First Status Seminar of the Helmholtz Alliance on Systems Biology

Kongresshotel Potsdam

June 22-24, 2008

HELMHOLTZ | ASSOCIATION

Alliance on Systems Biology

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Program

Sunday, June 22, 2008		
16:00	**Registration** (Kongresshotel Potsdam, Foyer)	1:00
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	Presentation of Network **SBCancer I**	
18:15	**SBCancer Overview** | Roland Eils and Ursula Klingmüller	0:10
18:25	**Distinct Functions of PTEN and SHIP1 in Negative Regulation of PI3K/Akt Signaling** | Marcel Schilling, DKFZ	0:20
18:45	**Modeling erythropoietin receptor endocytosis identifies essential ligand binding properties and rapid recovery of cell surface receptor** | Verena Becker, DKFZ	0:15
19:00	**Differential regulation of IL-6 signaling in tumor keratinocytes and fibroblasts** | Margareta Müller, DKFZ	0:15
19:15	**Signaling and transcription factor networks in a mouse model of inflammation-associated cancer** | Jochen Hess, DKFZ	0:20
19:35	**Spatial and temporal resolution of the IFN network** | Dagmar Wirth, HZI	0:20
19:55	**Dinner** (Foyer)	1:05
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Monday, June 23, 2008		
9:00	Presentation of Network **CoReNe**	1:30
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Measuring and optimizing synchronous oscillation in Delta-Notch signaling | Fabian Theis, HMGU		
Genetic networks active in mouse midbrain development | Nilima Prakash, HMGU		
Role of Dicer/microRNAs in neurogenesis of the adult mammalian brain | Witold Konopka, DKFZ		
Intuitive Modeling of Dynamic Systems with Petri Nets and Fuzzy Logic | Lukas Windhager, LMU München		
10:30	**Coffee Break**	0:30
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Mathematical modeling of neurodegenerative processes in Alzheimer's disease | Katja Rateitschak, University of Rostock		
Signal transduction and transcription networks relevant for cardiac hypertrophy and failure | Jana Wolf, MDC		
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14:25	**Analysing gene expression patterns in the metabolic network of neuroblastoma tumours with wavelet transform**s | Rainer König, University of Heidelberg	0:20
14:45	**Gene Network Dynamics Controlling Keratinocyte Migration** | Hauke Busch, DKFZ	0:20
15:05	**From molecular machines to gene-regulatory networks in mammalian cells** | Thomas Höfer, DKFZ	0:20
15:25	**Coffee Break**	0:35
	Presentations of **Technology Platforms**	
16:00	**Dissecting cellular networks with high-throughput RNAi screens** | Sandra Steinbrink, DKFZ	0:20
16:20	**Systematic Analysis of Human Protein-Protein Interactions** | Ulrich Stelzl, Max Planck Institute for Molecular Genetics, Berlin	0:20
16:40	**Structural Characterisation and Relative Quantification of Protein Phosphorylation involved in Signal Transduction** | Wolf-Dieter Lehmann, DKFZ	0:20
17:00	**Identifying partners essential for receptor endocytosis** | Carsten Schultz, EMBL	0:20
17:20	**From Distributed Knowledge to Qualitative Models** | Volker Stümpflen, HMGU	0:20
17:40	**Data Management for Systems Biology** | Jürgen Eils, DKFZ	0:20
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20:00	**Dinner**	

	Tuesday, June 24, 2008	
9:00	Presention of Network **Human Brain**	1:30
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	A consistency check of layer-specific connectivity estimates and applications to cortical network modeling | Tobias Potjans, RIKEN Brain Science Institute	
	On the relation of spike synchrony and the local field potential | Michael Denker, RIKEN Brain Science Institute	
	Coordinated neural activity in monkey visual cortex during free viewing | Sonja Grün, RIKEN Brain Science Institute	
10:30	**Coffee Break**	0:30
11:00	Presentations of Network **Contaminant Molecules**	1:30
	From contaminant molecules to cellular response: system quantification and predictive model development - Overview | Irina Lehmann, UFZ	
	The BaP-AhR-pathway: Spatio-temporal dynamics of the receptor-ligand-complex | Juliane Mai, UFZ	
	Deciphering the transcriptional regulatory network of the Ah receptor | Andreas Beyer, TU Dresden	
12:30	**Lunch**	1:30
14:00	Presentation of Network H**ZI** / Discussion	1:00
	From Systems to Synthetic Biology: Streamlining and Reprogramming Biocatalysts | Vitor dos Santos, HZI	
	Systems analysis of T cell receptor and interferon signaling in T cells and its regulatory networks | Hansjörg Hauser, HZI	
15:00	Closing Remarks	0:30
15:30	**Official End**	

The Helmholtz Alliance on Systems Biology

Introduction

The Helmholtz Alliance on Systems Biology is a centrally funded, joint initiative of several Helmholtz centers and external partners. The aim of the alliance is to exploit the outstanding expertise of the Helmholtz Association in basic, high-throughput and bioinformatics research and to transfer it to innovative "Systems Biology" type of approaches. Scientific focuses of the Alliance are various complex diseases with the overall goal to widen the understanding of the causes of these diseases and the development of new strategies for treating them.

The Alliance consists of interconnected networks, each led by a specific Helmholtz centre.

In order to promote the emerging discipline of Systems Biology, the Helmholtz Alliance will establish an educational program (workshops, Summer Schools, PhD programs) to transfer its knowledge and experience.

Participating Networks and Centers

SBCancer: Systems Biology of Signaling in Cancer

German Cancer Research Center (DKFZ), Heidelberg

The network on Systems Biology of Signaling in Cancer (SBCancer) concentrates on signaling pathways that play a pivotal role in the cellular decisions between proliferation, differentiation and death. Alterations in these signaling pathways and connected gene regulatory networks can change cellular decisions and thereby trigger the onset of tumor formation.

MSBN: The MDC Systems Biology Network

Max-Delbrück Centre for Molecular Medicine (MDC), Berlin-Buch

MSBN focuses on the regulation of disease phenotypes of progressive cardiovascular and neurodegenerative disorders. Both are characterized by long asymptomatic phases before they become manifest. The regulatory mechanisms that effect first compensation and later pathology are being investigated and compared, employing a well designed, comprehensive systems biology strategy.

From contaminant molecules to cellular response: system quantification and predictive model development

Helmholtz Centre for Environmental Research (UFZ), Leipzig

The systems biology network of the Centre for Environmental Research aims at understanding cellular responses to chemical stressors with a systems perspective. This involves analysis of intra-cellular transport of the chemicals, reaction with sub-cellular target sites and the response of the cell at the transcriptional and post-transcriptional level. Research is initiated focusing on the cellular response to polycyclic aromatic hydrocarbons, which are known to exert a wide range of toxic effects.

CoReNe: Control of Regulatory Networks with focus on non-coding RNA

Helmholtz Zentrum München - German Research Center for Environmental Health (HMGU)

The interdisciplinary network entitled CoReNe focuses on the integrative interpretation of regulatory networks involved in cell differentiation with focus on the interaction of non-coding RNAs (ncRNAs). Focus is put on the influence of ncRNAs on gene expression and thereby on the regulatory networks of the cell. The aim is to extend existing models of regulatory networks by integrating the influence of ncRNAs.

The Human Brain Model: Connecting neuronal structure and function across temporal and spatial scales

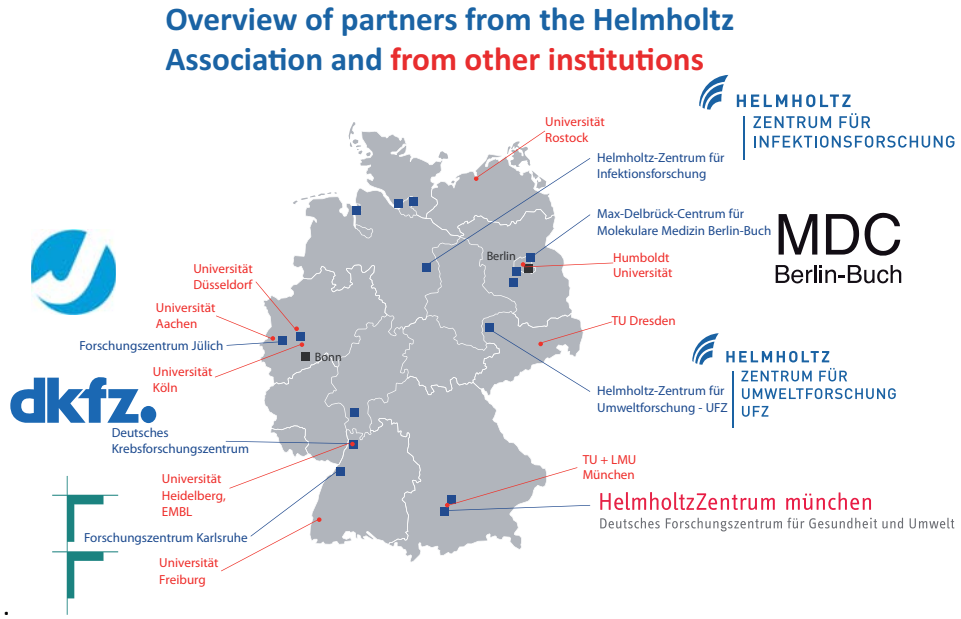
Research Centre Jülich (FZJ)

The network entitled "The Human Brain Model" investigates structure function relationships in the human brain as a multi-scale complex system. In particular, the structural, spatial and temporal interactions of different units of the nervous system are analyzed on different scales.

