



Otto Warburg Laboratory

Neurodegenerative Disorders

(Established: 02/02 as part of the Dept. of Vertebrate Genomics, independent since 09/08)

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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 respect, 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

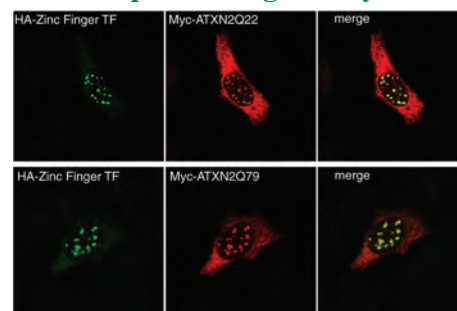


Figure 1: Over-expression of an interacting transcription factor (HA-Zinc Finger TF, green) causes recruitment of over-expressed ataxin-2 with a normal (Myc-ATXN2Q22, red) or an expanded (Myc-ATXN2Q79, red) polyglutamine stretch into nuclear bodies.

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 intracellularly 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 glyceraldehyd-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.

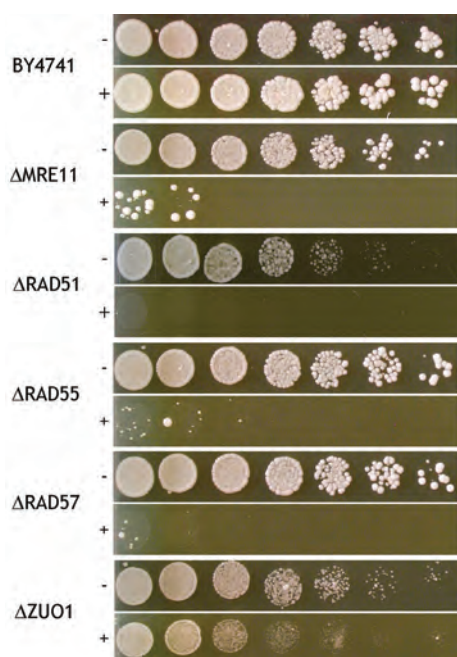


Figure 2: Selected results from a global drug screen in *S. cerevisiae*. Growth of wild type (BY4741) and deletion strains in the presence of solvent control (-) and chemotherapeutic drug (+).

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, Krobitch 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, Krobitch 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, Krobitch 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, Krobitch S (2007). *Dynamic re-routing of the carbohydrate flux is key to counteracting oxidative stress*. Journal of Biology 6(4):10.

Nonhoff, U., Ralser, M., Welzel, F., Piccini, I., Balzereit, D., Yaspo, M.-L., Lehrach, H., and Krobitsch, S. (2007). *Ataxin-2 interacts with the DEAD/H-box RNA helicase DDX6 and interferes with P-body and stress granule structures.* Mol. Biol. Cell 18 (4):1385-1396.

Taussig, M.J., Stoevesandt, O., Borrebæck, 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., Muyltermans, 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

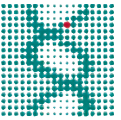
Ute Nonhoff (2007) *Untersuchungen zur Rolle von Ataxin-2 im zellulären mRNA-Metabolismus*, PhD Thesis, Freie Universität Berlin (supervisor: Sylvia Krobitsch)

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 Krobitch)

Susanne Weber (2009) *Identifizierung funktioneller Intrabodies für die Untersuchung von Protein-Protein-Wechselwirkungen in SCA2*, Diploma thesis, Technische Universität Braunschweig (supervisor: Sylvia Krobitch)

Mareike Schnaars (2007) *Funktionelle Charakterisierung der Proteininteraktionen zwischen Ataxin-2, LSM12 und Chromogranin B*, Diploma thesis, Universität Bremen (supervisor: Sylvia Krobitch)

Franziska Welzel (2006) *Funktionelle Charakterisierung der Interaktion von Ataxin-2 und DDX6*, Diploma thesis, Freie Universität Berlin (supervisor: Sylvia Krobitch)

Desagani N. Kumar (2006) *Investigations into molecular pathways involved in spinocerebellar ataxia type 2*, Diploma thesis, University of Skövde, (supervisor: Sylvia Krobitch)

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

Participation in Lecture Biophysikalische Chemie, Universität Hamburg, summer terms 06/07/08

Otto Warburg Laboratory

Bioinformatics / Structural Proteomics

(Established: 10/04)

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Patrick Slama*
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Hyeong Jun An

Scientific programmers

Jose Duarte (12/05-11/09)
Lars Petzold

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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

