (TVIPS) and aim to establish an automated image processing pipeline. Another focus lies on structural analysis of highly dynamic protein modules within the collaborative research centre 740 "From molecules to modules: Organisation and dynamics of functional units in cells". Here we aim to implement molecular electron tomography as a new tool for initial structure determination and the analysis of macromolecular assemblies with varying stability and/or only temporary associated subunits.

Internal cooperations

- Knud Nierhaus, Ribosome Group, *Structure determination of ribosomal complexes;*
- Bodo Lange, Department of Vertebrate Genomics, *Isolation and purification of protein complexes;*
- Rudi Lurz, Microscopy Group, *electron microscopy and electron tomography.*

External cooperations

- Prof. C.M.T. Spahn, Prof. K.P. Hofmann, Charité – Universitätsmedizin Berlin
- Prof. P.-M. Kloetzel, Prof. E. Krüger, Dr. U. Seifert, Charité – Universitätsmedizin Berlin
- Prof. R. Beckmann, Prof. P. Cramer, Genzentrum München, Ludwig-Maximilians-Universität (LMU), München

- Prof. H. Oschkinat, Prof. Bernd Reif, Dr. Christian Freund, Leibniz-Institut für Molekulare Pharmakologie (FMP), Berlin
- Prof. E. Wanker, Max-Delbrück-Centrum für Molekulare Medizin, Berlin
- Prof. N. Grigorieff, Brandeis University, Waltham MA, USA
- Prof. P. Penczek, Dept. of Biochemistry and Molecular Biology, The University of Texas - Houston Medical School, Houston, TX, USA
- Dr. G.F.X. Schertler, MRC Laboratory of Molecular Biology, Cambridge, UK

General information

Selected publications

Becker, T., Mandon, E., Bhushan, S., Jarasch, A., Armache, J.-P., Funes, S., Jossinet, F., Gumbart, J. C., Mielke, T., Schulten, K., Westhof, E., Gilmore, R. and Beckmann, R. (2009) *Structure of monomeric yeast and mammalian Sec61 complexes interacting with the translating ribosome*, Science 326:1369-73

Seidelt, B., Innis, C.A., Wilson, D.N., Gartmann, M., Armache, J-P., Trabuco, L.G., Becker, T., Mielke, T., Schulten, K., Steitz, T.A. and Beckmann, R. (2009) *Structural insight into nascent chain-mediated translational stalling*, Science 326:1412-15

Schuette, J.-C., Murphy, F.V. 4th, Kelley, A.C., Giesebrecht, J., Connell, S.R., Loerke, J., Mielke, T., Zhang, W., Penczek, P.A., Ramakrishnan, V. and Spahn, C.M.T. (2009) *GTPase activation of elongation factor EF-Tu by the ribosome during decoding.* EMBO J. 28, 755–765

Connell, S.R., Topf, M., Qin, Y., Wilson, D.N., Mielke, T., Fucini, P., Nierhaus, K.H. and Spahn, C.M.T. (2008) *A novel tRNA-intermediate revealed on the ribosome during EF4 (LepA)-mediated back-translocation.* Nature Structural & Molecular Biology 15, 910-915

Connnell, S.R., Takemoto, C., Wilson, D.N., Wang, H., Murayama, K., Terada, T., Shirouzu, M., Rost, M., Schüler, M., Giesebrecht, J., Dabrowski, M., Mielke, T., Fucini, P.M., Yokoyama, S. and Spahn, C.M.T. (2007) *Structural Basis for Interaction of the Ribosome with the Switch Regions of GTP-bound Elongation Factors*. Molecular Cell 25, 751-764

External funding

DFG, Collaborative research centre 740 (SFB 740): *From molecules to modules: Organisation and dynamics of functional units in cells*, 01/07-12/10

EFRE: *Anwenderzentrum*, 05/ 06 – 11/ 08

EFRE: *Ultrastructure Network*, 06/03 – 06/06

Teaching activities

Lecture as part of the program of study "Medizinische Physik", Charité-Universitätsmedizin Berlin.

Public relations

Practical demonstrations of the cryo-EM facility to the public within the "Long night of Science in Berlin"

Practical demonstrations to several visiting school classes from Germany and Austria

Miscellaneous Research Groups

High-throughput Technologies

(Established: 1992)

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Scientists
Dr. Michael Kube* (since 08/03)
Dr. Heiner Kuhl* (since 12/07)
Dr. Mbaye Tine* (since 11/08)
Dr. Bernd Timmermann (02/06-06/08)

Tecnicians
Grzegorz Wozniak* (since 04/02)
Anett Kühn* (since 05/07)
Silvia Schmoger* (again since 12/07)
Kathrin Huth* (since 09/08)
Aydah Sabah (since 11/08)
Beatrice Baumann* (again since 01/09)
Steffen Scheer* (since 04/09)
Janina Thiel* (again since 05/09)
Dirk Wendland* (since 09/09)
Julia Cekanov (since 09/09)
Sabine Gallert* (01/09-06/09)
Katja Heitmann* (98-04, 08/08-04/09)
Sabrina Ludwig* (09/08-03/09)
Beata Thalke* (11/08-02/09)
Thomas Przewieslik* (07/08-12/08)
Kristoff Stützner* (09/06-12/08)
Christian Warmt* (07/06-12/08)
Kathleen Barda* (09/08-12/08)
Franka Bläsing* (08/08-12/08)
Gabriele Bläss* (03/02-12/08, part time)
Christine Lübbert (08/08-12/08, part time)
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Birol Köysüren* (since 05/09)

Scientific overview

The htpt-group is a co-operation partner of the international human genome sequencing project consortium, as described by H. Lehrach (Dept. Vertebrate Genomics), member of various European projects and several national project financed by BMBF, DFG and MPG. The group has established a good infrastructure for large-scale genomic analysis projects such as sequencing, mutation analysis and mass spectrometry, as has been partially described in the section of the service group.