Systems Biology at the HZI

Helmholtz Centre for Infection Research (HZI), Braunschweig

Various projects at the HZI work on scientific problems using methodologies from systems biology. These projects include medical as well as biotechnological topics.



Talks

Systems Biology: WYDSIWYG (What You Don't See Is What You Get)

Jaroslav Stark

Department of Mathematics, and
Centre for Integrative Systems Biology at Imperial College *(*CISBIC*)*,
Imperial College London, London, SW7 2AZ, UK.

Modern high throughput and imaging experimental techniques are generating increasingly large and complex data sets. It is becoming more and more difficult to extract useful biological information from this data using conventional methods. There is growing agreement that to fully realize the potential of these new experimental technologies it is necessary to employ an interdisciplinary approach with a tight coupling of experiments and mathematical or computational modelling and analysis. The term *systems biology* is emerging to encompass a wide variety of such approaches. In this talk I shall begin by giving a brief introduction to my personal view of Systems Biology, and to the BBSRC funded Centre for Integrative Systems Biology at Imperial College (CISBIC).

I shall then present one particular strand of research within CISBIC on the analysis of high-throughput time-course data. Paradoxically, despite the vast abundance of data, key variables required to deduce biological functions can be difficult or impossible to measure. Thus for instance, DNA microarrays can measure transcript concentrations of a large number of genes simultaneously, but give no direct information on the activity of the transcription factors which control their production. Such transcription factors are commonly activated by phosphorylation and/or dimerization events which may be much more difficult to quantify experimentally. More generally, the identity of critical regulatory or signalling molecules may be unknown. Fortunately, it turns out that quantitative mathematical models may allow us to estimate such "hidden" variables and parameters. We shall describe the basic principles behind the solution of such "inverse" problems and then present an example from the analysis of DNA microarray time courses.

Keynote-Lecture

Distinct Functions of PTEN and SHIP1 in Negative Regulation of PI3K/Akt Signaling

Marcel Schilling[1], Bin She[1], Lu Wang[2,4], Carlos Salazar[2], Seong-Hwan Rho[3], Jens Timmer[3], Thomas Hoefer[2], and Ursula Klingmueller[1]

[1] Systems Biology of Signal Tranduction, German Cancer Research Center, Heidelberg, Germany
[2] Modeling of Biological Systems, German Cancer Research Center, Heidelberg, Germany
[3] Freiburg Center for Data Analysis and Modeling, University of Freiburg, Freiburg, Germany
[4] Center for Theoretical Biology, University of Peking, Beijing

The phosphoinositide 3 kinase (PI3K) / Akt signaling network is a ubiquitous pathway controlling cell proliferation, differentiation and survival as well as cancer development. This complex signaling network comprises phosphorylation and dephosphorylation events of proteins and lipids at the plasma membrane. We show by quantitative immunoblotting that stimulation with erythropoietin (Epo) induces distinct Akt kinetics in primary mouse cells at the colony forming unit-erythroid stage (CFU-E) compared to the lymphoid pro-B cell line expressing the EpoR (BaF3-EpoR). Despite shorter receptor signaling, CFU-E show a more sustained phosphorylation of Akt. To investigate this behavior, we quantified the stoichiometry of the signaling components and developed a mathematical model. As the erythropoietin receptor (EpoR) has multiple binding sites for adaptor and signaling proteins, this dynamic pathway model takes combinatorial complexity into account. The mathematical model quantitatively explains the differences between the two experimental systems based on the stoichiometry of the signaling components. To gain further insight, we overexpressed the two lipid phosphatases SH2 domain containing inositol-5-phosphatase (SHIP1) and phosphatase and tensin homolog (PTEN). We show by a combination of quantitative immunoblotting and mathematical control analysis that SHIP1 concentration determines signal duration while PTEN levels affect both signal amplitude and duration. We further demonstrate that SHIP1 is important for signal reduction at high stimulus levels, while PTEN critically reduces Akt phosphorylation at low input concentrations. This finding correlates with the tumor suppressive behavior of PTEN. We conclude that the stoichiometry of signaling components crucially controls the kinetics of Akt activation. Additionally, we show the different functions of the negative regulators SHIP1 and PTEN, with SHIP1 controlling signaling amplitude at high stimuls concentrations, while PTEN is important for the amplitude and duration of Akt even at low stimulations.

Network: SBCancer

Modeling erythropoietin receptor endocytosis identifies essential ligand binding properties and rapid recovery of cell surface receptor

V. Becker, M. Schilling, J. Bachmann, U. Baumann, S. Hengl, T. Maiwald, J. Timmer, and U. Klingmüller

To balance constant self-renewal and rapid adaptation to environmental changes in the hematopoietic system, cytokine receptors display several mechanisms to efficiently activate as well as terminate signal transduction. In contrast to receptor tyrosine kinases, for cytokine receptors such as the erythropoietin receptor (EpoR) only a minor fraction of receptor protein is localized to the plasma membrane whereas the majority is retained in intracellular compartments. Therefore, the contribution of endocytic downregulation of cell surface receptor for signal attenuation of cytokine receptors remains elusive.

Based on quantitative experimental data, we established non-linear ordinary differential equation (ODE)-based models describing ligand-independent and ligand-induced EpoR endocytosis, the key regulator of erythropoiesis. Model predictions were experimentally validated and revealed that signaling through the EpoR is tightly controlled by endocytosis-mediated ligand consumption, while the population of cell surface receptor is rapidly recovered. Sensitivity analysis showed that for parameters describing Epo-related processes the ligand association rate k_{on} is the parameter most sensitive to perturbations for ligand half-life and therefore the formation of signaling-competent ligand-receptor complexes. Furthermore, we could successfully exploit our model to determine k_{on} of the hyperglycosylated Novel Erythropoiesis Stimulating Protein (NESP) based on quantitative immunoblotting data. Therefore, this method presents a tool for efficient evaluation of Epo derivatives with enhanced bioavailability. Our approach demonstrates the potential of combining biochemical and mathematical analysis to establish new strategies for the development of refined clinical therapeutics and to elucidate design principles governing EpoR signaling and erythrocyte homeostasis.

Cytokine signaling through both the homodimeric EpoR as well as hetero-oligomeric interleukin (IL) receptors such as the IL-6R and IL-13R employ activation of the JAK-STAT pathway to induce target gene expression. To identify general design principles of these signaling networks, it is crucial to understand the underlying receptor architecture and ligand-receptor interactions. Thus, this study constitutes the basis for comparative analysis of EpoR and interleukin receptor activation.

Network: SBCancer

Differential regulation of IL-6 signaling in tumor keratinocytes and fibroblasts

Sofia Depner, Tobias Scherzinger, Marco Nici, Alexandra Just and Margareta Mueller

The activated progression promoting tumor microenvironment is initially induced by a network of tumor derived growth factors/cytokines that induce cellular responses in tumor and stromal cells. In a tumor transplantation model of HaCaT skin squamous cell carcinomas we could demonstrate the functional contribution of an IL-6 regulated growth factor network to tumor progression. The network induces tumor cells proliferation and migration as well as persistent angiogenesis and recruitment and activation of stromal cells. In response to ligand binding the IL-6R activates the JAK/STAT signaling pathway in stromal fibroblasts and tumor cells but pathway activation results in the induction of different target genes and triggers different cellular responses in both cell types. This differential target gene response is most likely mediated by a differential kinetics of expression, phosphorylation and nuclear localization of STAT proteins (STAT1 and 3) after IL-6 stimulation in both cell types. Additionally tumor keratinocytes and stromal fibroblasts respond with a different pattern of activation for MAP kinases such as Erk1/2. Blockade of one of these IL-6 induced growth factors (GM-CSF) in tumour keratinocytes led to alterations of the IL-6 induced STAT1 and 3 activation kinetics, indicating the existence of an autocrine positive feedback loop in the JAK/STAT signaling pathway between IL-6 and GM-CSF.

Network: SBCancer

Signaling and transcription factor networks in a mouse model of inflammation-associated cancer

Astrid Riehl[1], Christoffer Gebhardt[1,5], Julia Németh[1], Moritz Durchdewald[1], Daniel Haag[2], Hauke Busch[3], Benedikt Brors[3], Axel Szabowski[1], Meinhard Hahn[2], Bernd Arnold[4], Angelika Bierhaus[6], Peter Nawroth[6], Peter Angel[1], and Jochen Hess[1]

[1]Division of Signal Transduction and Growth Control, [2]Division of Molecular Genetics, [3]Division of Theoretical Bioinformatics, and [4]Division of Molecular Immunology, DKFZ Heidelberg, Germany; [5]Department of Dermatology and [6]Department of Medicine I, University Hospital Heidelberg, Germany

A hallmark of chronic inflammation and inflammation-associated cancer is a sustained activation of AP-1 and NF□B regulated gene programs, including expression of pro-inflammatory cytokines, angiogenic factors, and secreted proteases. Both transcription factors respond to a plethora of internal and external stimuli and are implicated in cellular decisions of differentiation, proliferation, and survival. However, the onset and the order of signalling events resulting in sustained AP-1 and NF□B activation remain undefined but will be an absolute requirement for future concepts of translational research. In the past, we analyzed one of the best-established in vivo models of inflammation-associated cancer in which tumour initiation of mouse back skin is achieved by the mutagen DMBA and tumour promotion is triggered by the potent pro-inflammatory phorbol ester TPA. Interestingly, mice deficient for the receptor of advanced glycation end products (Rage) showed impaired chemical-induced skin carcinogenesis due to a defect in converting an acute pro-inflammatory stimulus into sustained tissue activation. We found reduced proliferation and accelerated apoptosis in Rage-deficient tumour cells accompanied by reduced activation of AP-1 and NF□B. Global gene expression analysis with TPA-treated control and Rage-/- skin samples revealed a comprehensive list of differentially expressed genes, including pro-inflammatory mediators and cytokines. Notably, TPA-induced expression of numerous candidate genes is unaffected by the loss of Rage within the first hours, but major differences were measured at later time points. Our data suggest that the response of mouse back skin to TPA administration can be subdivided in two stages: a Rage-independent and a Rage-dependent phase. In order to proof this assumption, we established immortalized keratinocytes from control and Rage-/- animals to study principles of dynamic transcription factor activation in response to different modes of extracellular signalling.