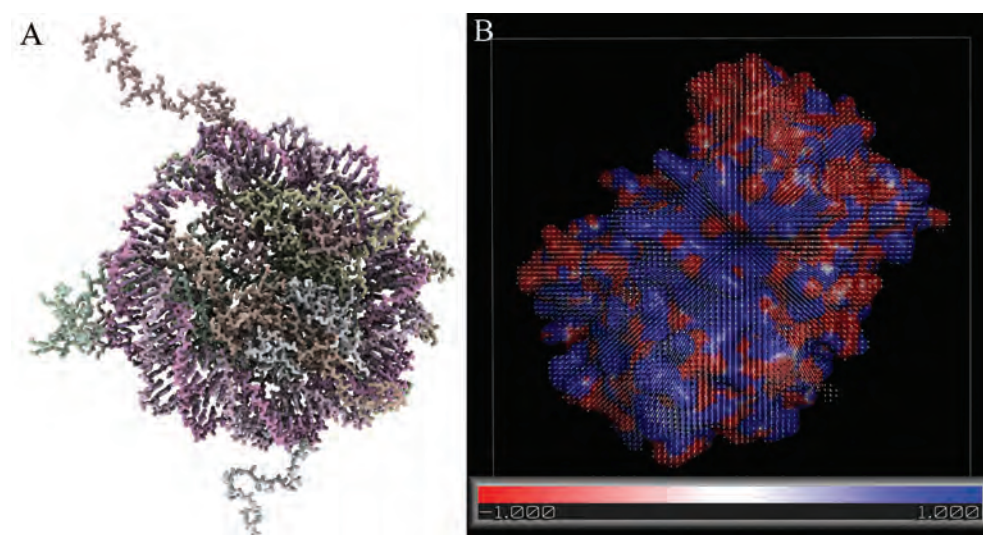


Figure 1: The structural basis of the epigenetic code (A) Overview of the nucleosome shows the histone octamer with the double-stranded DNA wrapped around it. The interface between DNA and the histone-core is called the lateral surface of the “wheel-like” core structure. Several Lysine and Arginine positions 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 remains impossible to satisfy all known experimental restraints simultaneously when the interacting proteins undergo conformational changes upon binding. Since most complexes are far too 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 2\AA 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\AA 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.

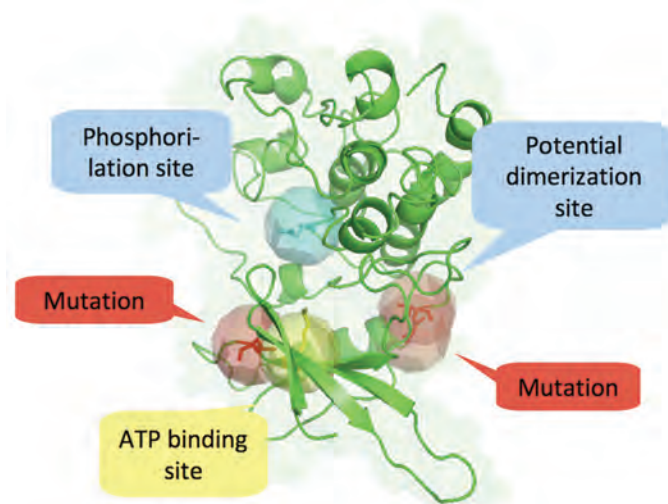
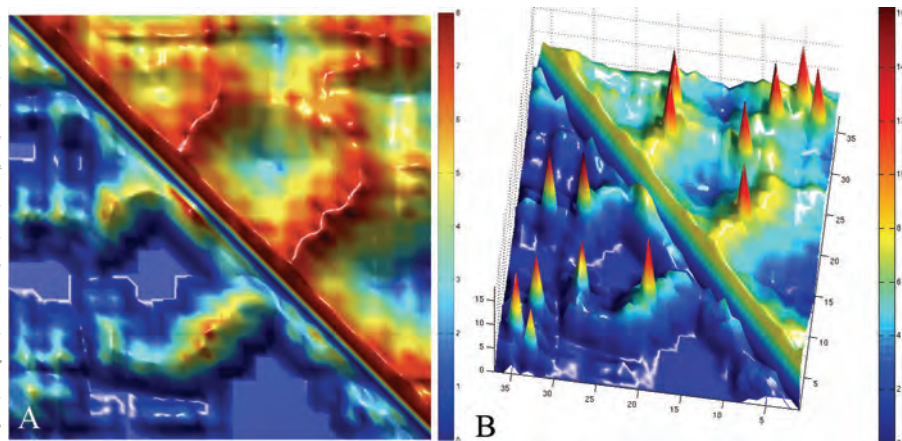


Figure 3: Mapping cancer mutations to protein structures. The epidermal growth factor receptor 2 (ERBB2) is implicated in aggravating tumor growth in breast cancer. The figure shows mutations found in cancer patients (red spheres) and important sites for the function of this protein. Proximity between 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 define the structure: a minimal reconstructable subset. We developed several algorithms capable of deriving extremely sparse subsets with approx. 4,8 Å C_{α} 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 flexible 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

Daujatz 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 May 13; 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

Paszkiwicz 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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Dr. Chung-Ting Han*
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Dr. David Meierhofer*

PhD students

Radmila Feldmann*
Susanne Holzhauser*
Christopher Weidner*

Engineers

Anja Freiwald*
Magdalena Kliem*
Claudia Quedenau*

Technician

Beata Lukaschewska-McGreal*

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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 PPAR γ ligands in mature adipocytes.