* externally funded

The recent publications of human chromosomes and of the completed human genome sequence were the ultimate successes of our major effort within the time period since 2000. Important steps on this successful way are the sequencing and final analysis of chromosome 21, the second finished human chromosome, still one of the most accurately analysed one, and our contribution to several regions of the human chromosomes 1, 3, 17, and X. Our next finalised projects along this line are the completed elucidation of chimpanzee chromosome 21, the ortholog to human chromosome 21 and major parts of the X-chromosome. The whole project was organised by a German-Asian consortium (X-chromosome only Germany), wherein MPIMG was responsible for the German part. These results, being summarised within the present report period, are not only important because chimpanzee is our closest relative, it is the first time that a large genomic arrangement, two complete chromosomes of man and chimpanzee, are comparatively analysed. Therefore, not only genes and variations within the coding elements are comparable, but also intronic regions and even more important, promoter elements are accessible for any comparative analysis and elucidation.

In addition, we have been involved in the analysis of model organisms such as mouse (chromosome 2 and 6), rhesus MHC and the complete analysis of the rat MHC (RT1) complex, which plays an important role in infectious diseases. The MHC region belongs to the most densely packed, gene rich regions and although it spans only over a 4 Mbases area, we have identified 220 genes, nearly as many as in the human chromosome 21 region, which is about 34 Mbases large.
Other launched projects concern contributions to the final sequence of chimpanzee chromosomes X and Y, with special interest to Xq28 and regions associated with mental retardation. We are also involved in national and international projects, from bacterial genomes to model organisms like the urochordate *Oikopleura* (M. Kube) and the European Sea bass (H. Kuhl), as listed below (project grants and MPG projects).

The early scientific interest related to *Oikopleura dioica* was focused on questions of systematics, the phenomenon of "marine snow" and of bioluminousity, research in *Oikopleura´s* nervous system, and ecological questions, like the influence on picoplancton. With a genome size of only around 75 Mbases (estimated number of 15.000 genes), smaller than *C. elegans*, and less than half that of *D. melanogaster*, the genome of *Oikopleura dioca* gives the chance for a closer look inside an early chordate genome. In addition, this organism has also other interesting features, making it a key system to understand the functions of human/ vertebrate genome. Within this line of interest is our scientific contribution to varies established EU project, especially our genome project on the European Sea bass, for the NoE **M**arine **G**enomics **E**urope (MGE).
Dicentrarchus labrax (European Sea bass) is a carnivorous teleost fish (genome size ~620 Mbases) with a natural distribution along the Atlantic and Mediterranean coasts of Europe, where it is a target for established fisheries and aquaculture industries. As a result of its economical importance *D. labrax has* been the subject of considerable basic and applied research in the past decade. With the formation of the MGE and the AquaFirst project, European Sea bass research has advanced to the age of genomics. Several EST-sequencing and a BAC end-sequencing project have been started recently at MPIMG, complementing the

Figure 1: European Sea bass (Dicentrarchus labrax)

whole genome shotgun sequencing as a combined approach of Sanger- and next-generation sequencing-techniques (present status: three chromosome nearly finished and annotated).

In addition, we have managed several NGFN-2 and NGFNplus projects listed below. The project for disease gene identification and systematic re-sequencing of candidate genes of genomic regions of interest should be specifically mentioned as a pre-project, to develop necessary infrastructure for NGFNplus, where data exchange between the clinical partners, also non-NGFN partners, is organized by a Web interface (*http://www.resequencing.mpg.de/*). Finished genomic sequence data are submitted to public data bases or/and are presented on our project Web pages.

Infrastructure of htpt-group

The htpt-group has established a good infrastructure for large-scale genomic analysis projects such as sequencing, medically related re-sequencing, mutation analysis and mass spectrometry, using most advanced methods and hardware systems. DNA samples are purified, using our patent related magn. beads methods and a novel PEG/org. solvent based precipitation methode (Heiner Kuhl). Our angular gel electrophoresis system (patented) is able to visualise 384 sample on a MTP-sized gel and to be automatly processed exploring a capacity of more than 15.000 samples per day. Besides these 'high-throughput highlights', our lab equipment involves all necessary items to run large scale projects, e.g. thermocycler, centrifuges, incubators, protein purification systems and sophisticated computer equipment.