Network: SBCancer

Spatial and temporal resolution of the IFN network

Dagmar Wirth, Mario Köster and Hansjörg Hauser

Gene Regulation and Differentiation, Helmholtz Centre for Infection Research, Inhoffenstr. 7, 38124 Braunschweig

Interferons are central players in innate immune responses towards a variety of pathogens. These cytokines exhibit a plethora of different effects mediated by induction of interferon stimulated genes which can act either direct (in an auto- and paracrine manner) or indirect through innate and adaptive immune responses on cells. For an advanced understanding of the well coordinated antiviral, antiproliferative or immunomodulatory functions of interferons in vivo we have generated sensitive reporter cell lines and transgenic mice to monitor the response to IFN in real time. The aim of this project is to analyze the spatial and temporal kinetics IFN activity in vivo during infection which is a basis for a mathematical description of the interferon system. To analyse the IFN signalling circuits in single cells promoter elements of key markers of the IFN system were combined with reporter genes. They allow to distinguish between auto- and paracrine activities. We confirmed specificity and co-regulation of the reporter constructs with the endogenous genes by qRT-PCR. Using time-lapse microscopy we determined the kinetics of promoter induction and nucleocytoplasmic translocation of the signaling molecules IRF7 and STAT1/2 after adding IFN or virus to the cells. These factors represent the IFN induction and response phase, receptively. The quantification of fluorescence intensity in individual cells over time shows significant differences in the induction strength and timing of the onset of promoter activation.

Viruses have developed diverse and potent mechanisms to interfere with the antiviral host response and thus can be considered as natural perturbators of the IFN system. To elucidate their potential, we quantitatively compare the systemic effects of different pathogens using these reporter systems. Further, viral genes are being employed for controlled perturbation using synthetic expression modules. Such modules are a potent tool both for a controlled genetic perturbation of cellular networks and for reconstruction of endogenous circuits. We have established several controlled modules for controlled expression of viral factors interfering with onset and response to IFN. They are targeted into specific chromosomal loci via recombinase mediated cassette exchange and provide predictable expression properties. Both gradual and binary (stochastic) modules have been developed and mathematically modeled. Having evaluated these modules in cells and mice they are currently employed for controlled intervention of the IFN network.

Together, these approaches will allow to establish an extended data-based model for IFN induction and action in individual cells as well as in mice.

Network: SBCancer

CoReNe the Munich Contribution the Helmholtz Systems Biology Alliance

Hans-Werner Mewes, F. Theis, V. Stümpflen

Gene regulatory networks have been a preferred subject of Systems Biology approaches. The onset and progression of multifactorial diseases is clearly related or reflected by changes in regulatory pathways influencing their targets and causing the development of pathophenotypes. Within the HZGU several institutes have a long standing experience in the investigation of models related to regulation such as stem cell development, neurogenesis, or the d-notch pathway (Lickert, Prakash, Beckers (HZGU), Schütz (DKFZ). The CoReNe consortium intends to develop models in these research areas to develop a generic way how interactions in functional modules and complex networks can be mapped to systems models. The first step for a comprehensive description is the development of qualitative models based on the available biological knowledge (Stümpflen, Mewes (HZGU)). These models serve as templates for the quantitative analysis as performed in the group of F. Theis (CMB, Computational Models in Biology) and the development of mathematical models (Lasser, HZGU). Our external partners (Zimmer, LMU) and Allgöwer (Univ. Stuttgart) bring in knowledge on Petri Nets and Fuzzy Logic approaches and the behavior of regulatory circuits.
Special emphasis of this CoReNe is directed to the action of non-coding RNAs on the regulatory networks investigated. Coming experiments will try to elucidate the critical role of miRNAs in developmental pathways.
I will present the structure of the network, early results and a perspective to the next steps in the programme.

Network: CoReNe

Measuring and optimizing synchronous oscillation in Delta-Notch signaling

Fabian J. Theis, Hendrik Tiedemann, Elida Schneltzer, Gerhard K.H. Przemeck, Martin Hrabé de Angelis

Oscillatory networks are a key motif in many gene regulatory systems such as cell cycle, circadian clock and somitogenesis, the focus of this contribution. How oscillation in this multi-cellular system is robustly maintained over more than 80 hours is a fundamental question in the field. It has been shown that Delta-Notch signaling is a key functional element in the synchronization of the oscillating Hes-genes. We study an extended version of the dynamical system put forward in (Tiedemann et al. J Theor Biol 248(1):2007), which models the oscillator as an ordinary differential equation of Hes1-mRNA and -protein concentrations in the two compartments nucleus and cytoplasm. The question is how to formulate and automatically determine parameters to allow for ongoing oscillation and efficient synchronization via the Delta-Notch pathway.
We propose a new measure for synchrony of the system that we then optimize via global optimization methods. The measure consists of three terms that are being weighted accordingly to guarantee approximately equal contribution: (i) each Hes1-oscillator is to produce minimally decaying oscillation with maximal amplitude, (ii) the oscillation frequency needs to be as close to 120 minutes (for mouse) as possible, (iii) synchronization of the coupled system is to be as fast as possible. Experimental data is used to determine some rates in the system. After minimizing the cost function in terms of the other parameters, we achieve synchronization within 2-4 oscillation periods (frequency 121 minutes) in a two-cell system. When extending this to a line of cells, we observe a similar synchronization behavior but only after 10-15 oscillations.
Quantifying synchronous behavior in somitogenesis in a robust and efficient way allows us to optimize a coupled cellular system for maximally fast synchrony. The employed global optimization method based on a combination of differential evolution and simulated annealing robustly avoids local minima with simple specification of biologically meaningful parameter boundaries.

Network: CoReNe

Genetic networks active in mouse midbrain development

Nilima Prakash, Dietrich Trümbach, Dominik Wittmann, Florian Blöchl, Dominik Lutter, Jens Hansen, Fabian Theis, Wolfgang Wurst

The mammalian midbrain harbors a vast variety of neuronal populations involved in motor coordination and motor learning, sensorimotor gating, visual and auditory processing, and simple motor control. These neuronal populations are distinguished by their position in the ventral (tegmentum) or dorsal (tectum) midbrain, their neurotransmitter phenotype, their connectivity within the brain and their particular transcriptional code during development. The most prominent midbrain population are the neurons synthesizing the neurotransmitter dopamine (DA), located in the midbrain tegmentum. The midbrain dopaminergic (mDA) neurons control voluntary movements and modulate cognitive and affective behaviors. Degeneration or dysfunction of these neurons leads to severe neurological and psychiatric disorders in humans, such as Parkinson's Disease, schizophrenia, drug addiction and depression.

The identity of the midbrain territory is established during early mouse embryo development by the activity of an important secondary organizer, the isthmic organizer (IsO). The IsO is established at the boundary between the midbrain and the hindbrain, the mid-/hindbrain boundary (MHB), during development. It is achieved by the mutual activation or repression of a set of genes encoding cell-extrinsic (secreted) signaling factors and cell-intrinsic (transcription) factors, which ultimately delimit and thereby establish the distinct domains in the mid-/hindbrain region that will generate the different neuronal populations mentioned above. Several of these secreted and transcription factors are also active later in development and contribute to the fate specification and differentiation of the corresponding progeny. Although little is known about the ncRNAs, especially miRNAs, participating in these regulatory networks in the mouse embryo, it is most likely that they make a significant contribution. We are currently modeling some of the regulatory networks active during mid-/hindbrain and in particular during mDA neuron development, and establishing the role of miRNAs in this process by both gene targeting approaches and expression profiling of selected miRNAs.