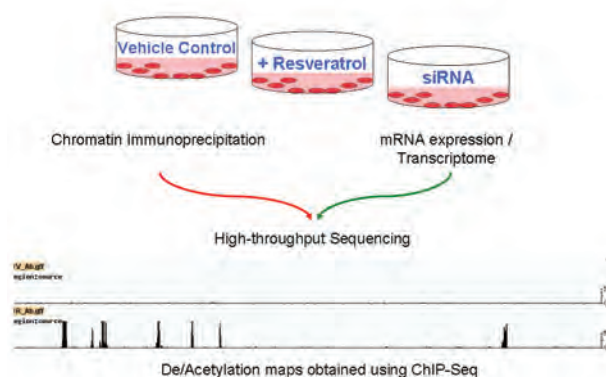


Figure 4: Genome-wide detection of histone modification caused by resveratrol treatment of cells.

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, Walitzka S, Sauer S, Warnke A, Remschmidt H, Gilsbach S, Schäfer H, Hinney A, Hebebrand J, Herpertz-Dahlmann B. *Familiarity 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 genotyping 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

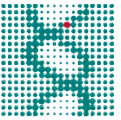
Organisation of scientific events

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 extracellular 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.

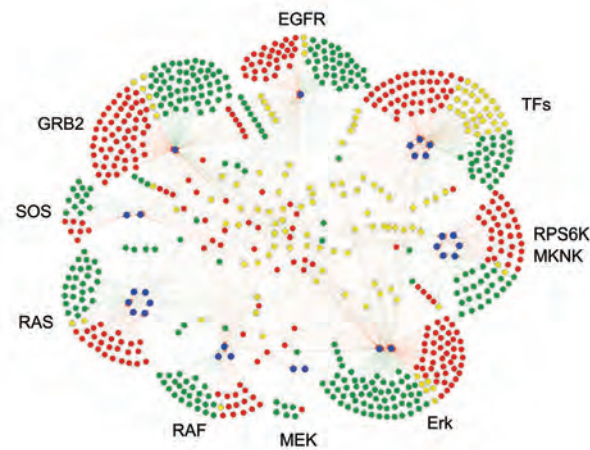


Figure 1: Model of a directed PPI signaling network. The EGF/Erk pathway and 733 direct interacting partners are shown. Blue nodes represent the 28 members of the EGF/Erk core pathway in a counterclockwise arrangement. Red and green nodes correspond to input and output nodes linked to the core pathway, 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

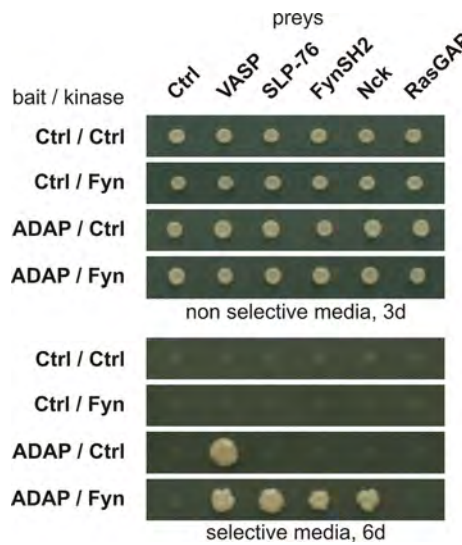


Figure 2: Analysis of P-dependent interactions involved in T-cell activation in a modified Y2H system. Diploid yeast colonies expressing indicated bait, prey and kinase constructs were assayed for growth on non selective and selective media, respectively. Y2H interaction between ADAP and VASP is independent 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 posttranslational 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)

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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 than the 30S initiation was unraveled [Schluzen et al., 2006, together with P. Fucini, MPIMG Berlin].

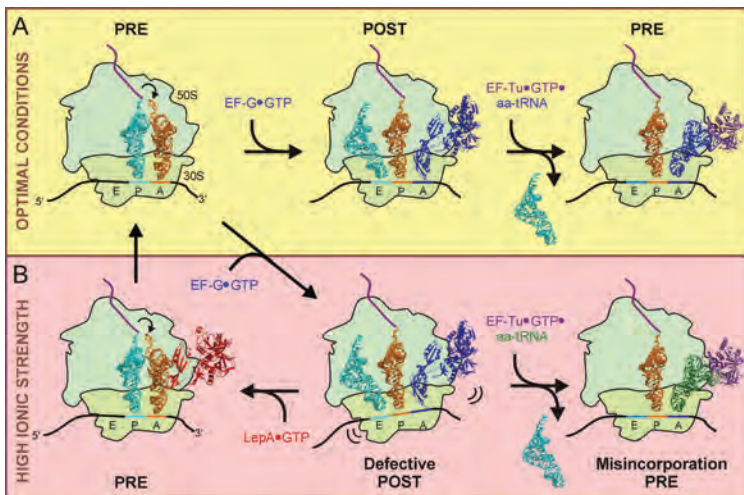
Evolution of the translational machinery

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 [Kaberina 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



268 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.

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 Giacomo 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].



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 non-cognate 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 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, 11.09.2008

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