Selected external cooperations

Academic

- MPI Bremen, *various bacterial genomic and metagenomic projects*
- MPI Marburg, *Metagenomics of rice and forest soil*
- J. W. Waegele, Uni. Bonn, *German-Deep Phylogeny-Consortium*
- H. Hofmann, EPFL, Lausanne, Switzerland, *Development of tailored magn. nanoparticles*
- NoE Marine Genomics consortium
- Centre National de Génotypage, France, *SNP-technology*
- B. Gerwick,University of California at San Diego, USA
- D. Gordon, University of Washington, USA
- A. Canario, Universidade do Algarve, Faro, Portugal
- J. Cerda, IRTA CSIC, Barcelona, Spain
- F. Bonhomme, University of Montpellier, France
- P. Prunet, INRA Rennes, France
- E. Lubzens IOLR Haifa, Israel
- A. Kaplan, Hebrew University, Jerusalem, Israel
- B. Wróbel, Polish Academy of Sciences, Warzsawa, Poland
- P. Holland, University of Oxford, UK
- L. Bargelloni, University of Padova, Italy
- E.Sarropoulou, HCMR Crete, Greece
- D. Reinhard, University of Fribourg, Switzerland
- F. Nielson, Institute of Marin Research, University of Bergen, Norway
- K.S. Jakobsen, University of Oslo, Norway
- L. Contreras-Porcia, Santiago, Pontificia Universidad Católica de Chile
- M. Thorndyke, Kristineberg Marine Research Station, Fiskebackskil, Sweden
- M. Achtman, University of Cork, Ireland
- D. V. Cantonnel, Universidad de la República, Uruguay
- B. Kloareg, SB Roscoff, France
- L. Lauro, SZN Naples, Italy
- P. Schmid-Hempel ETH Zürich, Switzerland

Industry

- Bruker Daltonik, Bremen and Leipzig
- micromod Partikeltechnologie GmbH, Rostock
- Scienion AG, Berlin
- Life Technology, Germany

General information

Complete list of publications (2006-2009)

2009

Raabe CA, Sanchez CP, Randau G, Robeck T, Skryabin BV, Chinni SV, Kube M, Reinhardt R, Ng GH, Manickam R, Kuryshev VY, Lanzer M, Brosius J, Tang TH, Rozhdestvensky TS. *A global view of the nonprotein-coding transcriptome in Plasmodium falciparum.* Nucleic Acids Res 2009 Oct 28. Epub

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Awards

Julia Cekanov

- Trainee award of the Max Planck Society 2008
- "Best trainee" award of the Chamber of Commerce and Industry, Berlin, 2008

PhD thesis

Kuhl, Heiner (03/2008) *Ein Verfahren für BAC-DNA Aufreinigung im Hochdurchsatz zur Genomkartierung von Dicentrarchus labrax*. PhD Thesis, Technical University of Berlin (supervisor: Richard Reinhardt)

Student Theses

2009

Alexander Migdoll (01/2009) *Vergleichende Genomanalyse der pflanzenassoziierten Bakterien A. brassicae und A. palmae*. Master Thesis, University of Applied Sciences, Berlin

2008

Katja Heitmann (10/2008) *Entwurf und Entwicklung eines kommerziellen Web-Portals mit Drupal*. University of Applied Sciences, Berlin

Anne Musahl (11/2008) *Vergleichende Analyse mitochondrialer Genome der Gastropoda unter besonderer Berücksichtigung der Pulmonata*. Bachelor Thesis, Humboldt University of Berlin

Steffen Andrew Scheer (12/2008) *SP3: Ein Workflow Management System für die parallele Sequenzdatenverarbeitung*. Diploma Thesis, Humboldt University of Berlin

2006

Mazen Hassan (06/2006) *Einfluss von chemischen und physikalischen Parametern auf die größenfraktionierte DNA Fällung in PEG/Salz-Gemischen*. University of Applied Sciences, Berlin

Patents

Kalkum M, Müller M, Nordhoff E, Reinhardt R, Eickhoff H, Rauth H.

- *Method and Device for processing extremely small substance quantities*, PCT/EP99/02964
- *Verfahren und Vorrichtung zum Prozessieren von Kleinstsubstanzmengen,* DE 198 23 719 A1

Rauth, H; Reinhardt, R; Nordhoff, E. *Verfahren zum Anbinden von Nukleinsäuren an eine Festphase. PCT/EP00/08807, DE, EU, US*

Rauth H; Reinhardt R; Nordhoff E: *Verfahren zur Umkehrphasenaufreinigung und -konzentrierung von Peptiden und Proteinen an magnetischen Partikeln*. PCT Nr. 60/175,958

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External funding

EU: *EURATools* (LSHG-CT-2005-019015), 03/06-08/10

EU: *AquaFirst* (FP6-513692)

EU: ASSEMBLE *Association of European Marine Biological Laboratories* (FP7-227799) 03/09-02/13

EU: LIFECYCLE *Building a biological knowledge-base on fish lifecycles for competitive, sustainable European aquaculture* (FP7-KBBE-2007-2A) 02/09-01/13

BMBF, NGFN Plus: Verbund: *Rnomics of Infectious Diseases* (BMBF 01GS 0805) 09/08-08/11

BMBF: *Infektionsnetz: Resistance and Suspectibility to intestinal infections, WP VI: Sequencing and annotation of selected cloned fragments or isolates relevant to intestinal homeostasis* (BMBF 01KI 0798) 07/09-11/10

DFG: Schwerpunkt: *Deep Phylogeny* SPP 1174

MPG/joint genome project with MPI Bremen: *Microbal Corrosion*

MPG/joint genome project with MPG/ SARS Bergen: *Oikopleura, ca. 75 Mbases working draft, BAC-ends*

EU: NoE *Marine Genomics* (EU- FP6-2002-GLOBAL-1 Member of Scientific Steering Committee) 03/04-08/08