Network: CoReNe

Role of Dicer/microRNAs in neurogenesis of the adult mammalian brain

Witold Konopka, T. Arnsperger, M. Novak, J. Rodriguez Parkitna, and G. Schütz

In mammals neurogenesis is a process which starts during development and is continued throughout adulthood. The adult mouse brain contains two main neurogenic areas: the subventricular zone (SVZ) located in the lateral wall of the ventricle and subgranular zone (SGZ) in the dentate gyrus of the hippocampus. Neural stem cells (NSC) located within the SVZ give rise to neuroblasts, which migrate subsequently through the rostral migratory stream (RMS) to the olfactory bulbs (OB), where they become mature neurons. The transition from NSC cells to neuroblasts is a highly regulated process that requires rapid changes in the expression level of different genes. MicroRNAs, a group of small non-coding RNAs, serve as potential regulatory factors, controlling translation of mRNA of many different genes. To study the role of microRNAs in adult brain neurogenesis we have ablated the Dicer gene specifically in adult neural stem cells. We have used the tailless (tlx) gene promoter to drive expression of tamoxifen-regulated CreERT2 protein. Upon induction of the Dicer mutation we have observed a decrease in newly formed neuroblasts in the SVZ-RMS-OB tract. This result was correlated with a decreased proliferation rate within the SVZ, measured by the Ki67 proliferation marker. In contrast neural stem cells were still present in the SVZ as shown by GFAP and Cre immunostaining. These data were confirmed by the use of neurosphere cultures in vitro. Compared to control neurospheres, mutants proliferated slower and did not achieve a similar size. Moreover mutant neurospheres behaved differently when transferred to differentiation conditions. These results indicate a crucial role of Dicer/microRNAs in differentiation of neural stem cells towards neuroblasts and mature neurons.

Network: CoReNe

Intuitive Modeling of Dynamic Systems with Petri Nets and Fuzzy Logic

Lukas Windhager, Ralf Zimmer

ODE based models of biological systems allow exact modeling of their dynamics. However, they require laborious efforts for detailed parameter estimation and often lack comprehensibility, especially for users without mathematical background. Other approaches, like Boolean networks, are successfully applied for qualitative analysis of systems but often cannot capture complex dynamics.

We propose a new approach to overcome such limitations by combining the graphical representation provided by Petri nets with the modeling of dynamics by powerful yet intuitive fuzzy logic based systems. The mathematical functions and formulations typically used to describe or quantify dynamic changes of systems are replaced by if-then rules, which are both easy to read and formulate. Precise values of kinetic constants or concentrations are substituted by more natural fuzzy representations of entities.

The main innovation of our *PNFL* (Petri Nets with Fuzzy Logic) approach is the use of elements from fuzzy logic theory to describe biological systems: Fuzzy sets describe arbitrary entities or properties of a system; Fuzzy logic systems define the dynamics of biological processes and dependencies between entities. Petri nets are used as a scaffold for the fuzzy logic based definitions of biological entities and processes.

Our new modeling approach *PNFL* allows a semi-quantitative modeling of biological systems such as signal transduction pathways or metabolic processes. A prototype system has been successfully applied during different developmental stages to several small test systems, like typical network motifs (e.g. feed-forward loops, switches) and several oscillator models (Higgins-Selkov, minimal mitotic, coupled oscillators). In addition, a larger model of the EGF signal transduction pathway was evaluated by replacing mass action kinetics with fuzzy logic systems.

Network: CoReNe

Survey of alpha-alpha protein repeats and application to Huntingtin

Gareth P. Palidwor[1], Sergey Shcherbinin[2], Matthew R. Huska[3], Tamas Rasko[3], Pablo Porras[3], Luis Sanchez-Pulido[4], Erich E. Wanker[3], Miguel A. Andrade-Navarro[1,3]

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2. Medical Imaging Research Group, British Columbia Univ., Vancouver, BC, Canada
3. Max-Delbrück Center for Molecular Medicine, Berlin, Germany
4. Centro Nacional de Biotecnología, Madrid, Spain.

Approximate tandem sequence repeats adopting a two anti-parallel helix fold are present in proteins from a diverse set of living organisms and involved in multiple functions. Several solved protein structures have already shown that they often form similar arrangements: the repeats stack together to form a flexible rod that displays a large surface for protein interaction. However, their sequence similarity is often low, even between repeats from the same protein. This makes it difficult to detect them by sequence similarity. Here, we present the hypothesis that they might be better detected by analysis of secondary structure features, for example using a neural network, a strategy successful for the identification of coiled coils and transmembrane helices. The results confirm this, since the method detects more instances of repeats per sequence that are detected by domain databases using multiple profiles. We identify these repeats in approximately 0.4% proteins in eukaryotic genomes. We then illustrate the results for all human proteins (identifying 86 genes, many of them with functions on chromosome segregation and condensation, mitotic spindle maintenance, and tubulin interaction, and identifying repeats for the first time in seven protein families). We also identify recent events of repeat duplication in species from Archaea, Bacteria, and Fungi. Finally, we demonstrate the usefulness of predictions in directing experimental work to dissect the HEAT-repeat domains of Huntingtin: using yeast two hybrid analysis of three domains of HEAT repeats we show that they interact. An implementation of the detection algorithm is available as a web server that provides simple graphical output. This can be further visualized using BiasViz, a graphic tool for representation of multiple sequence alignments. Accessibility: http://www.ogic.ca/projects/aarep .

Network: MSBN

Mathematical modeling of neurodegenerative processes in Alzheimer's disease

Katja Rateitschak 1, Vanessa Schmidt 2, Anne-Sophie Carlo 2, Anje Sporbert 2, Angelyn Lao 1, Olaf Wolkenhauer 1, Thomas Willnow 2

1 University of Rostock
2 Max Delbrueck Centre Berlin

Central to the pathology of Alzheimer Disease is the amyloid precursor protein (APP). The APP polypeptide encodes a 40 to 42 amino acid sequence called Aβ. When liberated from the precursor by proteolysis, Aβ aggregates to neurotoxic oligomers and to amyloid plaques, the pathological hallmarks of the disease.
Our project establishes a mathematical model describing the biochemical network regulating the decision process between the amyloidogenic and nonamyloidogenic pathway of APP processing. Intracellular protein trafficking has been recognised as a major regulatory element in this critical step.
Initially, we develope a mathematical model describing the physical transport and chemical kinetics of the biochemical network, including interactions between the sorting receptor SorLA and APP. This model will be validated with wet lab experiments, providing quantitative spatiotemporal data of respective network components (Step 1).
In collaboration with the group of Wanker/Stelzl, we will subsequently add newly identified proteins that participate in APP processing and down-stream signaling pathways, to our mathematical and experimental models (Step 2). Systematic expression profiling studies in Anrade group, using cell and animal models with induced genetic perturbations of components of the APP interactome, will provide proof of concept for the pathophysiological relevance of our predictions (Step 3).
Our interdisciplinary project is based on an iterative process from experiments to mathematical modelling, hypothesis generation, design of further experiments and model validation. Our objectives for mathematical modelling and model analysis are quantitative predictions about network dynamics, the development of strategies to design effective experiments in systems biology, and providing information on how one may be able perturb these networks in support of therapeutic strategies (Step 4). The later studies will be performed in close collaboration with groups at Research Center in Jülich that aim at investigating the global structure function relationship in the human brain (The Human Brain Model) as part of the Helmholtz Initiative on Systems Biology.

Network: MSBN

Signal transduction and transcription networks relevant for cardiac hypertrophy and failure

Jana Wolf and Claus Scheidereit

Recent molecular investigation has centered on the identification of the molecular pathways that initiate and perpetuate cardiac hypertrophy in view of rational drug design. These efforts have identified dozens of extracellular growth factors and intracellular signal transducers which, following biomechanical stress, evoke transcriptional activation of a fetal gene program, a hallmark of cardiac hypertrophy. Maladaptive hypertrophy entails not only quantitative changes but also qualitative alterations such as changes in signaling pathways and induction of fetal over adult genes, which inevitably instigate functional decrements at the complete organ level. This suggests that the underlying cardiac alterations that evoke heart failure are the product of cumulative genomic and epigenetic inputs, and should be addressed accordingly using a systems approach towards the signal transduction and transcription networks.
Despite a lack of detailed knowledge about the gene regulatory processes that bring about the genetic re-programming of the heart muscle during the development of heart failure, important hypertrophic signalling mediators have been identified by previous genetic studies, including the calcium-controlled NF-AT, as well as GSK3β, β-catenin and NF-κB. The pathological function of NF-κB in heart disease is one of the examples of long-term activation of the NF-κB system, where a switch in functional output can occur over time. We here want to address the question how the canonical and non-canonical NF-κB signaling contribute to the activation of the NF-κB system under long-term stimulation conditions using a combination of experimental and theoretical analyses. In particular, we will investigate how both NF-κB pathway branches are utilized in time and how their persistency and termination is regulated. The detailed analysis of the signalling dynamics will be combined with a temporal analysis of target genes in cardiomyocytes (collaboration with the groups Hübner/ Rajewsky) and a proteomic approach to identify yet unknown regulatory components (collaboration with the groups Wanker/ Stelzl).

Network: MSBN

Life/Death decisions in death receptor signaling

Leo Neumann[1,*], Carina Pforr[2,*], Joel Beaudouin[1], Peter H. Krammer[2], Inna N. Lavrik[2*], Roland Eils[*1]

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Stimulation of CD95 (Fas/APO-1) as a prototypic death receptor results in cell death. In addition, it can also lead to the activation of NF-κB. We established an integrated kinetic mathematical model of CD95-mediated life and death signaling. Systematic model reduction resulted in a surprisingly simple model well approximating experimentally observed dynamics. The model postulates a novel link between c-FLIP$_L$ cleavage in the Death-Inducing Signaling Complex (DISC) and the NF-κB pathway. We validated experimentally that CD95 stimulation resulted in binding of p43-FLIP to the IKK complex followed by its activation. Furthermore, we showed that the apoptotic and NF-κB pathways diverge already at the DISC. Model and experimental analysis of DISC formation showed that a subtle balance of c-FLIP$_L$ and procaspase-8 determines life/death decisions in a non-linear way. This is the first model describing the complex dynamics of CD95-mediated apoptosis and survival signaling.