BMBF, NGFN-2: SMP-DNA: TP-02 *Chimpanzee-X sequencing* (BMBF 01 GR 0414) 07/05-12/08

BMBF, NGFN-2: SMP-DNA: TP-05 *Ratte*, 01/05-12/06

BMBF, NGFN-2: SMP-DNA: TP-16 *Re-Sequencing* (BMBF 01 GR 0414) 07/05-06/08

BMBF, NGFN-2: SMP-Epigenetic: *Methylom project* (BMBF 01 GR 0497) 05/05-04/08

EU: *STAR-rat A SNP and haplotype map for the rat* (EU LSHG-CT-2004-005235) 01/05-12/06

EU: NEST-Sleeping Beauty *Dormancy of cells and organisms- strategies for survival and preservation* (EU- NESTFP6-2003-NEST-B-1) 09/05-08/08

MPG: *Umweltgenomic (*Institutsübergreifende Projektförderung, MPIs in Berlin, Bremen, Marburg) 05/04-06/09

BMBF: *BioChancePlus* 12/06-05/09

Teaching activities

Courses

Generation of cDNA libraries by Primer Extension, within the SP1174 Deep Metazoan Phylogeny Project, 2006, 2007

cDNA libraries by primer extension course, PhD program at the MPIMG within the Marine Genomics Europe FP6 project, 2006, 2008

Pratical aspects of RNA extraction: "before and after" in cDNA Library construction, PhD program at CCMAR Faro, Portugal within the Marine Genomics Europe FP6 project, 2007

Organization of scientific events

Sleeping Beauty Workshop, Harnack House Berlin, 18.-20.05.2008

Guest scientists

2009

Nadav Denekamp, Esther Lubzens, IOLR, Haifa, Israel, 25.01.-01.02.2009

Prof. Dr. Klaus Geider, BBA Dossenheim, Germany, 22.02.-24.02.2009

Caterina Pellizzari, University of Padova, Italy, 02.03.-14.04.2009

Isabel Gehring, JKI Dossenheim, Germany, 09. -28.03.2009

Lars Wöhlbrand, Uni Oldenburg, Germany, 04.-20.05.2009

Duduk, Bojan, Prof.Bertaccini, DiSTA Plant Pathology, Bologna, Italy, 02.-05.07.2009

David Goncalves, Eco-ethology research unit-ISPA, Lisbon, Portugal, 13.-26.09.2009

2008

Tine Mbaye, Université de Montpellier 2, France, 23. -26.05. and 31.07.-31.10.2008 (Aquagenome short visit Research Program)

Sisco Monjo, Barcelona, Spain, 29.05.-18.07.2008

Nadav Denekamp, IOLR , Haifa, Israel, 04.-15.08.2008

Liliana Anjos, Center of Marine Science, University of Algarve, Faro, Portugal, 19.11.-16.12.2008

2007

Liliana Anjos, Center of Marine Science, University of Algarve, Faro, Portugal, 14.01.-14.02.2007

Prof. Dr. Klaus Geider, BBA Dossenheim, Germany, 18.-23.08.2007

Sónia Massa, CCMAR , Universidade do Algarve, Portugal, 18.11.-06.12.2007

Dr. Serkan Uranbey, Ministry of Agriculture and Rural Affairs,Ankara, Turkey, 24.11.-23.12.2007

Paola Gomez, MPI MB Bremen, Germany, 03.-05.12.2007

2006

Annika Busekow, Martina Ackermann, Steffi Arend, MPI Plön, Germany, 16.-20.01.2006

Prof. Dr. Klaus Geider, BBA Dossenheim, Germany, 13.-15.03.2006

Vasso Terzoglou, HCMR Heraklion, Greece, 16.-26.03.2006

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Marianne Hartwig
Eleonora Volcik

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Karsten Krause (part-time)
Ute Müller
Rita Röfke-Bohnau
Kerstin Steudtner

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Karsten Krause (head, part-time)
Jürgen Joch
Olaf Kischkat
Dominik Buggenhagen

Reception, post office
Sara Aziz (part-time)
Monika Schweizer-Annecke (part-time)

Driver
Claus Langrock

The high amount of external funding determines the central services' work considerably. Almost half of the MPIMG's total budget is funded by external sources. This is illustrated for instance by the institute's participation in the "Nationales Genomforschungsnetz" (NGFN), solely contributing to almost 48 m EUR during 2001 - 2011. As a consequence, fluctuation of personnel is extensive. A significant amount of the institutional funding is needed for bridging gaps in externally funded posts. Similarly, cash outflow is fluctuating extensively. The aforementioned examples make clear that controlling the institute's budget is therefore possible only to a limited extent. Furthermore, providing an adequate technical infrastructure is increasingly difficult since needs are changing rapidly. Without the growing share of overhead funds and without the Max Planck Society's flexible budgetary rules these challenges could not have been met.

Another challenge during the last years results from the rapid technological advance. Much of the large equipment is outdated already after three years, after five years at the latest. This is why a considerable amount of the consumables are rededicated to complement the short equipment budget. Furthermore, data output of scientific appliances is growing vastly, e.g. for high-throughput image analyses. Even other dimensions of data output are connected with next generation sequencing. In 2009, as a consequence, the institute's cooling system as well as the emergency power supply had to be expanded by means of a container system. Closely related is the steep rise in energy costs (about 80 % during the last five years), caused not only by price increase but more significantly by an increase in consumption.

The new collective labour agreement for the federal public service (TVöD - Tarifvertrag für den Öffentlichen Dienst) effective since October 2005 still bears many structural deficits. Moreover, performance related payments are still not possible within the Max Planck Society since an agreement between the joint works council and the management could not yet be reached. Partial relief brought the possibility to award additional bonuses for scientists and technical staff. Unfortunately, the administrative and technical service units are not eligible. Hiring qualified new staff therefore is still very difficult.

The German law on temporary employment contracts in science (Wissenschaftszeitvertragsgesetz) which came into effect in April 2007 turned out to be mostly helpful to employ young scientists for a limited time-period. Overall, the law is rendering more contractual freedom irrespective whether contracts are financed by institutional or external funds.

As mentioned before, without the flexible budgetary rules the Max Planck Society adopted since 2005, the institute could not have met the challenges during the last years. However, during the last two years special reporting obligations to the funding bodies are increasing. As a result, the budgetary flexibility and control are impeded again. Some of the advantages gained during the last years might get lost.

The cost accounting system of the Max Planck Society, implemented at the MPIMG in 2004, is now routinely used e.g. for calculating and charging special services for internal as well as external users. In 2009, the institute developed a full cost accounting system for the next generation sequencing unit. In 2010 the reporting system will be improved and harmonized for all Max Planck Institutes.

Further improvement is still required for the SAP-driven personnel administration system (PVS), although the system is running since 2003. Reporting procedures and the handling of time limited contracts is still cumbersome.

In 2006, the MPIMG implemented an electronic ordering system for the storeroom so that the goods are delivered twice a day directly to the lab. The system is running well and allows reducing stock-keeping costs considerably. In addition the institute reorganized the purchasing process and takes part in the electronic procurement system (e-procurement) of the Max Planck Society since 2007. Goods can be ordered electronically from any work place in the lab or office using a Max Planck specific catalogue for most of the standard goods or by transmitting any other offering electronically to the purchasing department. From the administrative point of view the system's most important advantage might be that substantially more time is provided for dealing with service contracts and large procurements. Only a less significant part of the purchasing activities deals with consumables and standard products; most of the efforts flow into contracts for complex services, a growing part of it are scientific services.

The institute's efforts in vocational education have resulted in 15 graduations since 2003 (10 animal keepers, two office clerks, one laboratory assistant and two IT specialists for system integration). Three trainees were awarded prizes as brilliant or best graduate of the year (another one in 2002). The animal house was awarded a prize as one of the best training sites of the Max Planck Society in 2008.

The MPIMG takes an active roll in supporting the institute's members who have children. The institute cooperates with two day-care centres; in addition, the institute seeks to offer child care during conferences. Within the institute's premises two rooms have been dedicated for small children and school aged children. Flexible working hours and teleworking are common in most groups. According to the German law on temporary employment contracts in science (Wissenschaftszeitvertragsgesetz), temporary contracts during the so-called qualification period ("Qualifizierungsphase") are extended automatically for times of parental leave. Furthermore, the institute is making efforts to offer this also to externally funded employees whenever possible. The "family service", provided by the Max Planck Society to all institutes, offers a broad range of services from child- and elder care to housekeeping. The Christiane Nüsslein Volhard Foundation supports talented young female scientists by financial grants to pay for assistance in household chores and for additional childcare. These initiatives are welcomed by the MPIMG.

For the following years, construction of tower III and the refurbishment of towers I and II will be the most important task. This will enable the institute to close down the premises on Fabeck Street occupied since 2002. Beyond that the growing number of damages in the institute's technical infrastructure puts pressure to finalise these measures as soon as possible.

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Technical Management & Workshops

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In 2003, the institute received the "go ahead" from the President of the Max Planck Society to build tower 3 and to completely renovate towers 1 and 2, built in 1968 and 1970, respectively. The necessary planning funds were approved in early 2006. By August of 2006 the planning stage should have been completed. The actual construction date was set for 2007. However, during the planning stage of 2006 the process for the approval and implementation of building projects of the Max Planck Society was totally revised and changed fundamentally.

The “Gemeinsame Wissenschaftskonferenz” (GWK), the Joint Science Conference of the German Federal and State governments eventually gave its approval for the construction in August 2009. Much of the actual initial planning must now be revised because of partial changes in the requirements that have developed during the past years.

Even more important are legal changes such as new energy saving laws affecting the planning. At present there is no time schedule in place. It is uncertain whether construction will begin before the middle of 2010. The renovation of towers 1 and 2 will not be completed, as originally planned, before the appointment of new heads of department, which weighs heavily on the institute.

The new tower 3 will be used by the Vingron Department, other theoretical research groups, and the IT service group. It will be the central entry foyer for the institute and the designated seminar rooms on the ground floor will be used for events. In addition a new server room will be built in tower 3. The construction work is estimated to last for approximately two years, followed by the renovation of tower 2 and tower 1 respectively. Since the work cannot take place while research is being conducted, it will be necessary to move into the freed up space in other towers and in our satellite offices and laboratories on Fabeck Street. The renovation of the towers 1 and 2 will be completed in approximately three years. Thus the total construction time including building Tower 3 will last roughly 5 - 6 years.