Network: SBCancer

Merging experimental and computational approaches to understand the interplay of autophagy and apoptosis

Nathan Brady

Programmed cell death (PCD) research is aimed at elucidating the multitude of mechanisms which positively and negatively regulate the activation and execution of cell death. Classically, PCD research focuses on the apoptotic pathways, which are triggered by either extrinsic (death receptor) or intrinsic (mitochondrial) signals. Autophagy, the process of protein and organelle lysosomal degradation, has recently been implicated as a major factor in both preventing and contributing to human disease. For example, autophagy is a potent survival mechanism in many neurodegenerative conditions and cardiac ischemia/reperfusion injury, and can either prevent or enhance cancer cell death.

We are investigating the dynamics of pro-survival and pro-death signaling which occur as the autophagic and apoptotic pathways converge at the mitochondrion and endoplasmic reticulum (ER). The apoptotic and autophagic pathways are linked at two levels: (i) the pro-autophagy Beclin1 is a BH3-only protein capable of binding Bcl-2 and Bcl-xL. The interaction of anti-apoptotic Bcl-2 proteins with Beclin1 may suppress Beclin1 signaling formation of autophagosomes. Furthermore, (ii) as calcium is an essential activator of autophagy, Bcl-2 family interactions at the ER plays an additional role in mediating autophagy activity via control of calcium homoeostasis. Thus, the apoptotic decision-making process is balanced between the mitochondrial, ER, and autophagosomal compartments, via interconnected calcium and Bcl-2 signaling pathways.

I will discuss our attempts to gain insight into the complexity of PCD by combining technologies in quantitative biology and computational network analysis.

Network: SBCancer

Analysing gene expression patterns in the metabolic network of neuroblastoma tumours with wavelet transforms

Gunnar Schramm[1], Andrea Gaarz[1], Marcus Oswald[2], Roland Eils [1,3] and Rainer König[1]

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Neuroblastoma tumours show a very heterogeneous clinical picture ranging from rapid growth with fatal outcome to spontaneous regression or differentiation into benign ganglioneuroma. Therefore, diagnosis and specific treatment is crucial and can be supported by understanding the molecular functionality of the tumour. Besides this, gene expression profiling with microarrays has produced data on a genomic scale for this variety of neuroblastoma tumours. The information needs to be funneled into functionally meaningful patterns and applications. We mapped gene expression data of neuroblastoma tumours onto the metabolic network to define biochemical pathways that show a discriminative regulation behaviour between the different tumour types. These pathways may well suit for further analysis by e.g. knock down experiments in the laboratory to define drug targets for the aggressive tumours.
Results: We applied an established method (König et. al., BMC Bioinformatics, 2006) that uses Haar wavelet transforms which is normally used for pattern recognition on images. Gene expression data from favourable and unfavourable tumours was mapped on two-dimensional adjacency-matrices of metabolic sub-networks taken from the pathway maps of the KEGG database. With this we were able to evaluate all KEGG maps in respect to their ability to discriminate neuroblastoma tumours of patients with favourable and unfavourable outcome. The most significant patterns were found for purine-, glutamate-, and pyrimidine-metabolism, indicating increased nucleotide production for proliferation (purine and pyrimidine metabolism), and a switch in the glutamate metabolism. Our findings may serve for more detailed experimental investigations, especially to treat the glutamate and one carbon pool metabolism.

Network: SBCancer

Gene Network Dynamics Controlling Keratinocyte Migration

Busch Hauke, Camacho D, Rogon Z, Breuhahn K, Angel P, Eils R, Szabowski A

Translation of large-scale omic data into a coherent model for cellular regulation allowing to simulate, predict and control cellular behavior is far from being resolved. Global regulation of cell homeostasis and cell fate requires a complex and controlled interplay of protein signaling and gene regulation. Combining these processes into one model is inherently difficult, as they occur on different time scales in the range of minutes to hours, respectively.
We propose a complexity reduction approach to capture cell-fate decisions based on the slaving principle, which states that the long-term macroscopic behavior of a system is controlled by its slowest evolving variables. In biological terms this means that long-term phenotypic behavior of a cell on the time scales of hours is reflected in its gene expression kinetics.
Using Hepatocyte Growth Factor-induced migration of primary human keratinocytes as an example, we infer a dynamic gene regulatory network model from time series measurements of DNA micro-array data.
Key genes are identified based on their kinetic profile as well as their biological function. Using a genetic algorithm combined with a search for robust system solutions, we show how to build a phenomenological model that can predict in silico the necessary and sufficient time-ordered events that initiate, maintain and stop migration, all of which are verified in vitro. The data analysis and modeling may provide a new way of obtaining a bird's eye view on the dynamic orchestration of various pathways and a broad degree of interdependency that control cell migration and cellular decisions in general.

From molecular machines to gene-regulatory networks in mammalian cells

Thomas Höfer

The complexity of regulatory networks in mammalian cells – exemplified by the vast number of components and their dynamic interactions on a wide range of time and space scales – requires mathematical models at different levels of organization. I will discuss experimentally-based models directed at understanding: (i) gene-regulatory networks in T-cell differentiation, and (ii) the multi-protein machinery that recognizes and repairs UV-damaged DNA. Iterative theoretical and experimental analyses of network dynamics have led us to uncover novel regulatory interactions and to identify fundamental properties for the action of chromatin-associated molecular machines.

Network: SBCancer

Dissecting cellular networks with high-throughput RNAi screens

Sandra Steinbrink, Michael Boutros

Signaling and Functional Genomics (B110) - German Cancer Research Center (DKFZ)

A number of highly conserved signaling pathways and regulatory networks are essential for the development and homeostasis of all multicellular organisms. By transmitting signals in and between cells, these pathways are important mediators of cellular communication. Thereby, signaling pathways control most biological processes, such as the immune response, cell differentiation, proliferation and death. Dysregulations in the signaling networks can cause developmental defects and diseases, such as chronic inflammation, cell loss disorders and even cancer. For a better understanding of these diseases and the development of potential therapeutic approaches, it is crucial to gain a more complete knowledge of the pathway mediators involved.
The sequencing of the human genome and the genomes of different model organisms enabled large-scale reverse genetic screens, identifying gene functions for so far uncharacterized genetic sequences. Using RNA interference (RNAi), genes can now be silenced on a genome-wide scale and the resulting phenotypes can be used to deduce their physiological function. Similarly to screens in invertebrate model organisms, we have developed and currently apply high-throughput systematic RNAi-approaches in human cultured cells with the focus on conserved and disease relevant signaling pathways.

Network: SBCancer /Technology Platform

Systematic Analysis of Human Protein-Protein Interactions

Erich Wanker, Ulrich Stelzl

[1]MDC Berlin-Buch; [2] Max Planck Institute for Molecular Genetics, Berlin

In this subproject of MSBN, systematic protein interaction studies will be improved and further developed in order to provide comprehensive and quantitative PPI information as a basis for systems biology modeling approaches. The focus of this project will be on the generation and analysis of an Alzheimer's disease (AD) protein interaction network (together with MSBN-P2). We will also test a set of protein targets involved in cardiac signaling together with MSBN-P1. The main task will be to integrate other data types (e.g. obtained in MSBN-P4) to create cell type and disease specific networks to investigate differences and commonalties of major signaling proteins in the two disease processes. Specific sub-networks should contribute basic connections for mathematical modeling.
The current status of the project is presented here. This includes the development of an empirical framework that provides various quality measures of HTP PPI data and the application of novel methods to address specific questions in PPI research.

Network: MSBN /Technology Platforms

Structural Characterisation and Relative Quantification of Protein Phosphorylation involved in Signal Transduction

Hahn Bettina, Winter D, Kienast A, Korf U, Klingmüller U, Lehmann Wolf-Dieter

While in cancer biology the structure of several signaling pathways based on reversible phosphorylation/ dephosphorylation is well understood, quantitative data in this field are still scarce. Electrospray tandem mass spectrometry coupled with Ultra Performance Liquid Chromatography (UPLC) is a powerful tool for performing quantitative studies, e.g. to measure position-specific phosphorylation degrees in phosphoproteins.
The analytes in this study were a set of commercially available phosphoproteins employed for protein chip preparation. First, these phosphoproteins were structurally characterized by a standard analytical proteomics stategy consisting of 1D gel electrophoresis, in-gel digestion and UPLC-MS/MS using a Q-TOF instrument. Identification of phosphorylation sites was done automatically using the search engine Mascot supported by additional manual interpretation of the MS/MS spectra. In this way the phosphorylation sites of AKT1, ERK2, JNK1, JNK2, p38alpha, p38beta, STAT3 and GSK-3beta were pinpointed, which led to the identification of a total of 21 sites.
Estimation of the position-specific degree of phosphorylation can roughly be done by comparison of the signal intensities of phosphorylated and nonphoshorylated peptide analogs within a single UPLC-MS run. In our study, the phosphorylation degrees found varied from low to high levels.
A more accurate determination of the phosphorylation degree is possible by taking into account the individual electrospray ionization efficiencies of peptide/phosphopeptide pairs and by correcting for their possibly individual recovery during the UPLC step [1]. This label-free strategy for accurate relative quantification of phosphorylation is demonstrated for the tryptic p38beta phosphopeptide containing pSer188 and pTyr190, as an example. Synthetic peptides are used for determination of the correction factors. A molar 1:1 mixture is prepared to measure the electrospray ionization correction factor. This mixture can also be employed for measurement of the UPLC correction factor. Using both correction factors, the ion intensities measured by UPLC-MS can be converted into relative molar ratios within peptide/phosphopeptide pairs in the sample solution, which represent the desired accurate degrees of phosphorylation.
[1] Winter D, Schilling M, Klingmüller U, Lehmann WD. Proceedings of the 56th ASMS Conference on Mass Spectromery, June 1-5, 2008, Denver, USA; Poster TP587. Label free relative quantification of MAP kinase phosphorylation degree by UPLC-MS.