The construction of tower 3 and the renovation of towers 1 and 2 is very urgent due to frequently arising technical issues in the infrastructure. It is becoming increasingly difficult to keep pace with the changing scientific demands. This becomes especially evident in the lack of energy supply for the cooling system and the emergency generator. Consequently, numerous additional small cooling systems had to be installed. To respond to emergency energy needs a container generator had to be installed. In total four emergency generators are being used now. The purpose of the renovations to the technical infrastructure is to resolve the aforementioned issues.

Currently extensive reconstruction is taking place at the animal facility. The animal facility is no longer divided into two areas. The mice are kept under specified pathogen free conditions in areas with restricted access only. Additionally new labs are being built. Defects identified on several occasions by the authorities are being addressed and resolved. By extending the ventilation and cooling system, the essential technical requirements are met to purchase a new cage system. This will increase the capacity of the cage system by 35 % and amount to almost 2,000 additional cages.

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Melanie Bienek (03/07-03/09)
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Sven Knaack (03/07-09/08)

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Özlem Demirel (since 06/08)
Silvia Kiesel (since 06/09)
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Stefan Heider (09/04-08/07)

Introduction

The scientific service group is active in the fields of DNA template preparation, purification, sequencing and sequence analysis, protein purification and analysis by Edman sequencing (terminated by Mai 2009), MALDI-MS methods (terminated by August 2009), enzyme preparation and purification as well as synthesis of highly specific oligo-nucleotides like Energy-Transfer primers etc.
Next generation sequencing (NGS) has been established since December 2006, starting with a Solexa GA Ix and a Roche GS 20 system and has become the fastest growing part of the service group, besides the needs for computational analysis. Since January 2008, the NGS activities of the institute have been continued in several groups, one run by Bernd Timmermann (using Illumina GA II and Roche 454 instruments), and two groups within the Department of Vertebrate Genomics (H. Lehrach), headed by Andreas Dahl, mainly working with SOLiD systems, and

Alexey Soldatov, using both Illumina and SOLiD systems. For my own scientific projects, NGFNplus, DFG- and EU-projects, internal MPG-services and co-operations, we are using Roche 454 and SOLiD systems for *de novo* sequence analysis of nc µRNAs and methylation analysis etc. Specific reports are presented by B. Timmermann and within the Department of Vertebrate Genomics.

Automation of procedures in any of these methods plays an important role. Another very important feature of our work is the miniaturisation, e.g. the downscaling of reaction volumes and costs per reaction. Within the framework of a BioChance-plus project, we have developed a nano-liter amplification system, based on a 454-DLX pico-titerplate and a piezo-dispenser, working with only few nl reaction volume. With a newly developed piezo-system (Scienion AG), we are optimistic to increase the speed of sample and volume transfer and to minimize the loss of sample after picking up the reaction products.
The group has a good infrastructure for mutation analysis and DNA-sequencing, with a special focus on the analysis of medically related re-sequencing projects. In parallel to conventional and NGS sequencing, we have examined approaches designed for improving the efficiency of large-scale projects, like MS-MALDI methods for base determination, SNP detection and mini-sequencing (terminated).
The service costs for our main issues are calculated and those requesting the service are charged of an individually assembled cost calculation. Besides the service aspects of our work, the group is a cooperation partner of the international HUGO project, European based projects, the national DFG and NGFN projects together with the Departments of Hans Lehrach, H.-Hilger Ropers and Martin Vingron. For this purpose, several software tools were optimised or developed in close cooperation with the computing people of the group, using advanced LINUX/UNIX based clustered hardware. For further needs we will extend new clustering strategies (multi-processor PC-based LINUX-cluster) for the automated assembly of very large data sets, automated checking and editing steps and web based software tools for co-ordinating projects with external partners. Most of this will be done in close co-operation with the Department of Computational Molecular Biology (M. Vingron) at the institute, the Sanger Centre (UK) and the University of Washington (USA).

Infrastructure analytic group

The analytic group has established a good infrastructure for large-scale genomic analysis projects as sequencing, mutation analysis and mass spectrometry, using most advanced methods and systems for protein purification and sequencing (ABI 394 and Bruker 2-D-MALDI (terminated)), SNP-detection and DNA-sequencing, including NGS (Roche 454 and SOLiD), mutation- and genetic variation analysis (WAVE-systems, ABI 3730XL and automated MegaBace 4500 (terminated 03/09). With the aim of miniaturisation, highly automated procedures including varies kinds of robotics are continuously developed, e.g. automated PCR, plasmide, fosmide and BAC template preparation and sequencing site, equipped with a CRS robotic arm on a track line, automated precipitation line incorporating a CRS arm, 96 and 384 Beckman Multimek pipetting systems, MTP centrifuges and a MTP-UV detection system. Separately, we have installed an automated clone hit picking device, designed to handle more than 200 MTP's in one run, incorporating an arm. Routinely available are MTP centrifuges, Tecan Twister freedom evo device to use 1536er MTP's and varies automated 384er Hydra/Twister mini systems to handle DNA samples to be purified, using our patent related magnetic beads methods and a novel PEG/org. solvent based fractionated precipitation method (thesis Heiner Kuhl). Our angular gel electrophoresis system (patented) is able to visualise

384 sample on a MTP-sized gel and to be automatically processed exploring a capacity of more than 15.000 samples per day. An 'Illumina bead station platform' provides the latest generation of µ-array technology for expression profiling and genotyping for human and mouse samples. Besides these 'high throughput highlights', our lab equipment involves all necessary items to run large scale projects, e.g. thermocycler, centrifuges, incubators, protein purification systems and sophisticated computer equipment.

Infrastructure IT-group

The IT-group responsible for the operation and development of the whole IT-infrastructure of the institute, which includes workstation and server systems, wire based and wireless LAN, Internet access, Internet services and remote access *via* modem, ISDN and DSL, security devices (anti-virus and anti-SPAM software, data backup-, fire-wall). Additionally, the maintenance of the biological databases and corresponding software tools are served also by the IT-group.
Our online storage capacity on disk-based file servers exceeds 700 TB of data, while the monthly backup volume has increased due to the needs of new projects to about 60 TB (without NGS systems!). Presently we serve about 450 Window based PCs and 600 Linux/Unix systems with a variety of hard- and software components and about 80 MAC systems. A variety of WEB-server are protected by our fire-wall installation, about 60 WEB-server are active and maintained by us, including hard- and software development and are serving the scientific departments as well as the service and administration groups.

Since 2008, the new requests of the NGS technology have dramatically increased the effort of the IT-group to serve the computational and storage needs for data processing and analysis. Within the last 18 month, we have established a new concept for short and long term data storage based on disk-arrays, installed additionally a storage capacity of more than 1500 TB and have increased the computational power, based on UNIX systems, by more than a factor of 100. The connection of the distributed NGS systems, one SOLiD is installed in Fabeckstrasse, is based on a separated fiber-glass ring to minimize the interference with the standard institute connections and to guarantee a contiguous connection and data flow. The back bone is based on 10-Gbit technology between the computational and disk servers, while each sequencer itself is connected *via* Gigabit. The data flow for one sequencing run is between two to five TB, depending on the protocols and sequence demands, while the final data storage after image analysis and subsequent data reduction is still in the range of 500 GB, including the quality files. This needs to be further down sized, but international efforts is necessary for standardisation.

To manage and control the flow of data, our fibre based 10-GigaBit-LAN, connecting laboratories in Fabeck- and Harnackstrasse to the campus Ihnestrasse, is segmented by about 100 manageable switches giving us the ultimate flexibility to control each segment and if necessary to configure each switch port individually. These additional needs have put extreme pressure on the personal capacity of the IT-group, as one and a half persons are completely absorbed by the NGS infrastructure. Thus, there is the urgent need to increase the personal power of the group to maintain the other requests of the scientific groups and to keep updated with the rapidly growing demands for security requests.

Both, analytic- and IT-group are very active in the training and education of young technicians, students, trainees and apprentices.

Research Support & Central Units
Next Generation Sequencing

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Overview

The Next Generation Sequencing group at the MPIMG was founded at the end of 2007 and is a central service unit open to all groups of the institute. The group was established to help researchers process DNA samples in an efficient and economical manner. By centralizing equipment and expertise, we have dramatically reduced the overall expense to the institute, while increasing the efficiency and quality of the data generated. At the moment we are using two different technical platforms: Roche/454 FLX and Illumina GAIIx systems.

Over the last two years protocols for the next generation sequencers were established and permanently improved. Both platforms, the Roche/454 and the Illumina GAIIx system, have been extensively used for different applications (a number of them in collaboration with other MPG institutes).

- *de novo* sequencing of eukaryotic and procaryotic genomes (e.g sequencing of the canaris genome in collaboration with Prof. M. Gahr, MPI for Ornithology);
- large-scale resequencing of human genomes (e.g. in the 1000Genomes Project with Dep. Lehrach);
- analysis of individual eukaryotic chromosomes (Dep. Ropers);
- expression profiling (Dep. Herrmann and Dep. Lehrach);
- microRNA Sequencing (with S. Sperling, Dep. Lehrach);
- ChIP-Sequencing analysis (Dep. Herrmann and Dep. Lehrach);
- methylation studies (Dep. Herrmann and Dep. Lehrach);
- mapping translocation breakpoints (Dep. Ropers);
- resequencing of enriched candidate genes and target regions (collaboration with M. Schweiger and L. Bertram, Dep. Lehrach).

Within the institute all departments having wet labs (Dep. Herrmann, Dep. Lehrach, Dep. Ropers, Research Group Mundlos and Otto Warburg Laboraties) are using the NGS facility.

The Next Generation Sequencing Group is currently acting as international reference side for Roche and as beta test side for Illumina. This provides access to technical improvements of the instrument early on. In our cooperations with Roche we have developed work flowes for using of human whole exome enrichment arrays in combination with Titanium sequencing kits. As beta test side of Illumina we are currently testing new polymerases for performing longer sequencing reads.

Research Support & Central Units
Animal Facility & Transgene Unit

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Service
Edward Somera

The Animal Facility was completely brought into service in the year 2003. It provides an optimal research environment in the field of Laboratory Animal Science which includes the basic animal breeding and maintenance service for approximately 300 genetically modified and 30 wildtype mouse strains and technical services with a highly motivated staff. The mouse strains are kept under specified pathogen free (SPF) conditions in areas with restricted access. By using several physical barriers and standard working protocols we have been strongly committed ourselves to keep our rodent colony free of rodent pathogens. All strains are housed in individually ventilated caging systems and are handled under sterile conditions (approximately 6,000 cages).