Network: SBCancer

Identifying partners essential for receptor endocytosis

Carsten Schultz, Vibor Laketa

EMBL Heidelberg

The signaling activity of receptor tyrosine kinases (RTKs) and other membrane receptors is in part limited by rapid internalization of the proteins after ligand binding. Usually the receptors are subjected to ubiquitination and subsequent destruction in the lysosome. We recently demonstrated that some phosphoinositides are also inducing RTK endocytosis, however, leading to full recycling of the receptor. In order to identify the proteins involved in EGF- and PIP3-induced receptor internalization, we conducted a siRNA screen against the 700 proteins of the human genome equipped with a lipid binding domain. We find that some clusters of signaling molecules are essential for receptor internalization while others are specific for ligand- or PIP3-induced endocytosis.

Network: SBCancer

From Distributed Knowledge to Qualitative Models

Volker Stümpflen, K. Nenova, T. Barnickel

The qualitative understanding of models in systems biology is fundamental for any further investigation of complex systems. Having relevant knowledge about potential associations and mutual interactions between different individual biological entities available is certainly one of the keys for accurate quantitative models and systematic experiments. The nature of the systems biological approach demands however for the association of many different knowledge resources starting from genomic databases over resources for biological networks up to knowledge hidden in literature.

In the past the most widely utilized solution for the combination of domain specific information were data integration technologies based on the import of relevant information into a new database. However this becomes with the exponential growth of available information more and more time and resource consuming (redundant data, updates, ….). Furthermore due to the underlying relational database technology the one of the most important information – the nature of association between biological entities – is normally not accessible in a coherent way. This is however a general problem not only within the biomedical community and on a Web-wide scale addressed with so-called “Semantic Web Technologies”.

We will discuss state-of-the-art semantic methods and approaches capable to bring distributed knowledge into systems biological models. This will include technologies to access distributed resources, recent developments in textming as well as new approaches to combine the different knowledge with semantic approaches in a uniform way.

Network: CoReNe / Technology Platform

Data Management for Systems Biology

Jürgen Eils

The ultimate goal of researchers in the interdisciplinary field of systems biology is to solve biological problems at the level of an entire system. Achieving this goal requires supporting the efforts of both experimental biologists as well as computational modelers. The phases of planning, actual experimentation and data analysis as well as model development, testing and validation should all be supported by an integrative data management.

The issue of data management using one comprehensive database solution will be discussed. Integrative data management by combining several standardized solution in each field of systems biology and combine them in an integrative way will be highlighted as one alternative.

In the second part of the talk, our in-house database solution iCHIP will be presented as an exemplary database solution the maintenance of experimental data. The iCHIP database (http://www.ichip.de) was originally developed to operate as a gene expression database. Given the rapid development of new technologies in molecular biology, the amount and heterogeneity of available data has increased dramatically. The functionality of iCHIP has therefore been extended to include proteomics, matrix-CGH and microscopy images. A comprehensive user and project management is implemented, which allows user-dependent rights for reading and writing of specific data and several application areas. In particular the public user can be defined with very restrictive access to data and masks. Furthermore, standardized exchange formats have been included and standardized interfaces for communication with third party products implemented.

This general accessibility, the broad functionality and technological enhancements of iCHIP are the facts which channel the iCHIP extension towards systems biology.

Network: SBCancer

Why Are Computational Neuroscience and Systems Biology So Separate?

Rolf Kötter

Despite similar computational approaches, there is surprisingly little interaction between the Jülich initiative "The Human Brain Model" and the other initiatives within the "Helmholtz Alliance on Systems Biology". In this talk I briefly reflect the history of what appears as two distinct disciplines and why they may have grown up apart based on thoughts expressed by Erik De Schutter in a recent article (PLoS Comput Biol 4(5): e1000078. doi:10.1371/journal.pcbi.1000078). It seems that systems biology is a better organized community which is very effective at sharing resources, while computational neuroscience has more experience in multiscale modeling and the analysis of information processing by biological systems. While De Schutter speculates that neuronal modelling on the cellular level may gradually be turning over to systems biology I will argue that computational neuroscience could embrace the field of neuroinformatics more tightly in order to improve its empirical data base and be ready to interface with bioinformatics once the field of systems biology realizes that there is an organ to be discovered beyond the cell.

Network: Human Brain

A consistency check of layer-specific connectivity estimates and applications to cortical network modeling

Tobias C Potjans and Markus Diesmann

The complex architecture of the cortical tissue provides the substrate for brain function. We employ large-scale network models to investigate the dynamical basis of information processing implied by the neural circuitry. In order to render our model compatible with the variety of existing connectivity data, we check the consistency of the two most comprehensive and quantitative data sets in the literature [1, 2].

Cortical connectivity estimates based on anatomical reconstructions [1] and physiological recordings [2] show apparent inconsistencies that reflect the methodological differences. Nevertheless, we arrive at a framework to formulate an integrated data set unraveling distinct methodological restrictions. In a first step, we identify invariant measures based on layer- and type-specific connection matrices and demand their conservation. Second, we utilize detailed knowledge of the experimental procedures to constrain the lateral connectivity model. Finally, we assess the significance of target-type specificity and find that functional inter-layer projections target specific neuron types. As a result, we propose an integrated data set that consistently summarizes the present knowledge in compliance with target-type specific connectivity.

The dynamical properties induced by layer- and type-specific connectivity are investigated by means of simulations of a local cortical module consisting of 80,000 neurons. In so doing, we link the structure of the cortex to its activity.

1. Binzegger T, Douglas RJ, Martin KAC (2004) J Neurosci, 24(39):8441-8453
2. Thomson AM, Lamy C (2007) Front Neurosci, 1:19-42

Network: Human Brain

On the relation of spike synchrony and the local field potential

Michael Denker, Sebastien Roux, Markus Diesmann, Alexa Riehle, Sonja Grün

Oscillations of cortical local field potentials (LFP) are commonly assumed to reflect the summed synaptic currents in the local volume. Consequently, oscillatory excursions in the LFP should reflect to a large extent synchronized spiking activity. Here we provide for the first time direct experimental evidence that this prediction holds.

In order to test the prediction we combine multiple single-unit recordings with simultaneous recordings of the LFP in monkey motor cortex and assess the degree of phase synchronization between these two signals as a measure for the entrainment of spike times to the LFP oscillations.

We observe that: (1) the coupling of spikes to the LFP increases with the oscillation amplitude and (2) spikes that coincide with others are better locked than asynchronous spikes. The prediction is therefore confirmed in two ways, exploiting different aspects of the mechanism. While the first analysis considers in how far a property of the LFP is conclusive for the relationship of spikes and LFP, the second relies on characteristics of the parallel spike train data.

Furthermore, we demonstrate that synchronous activity reflected in the LFP does not originate from arbitrary sources. If the number of spike coincidences in certain time intervals exceeds the chance level, these coincidences, termed Unitary Events, are better locked to the LFP than coincidences outside these intervals. However, the statistical properties of the LFP inside and outside of the time intervals exhibiting significant synchronization are identical. This may indicate that in other time intervals the synchronized activity of neuronal subnetworks not under observation is reflected in the LFP. Our findings suggest that transient excess synchrony contributes a temporally organized major component to the LFP dynamics.

Network: Human Brain

Coordinated neural activity in monkey visual cortex during free viewing

Sonja Grün, J. Ito, P. E. Maldonado

When inspecting visual scenes, primates perform on average four saccadic eye movements per second, which implies that identification of image components is accomplished in less than 200 ms. Thus, individual neurons may contribute only with a few spikes for these complex computations, suggesting that information is encoded not only in the firing rate but also in the timing of spikes. We tested this hypothesis by analyzing multi-electrode spike recordings from primary visual cortex of monkeys freely viewing natural scenes. By employing the unitary event analysis method we were able to analyze the data for excess spike synchrony (UEs, Grün et al, 2002a,b) in relation to eye movements. UEs occur in a phasic manner after fixation onset, whereas the firing rates have a much slower temporal profile and peak only after the UEs (Maldonado et al, submitted). Additional controls, like intentional destruction of synchronous activity by spike dithering (see also Pazienti et al), or comparison to statistical models clearly showed that the temporal profile of the synchronized activity is not an epiphenomenon. The population activity in V1, as measured by the local field potential (LFP), exhibits a strong phasic oscillation (beta-band) locked to saccade onset, persisting also during the succeeding fixation. The increase of the firing rate after fixation onset, however, rather seems to reflect the neuronal response to visual input during fixations. To explore the detailed relationship between the spiking activity and the LFP, we analyzed the relation of individual spikes to the phase of the LFP as measured by the phase locking value. Spikes entering V1 after fixation onset, and in particular the very first spikes, appear to be well locked to this oscillation (Ito et al, 2008). A possible interpretation of our observations is that the LFP oscillations reflect an intrinsically generated reference signal, enabling precise locking of early spikes of the visually evoked activity. This phenomenon provides a mechanism to accurately time spikes for further processing, as would occur in spike synchronization.