The Animal Facility provides high standard services which includes:

- experimental work and colony maintenance;
- cryopreservation of mouse embryos and sperm freezing;
- *in vitro* fertilisation (IVF);
- Sterile Embryotransfer;
- tissue biopsies;
- blood and organ collection;
- assistance in experimental design and techniques;
- training for researchers, caretakers and trainees;
- rederivation;
- import/export of animals.

For the management of these mouse strains and the offered services, a mouse-colony management software program (PyRAT®) was established. By using this software all mouse data are easily accessible for scientists.

The *Transgene Unit* of the Animal Facility was established in the year 2004 to enable the successful and efficient generation of genetically modified mice for the scientific staff of the institute. It provides a centralised resource and state-of-the-art technology in generating knockout mice by injection of embryonic stem cells into mouse embryos or by aggregation of diploid and tetraploid embryos and transgenic mice by injection of DNA into the pronucleus of fertilised mouse oocytes. Above that the service provides cleaning, freezing and thawing of mouse embryos, sperm freezing and in-vitro-fertilisation.

Already more than 200 DNA constructs were used for pronuclear microinjection to generate transgenic mice and 80 ES-cell clones were used for blastocyst microinjection to generate knockout mice.
In addition to this more than 100 mouse strains were cryopreservated for backup of important transgenic mouse lines of scientists of the institute and stored in liquid nitrogen.

The Fish Facility of our institute is set up to raise and keep up to 15,000 zebrafish *(Danio rerio*). The aquatic system is located in the animal house and consists of approximately 150 single tanks. It is available to all researchers at the institute, either to keep fish, or to provide eggs, embryos and larvae. For embryonic manipulations the pressure-driven microinjection technique for mRNA or DNA is available.

Research Support & Central Units
Library

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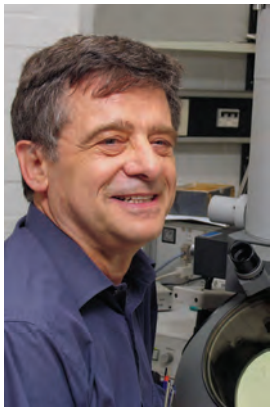
- Ralf Sudbrak, EU coordinator
- Ralf Spörle, Dept. Developmental Genetics
- Sigmar Stricker, Research Group Development & Disease
- Knud Nierhaus, Ribosome Group
- Michael Lappe, Otto Warburg Laboratory

The library supports the scientific work of the institutes *via* an optimal supply of literature and information. Its main function is to have the scientific literature as up-to-date and as complete as possible in a reference library.

The library holds about 60 000 volumes and subscribes to over 80 scientific journals and series. It still reduces the holdings of print journals but improves the electronic spectrum of scientific information and e-books. In addition to the web catalogue, an increasing number of databases as well as electronic interlibrary loan service are offered. Online workshops are available to staff and guests of the institute to provide an overview and a better understanding of important scientific information resources and tools for literature searching and bioinformatics-related research on scientific information resources and methods. The team also offers introduction courses in how to use the library, databases and other library services. Seminars with guest speakers about current subjects like electronic information systems, Open Access and E-science are offered for the scientists. All publications of the institute are edited and submitted into the yearbook of the Max-Planck-Society. The library team is part of the pilot program within the Max-Planck-Society projects “PubMan” and “OpenAccess”.
The goal for further development of the library is still to improve the “Virtual Library”, a network of knowledge systems ensuring the delivery of information to researchers’ desktops wherever and whenever needed.

Research Support & Central Units
Imaging



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The microscopy group is a central scientific service unit for the institute. We provide a broad variety of techniques, support, service and maintenance in both, light microscopy and electron microscopy.
In light-microscopy, the group is responsible for the service, maintenance and training of an increasing number of microscopes and user. Meanwhile, we support three confocal microscopes (Zeiss LSM510, LSM510meta and LSM700) and two fluorescence microscopes (an upright Zeiss AxioImager, an inverted Olympus microscope), covering a broad range of cell-biological applications. These instruments are used as shared equipment within all departments of the institute. Our group services and maintains the instruments. We introduce and train new users to the different instruments and techniques. Furthermore, we support all users according to their biological questions and implement new techniques and applications.

Monica Shevack
(part time)

The scientific illustrator closely cooperates with the scientists to prepare publications and presentations. Most of the artwork is computer generated (PC and Mac) using programs such as Photoshop, Adobe Illustrator, Freehand, Powerpoint and other interactive tools. In addition, conventional hand drawn illustrations can be prepared on request. Recently the textbook "Systems Biology in Practice" was illustrated for the Kinetic Modeling group.

Katrin Ullrich
(part time)

The classical wet darkroom is replaced by digital methods. Main tasks involve scans for presentations and photographic documentation including the finishing of the digital images in Photoshop. There is a broad range from photo reproduction of colonies or well plates, update the online catalogue to portraits of the groups and documentation of events in the institute. Various scanners and cameras are provided as general equipment.

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Underground / U-Bahn
- U3/Oskar-Helene-Heim
- U3/Thielplatz

Urban rail system / S-Bahn
- S1/Sundgauer Str.

Bus 110, M11
- Bitscher Str.

Bus 115, X10
- Schützallee or
- Leichhardtstr.

Bus 101, M48
- Holländ. Mühle or
- Winfriedstr.