Network: Human Brain

From contaminant molecules to cellular response: system quantification and predictive model development - overview

Irina Lehmann

[1]Department of Environmental Immunology, Helmholtz-Centre for Environmental Research – UFZ

This network aims at understanding cellular responses to chemical stressors with a systems perspective, which involves intra-cellular transport of the chemical, reaction with sub-cellular target sites and the cell's response at the transcriptional and post-transcriptional level. Systematic large-scale cell-based experiments will be coupled with the integrated simulation of the intracellular transport and regulatory networks. We selected benzo(a)pyrene (BaP), as the model chemical to initiate this research and investigate the interaction of BaP with the aryl hydrocarbon receptor (AhR) signalling pathway and resulting changes on genome and proteome level. BaP represents an important class of environmental contaminants. The AhR pathway, which is affected by BaP, is a central route for the toxic effects of many organic chemicals. Therefore, results obtained from this model might help to unravel the effects of other toxic agents as well.

For a subset of conditions we will analyse the aspects of entrance and distribution of BaP in the cells, the binding to the arylhydrocarbon receptor (AhR) and the effects on gene transcription and protein expression. Highly resolved experimental data on the spatial and temporal intracellular distribution of BaP molecules and on the interaction of BaP with the AhR pathway will be generated by live-cell imaging. Basing on this microscopy data cell geometries will be reconstructed and the intracellular movement of B[a]P and the B[a]P-AhR complex will be modelled using reaction-diffusion-equations and random-walk-processes. A consistent data set of a time course of regulated genes and proteins will be provided for the description of regulatory networks. An integrated model for the entire signalling pathway from extracellular exposure of the cell with BaP to cell response is the final aim.

The data and the models developed in this study will serve as a prototype for elucidating other stress response pathways in the future.

Network: Contaminant Molecules

The BaP-AhR-pathway: Spatio-temporal dynamics of the receptor-ligand-complex.

Juliane Mai[1], Gabriel Wittum[2], Sabine Attinger[1]

[1]Department Computational Hydrosystems,Helmholtz-Centre for Environmental Research – UFZ,
[2]Interdisciplinary Center for Scientific Computing, University of Heidelberg

One major part of our project focuses on the description of the kinetics of the entry of a chemical contaminant into the cell, its intracellular distribution and interaction with intracellular components. Furthermore, the interaction of this chemical ligand with the arylhydrocarbon receptor (AhR) and the translocation of the receptor-ligand complex into the nucleus will be under investigation. Benzo[a]pyrene (BaP) is used as a model chemical due to its fluorescent properties. The usage of a fluorescent ligand and a fluorescence tagged AhR receptor gives us the opportunity to image both, the intracellular distribution of the ligand and the ligand-receptor complex.
Basing on live-imaging techniques spatial and temporal highly resolved data will be generated. Characteristic parameters like diffusion coefficients and binding rates will be assessed by the technique of Fluorescent Recovery After Photobleaching (FRAP).
Microscopy data are used to reconstruct the complex cell geometries basing on filters and contour extracting algorithms. In a next step, the intracellular movement of the contaminant B[a]P will be modelled using reaction-diffusion-equations and random-walk-processes. Since imaging data for B[a]P are not available so far, we currently simulate FRAP experiments with different kinds of motion and/or binding behaviour and look at the (qualitative) effects on the recovery of the fluorescence. To change these initially qualitative conclusions into quantitative we plan to derive analytical solutions for the FRAP recovery. The input of experimental data will later allow us to calculate the so far unknown parameters of motion and binding.

Network: Contaminant Molecules

Deciphering the transcriptional regulatory network of the Ah receptor

Andreas Beyer, Jacob Michaelson

Biotechnology Centre, TU Dresden

The aryl hydrocarbon receptor (AhR) is a cytoplasmic receptor that after activation by ligand binding enters the nucleus and acts as a transcriptional regulator. AhR is known to play a crucial role in mediating the toxic response to many xenobiotics such as dibenzo-*p*-dioxins and aromatic hydrocarbons.
In order to better understand the transcriptional network regulating the expression of AhR itself as well as its target genes we combine genome-wide transcription data from different tissues measured in different mice with variable genetic backgrounds. By correlating the genotypes of the mice with the expression responses we can identify expression quantitative trait loci (eQTL). Such eQTL comprise genomic regions (loci) that are thought to contain direct or indirect transcriptional regulators of the respective target gene. This computational framework can be used for identifying new putative target genes of AhR, thereby complementing our insight into the AhR pathway. We demonstrate that eQTL related to AhR are highly tissue specific, underlining that the cellular context and transcriptional co-factors play a crucial role for understanding the regulatory network of AhR.

Network: Contaminant Molecules

From Systems to Synthetic Biology: Streamlining and Reprogramming Biocatalysts

Audrey LePrince, Jacek Puchalka, Miguel Godinho, Piotr Bielecki, Vitor Martins dos Santos

The pursuit of Synthetic Biology is both the design and fabrication of biological components and systems that do not exist in the natural world as well as the re-design and fabrication of already existing biological systems. I will provide a short overview on the current scope of Synthetic Biology for Industrial and Medical Biotechnology, on its challenges and perspectives, and will illustrate these through cases studies.
Specifically, I will present two transnational projects (EU, ERA-NET) on Systems and Synthetic Biology that aim at constructing a functioning, streamlined bacterial cell devoid of part of its genome and endowed with a series of highly coordinated, newly assembled genetic circuits for the biotransformation of a range of high added value compounds and that include circuits for synchronized behaviour, noise minimisation and low-temperature biocatalysis. By achieving such constructs as a proof-of-principle, it is aimed at establishing a solid, rational framework for the streamlining and engineering of cells performing effectively and efficiently specific functions of biotechnological and medical interest. The added value comes from obtaining a streamlined bacterial factory, devoid of unnecessary gene complements and undesired cross-talk (and in which the internal wiring is well characterised through Systems Biology approaches), thereby enabling a higher degree of control and hence re-programming, by plugging-and-playing at will.

Network: HZI

Systems analysis of T cell receptor and interferon signaling in T cells and its regulatory networks

Hansjörg Hauser

B. Schraven, A. Kel, R. Weissmantel, J. Lindquist, D. Wirth, M. Köster, A. Kröger, L. Jäntsch, M. Probst-Kepper

The mediators of immune regulation act as stimulators which trigger specific cell surface receptors. These signals are further propagated into the intracellular environment where they initiate multi-component signalling cascades that trigger new gene expression patterns. While the vertical sequences from the cell surface to the nucleus of a variety of signalling cascades are well understood, little is known how individual signalling pathways are horizontally interconnected with each other (cross-talk). This knowledge is necessary for a global understanding of how immune cells work and why immune cells sometimes do not work as they should. T cells play a central role in autoimmune disorders and infectious diseases. Key players in the maintenance or breakdown of self-tolerance in autoimmunity and infection are regulatory T (Treg) cells.
One team of this FORSYS group has established a quatitative systems biology description of the T cell receptor (TCR) signalling. This activity is complemented by teams that extends this work in two directions. One concerns the cross-talk of a signalling pathways that is induced by type I interferon (IFN) signalling through the respective receptor (IFNAR) with TCR mediated signalling. The other concerns a comparison of the TCR signalling in peripheral Tcells with the signalling of regulatory T (Treg) cells.
The goal of this initiative is to develop a model for interferon induced signalling in T cells and to experimentally validate the model. It is further aimed to define the level and extent of cross-talk between the signalling triggered by T cell receptor agonists and interferon. Finally, we will compare these signalling networks in peripheral T cells and regulatory T cells and define key events that distinguish them.

Network: HZI

Poster Abstracts

A: SBCancer

Poster-1: Role of CD95 receptor organization on procaspase-8 activation

Joel Beaudouin, Roland Eils

Death receptors that are linked to their specific ligand induce apoptosis by activating initiator caspases like procaspase-8. This procaspase is normally a monomer and its activation occurs thanks to dimerization after recruitment on the receptor. After dimerization the active enzyme can cleave itself as well as other caspases that effectively execute apoptosis. In parallel to this dimerization clustering as well as internalization of the receptor has been observed after activation. Nevetheless it is not known if and how these processes regulate procaspase-8 activation. To characterize this potential regulation we want to analyze experimentally and theoretically the input-output function of procaspase-8 activation depending on ligand concentration. We focus on CD95 receptor, which recruits the death inducing signalling complex (DISC) proteins when activated. We study the process in HeLa cells, either wild-type or stably expressing different amount of DISC proteins. We have notably generated lines that express fluorescently tagged DISC proteins that allow the visualization of DISC in living cells. We also developed probes that change localization when cleaved by caspases, allowing the observation of multiple probes within one cell over time. Probes were targeted to different cellular localizations to observe the intracellular localization of caspase activity over time. We notably observed that while soluble probes are cleaved at the same time as probes targeted to the plasma membrane, probes linked to cytokeratin are not as efficiently cleaved, suggesting that the activity is first confined to the plasma membrane. In parallel we also investigate the organisation of receptors on the plasma membrane using immunofuorescence, high-resolution fluorescence microscopy and image analysis. HeLa cells contain few receptors that can be resolved as dots on the plasma membrane. As we can count their number and measure their intensity, we can use this approach to test the classical hypothesis that recepors are organized as trimers on the plasma membrane.

Poster-2: Kinetic Modeling of Nucleotide Excision Repair

Gesa von Bornstaedt, Martijn Luijsterburg, Roel van Driel and Thomas Höfer

Nucleotide Excision Repair (NER) is a multi-protein and multi-step repair system for DNA damage caused by UV light.
In our model the main action is performed by five enzymatic reactions: the initial and full unwinding of the DNA helix; double incision of the damaged strand on both sides of the lesion; the re-synthesis of the gap and finally ligation of the repaired DNA.
Those five enzymatic steps separate six repair intermediates. The first one represents the recognition of the damage, which is performed by the strict sequential binding of two proteins. In the other repair intermediates located between the enzymatic steps, we assume random complex assembly in contrast to sequential binding. The right complexes have to assemble, since certain protein complexes are needed to perform the enzymatic reactions. For the repair intermediate of incised DNA not less than seven individual diffusing components have to assemble to form such multi-protein complexes.
We assume that this formation is highly random and the proteins exchange rapidly, consistent with in vivo measurements of the exchange rates by FLIP (fluorescence loss in photobleaching). In our model, the observed long lasting steady state levels of some repair proteins are due to the time spend for having the right complex assembly.

Poster-3: Mathematical Modelling of DNA Replication

Anneke Bruemmer, Vittoria Zinzalla, Carlos Salazar, Lilia Alberghina, Thomas Hoefer

The complex machinery of DNA replication ensures that the whole genome is duplicated exactly once during a limited time of the cell cycle. In the *S. cervisiae* genome, 190 early origins of replication are licensed in G1 phase by loading part of the replication machinery and, during the G1-S transition, are fired rapidly through recruitment of DNA polymerase. All 190 origins have to fire exactly only once and as synchronous as possible. Recently it has been discovered that origin firing is controlled by S-CDK through the multiple phosphorylation of the S-CDK substrates Sld2 and Sld3. To elucidate the functional design for coherent and robust origin firing, we developed a mathematical model of the molecular network of DNA replication initiation in budding yeast.
A core part of the model is the multiple phosphorylation of Sld2, randomly at 6 serine residues and sequentially at a threonine, leading to the formation of the activator complex, Sld3-Dpb11-Sld2. Analysis of the model shows that this complex acts as a catalyst for the ultimate recruitment of polymerase to origins and thereby can trigger origin firing.
High temporal coordination is required for origin licensing and firing in order to complete replication from all 190 origins almost simultaneously within a short time and to ensure functioning of re-replication prohibitory mechanisms. Simulations of experimental perturbations of S-CDK reveal that coherent origin firing can be robustly regulated by S-CDK. Sensitivity analysis of individual parameters shows how this feature is supported by the structural design of the molecular network. The model is used to rationalize how deregulation of origin replication may cause genomic instability.

Poster-4: Differential regulation of IL-6 signaling pathway in HaCaT A5 benign tumor keratinocytes and fibroblasts

Sofia Depner, Tobias Scherzinger, Marco Nici, Alexandra Just and Margareta Mueller

The activated progression promoting tumor microenvironment is initially induced by a network of tumor derived growth factors/cytokines that induce cellular responses in tumor and stromal cells. In a tumor transplantation model of HaCaT skin squamous cell carcinomas we could demonstrate the functional contribution of an IL-6 regulated growth factor network to tumor progression. The network induces tumor cells proliferation and migration as well as persistent angiogenesis and recruitment and activation of stromal cells. In response to ligand binding the IL-6R activates the JAK/STAT signaling pathway in stromal fibroblasts and tumor cells but pathway activation results in the induction of different target genes and triggers different cellular responses in both cell types. This differential target gene response is most likely mediated by a differential kinetics of expression, phosphorylation and nuclear localization of STAT proteins (STAT1 and 3) after IL-6 stimulation in both cell types. Additionally tumor keratinocytes and stromal fibroblasts respond with a different

pattern of activation for MAP kinases such as Erk1/2. Blockade of one of these IL-6 induced growth factors (GM-CSF) in tumour keratinocytes led to alterations of the IL-6 induced STAT1 and 3 activation kinetics, indicating the existence of an autocrine positive feedback loop in the JAK/STAT signaling pathway between IL-6 and GM-CSF.

Poster-5: Understanding Crosstalk Between Apoptosis and Autophagy Using Data-Driven Modeling

Marti Bernardo Faura, Nina Jennewein, Phillip Hundeshagen, Anne Hamacher-Brady, Roland Eils, Nathan Brady

Programmed cell death (PCD) research is aimed at elucidating the mechanisms which control and execute cell death. Disruption to the homeostasis of the PCD pathways is recognized as a major factor in the pathogenesis of many disease types, including ischemic injury to the heart and cancer. Recent studies have implicated autophagy (self-eating) as an important, yet paradoxical factor in many PCD-linked pathologies. Under certain conditions autophagy can enhance cell death and under other conditions autophagy can prevent apoptosis.

The complexity of PCD signaling, in terms of the number of participants and extensive crosstalk between pro-survival and pro-death pathways requires the use of novel comprehensive experimental and analytical methods. The MCF-7 breast cancer cell line was submitted to combinations of conditions which either both promote or block apoptosis and autophagy. Quantitative Western blotting was performed to measure the activity of the pro-death BH3-only protein, Bid. Flow cytometer was used to quantify autophagic activity. These datasets were analyzed using PCA (Principal Component Analysis), a technique which reduces the number of dimensions of the dataset to a principal component space, allowing more intuitive interpretation of the data. PLS (Partial Least Squares) regression modeling allowed the prediction of responses to new datasets, as well as the formulation of hypotheses that relate key elements. PLS also allows to objectively determine the contribution of the mentioned key players to the cellular response. The goal of such a systems level approach is to reveal the nature of the crosstalk between autophagy and Bid during PCD.

Poster-6: Analysis of T Lymphocytes Signaling and Proliferation Using CFSE Data

Dorothea Busse, Michael Floßdorf, Maurus de la Rosa, Kevin Thurley, Kirstin Hobiger, Max Löhning, Alexander Scheffold, Thomas Höfer

The cytokine IL-2 is known to be an important regulator of the proliferation behavior of T lymphocytes. To define the signaling dynamics of IL-2 and its impact on T cell proliferation, we simultaneously measured the activation of the IL-2 signaling pathway and the resulting cell divisions in primary murine T cells using flow cytometry. By mathematical modeling we uncovered complex dynamics of the IL-2 cytokine network that provide a mechanism for a discrete proliferation threshold of helper T lymphocytes and its tuning through competitive interference by regulatory T cells. In order to further study the proliferation behavior of activated T cells, we then used different proliferation models to directly fit the CFSE profiles of the in vitro and in vivo data. We thereby used different statistical techniques to extract the maximum possible information from the CFSE profiles, including an error analysis.

Poster-7: Uncovering the quantitative role of the c-FLIP isoforms in CD95 signaling

Nicolai Fricker, Leo Neumann, Joel Beaudouin, Christine Falk, Martina Schnölzer, Roland Eils, Peter H. Krammer, Inna Lavrik

CD95 is a member of the TNF receptor family, which can trigger apoptosis upon binding to its ligand but can also promote cell proliferation if cells are stimulated with a low amount of CD95 (Lavrik et al, 2007). Activation of the receptor leads to the formation of the death-inducing signaling complex (DISC) which consists of CD95, FADD, Caspase-8/10 and c-FLIP. There are two short and one long isoform of c-FLIP known. The short isoforms (c-FLIPR, c-FLIPS) compete with procaspase-8 for binding to the DISC and thereby inhibit the signaling. The long isoform (c-FLIPL) can promote procaspase-8 processing and activation at low concentrations but can also inhibit procaspase-8 binding to the DISC if expressed in high amounts. Cleavage and activation of procaspase-8 at the DISC leads to the initiation of apoptosis. CD95 stimulation can also lead to the induction of survival pathways: It induces Erk, IκBα and JNK phosphorylation. Therefore the composition of the DISC that means the amount of c-FLIPS/R, c-FLIPL and procaspase-8 is crucial for the life-death decision.

In this study the role of the different isoforms of c-FLIP will be incorporated into a mathematical model of CD95 signal transduction. We have established several HeLa cell lines which express different amounts of the c-FLIP isoforms. The response of these cell lines upon CD95 stimulation will be measured with quantitative immunoblots and bioplex. Further, the total amount of the main molecules involved in this signal transduction will be measured with mass spectrometry. This data will then be used to build a mathematical model of CD95 signaling which will allow a better understanding of this pathway.

Poster-8: Systematic approach for studying crosstalk between autophagy and apoptosis

Phillip Hundeshagen, Roland Eils and Nathan Brady

Autophagy is a process of self-digestion that serves to maintain cellular homeostasis through bulk protein and organelle removal via the lysosomal pathway. Defective autophagy has been put in relation to a variety of diseases. This includes neurodegenerative diseases (such as Alzheimer or Parkinson disease), ischemia/reperfusion and cancer. Autophagy has been strongly linked to tumorigenesis, as key players of the autophagic machinery are also involved in tumor development and have been shown to act as tumor suppressor genes.

We here present a novel technique for detecting regulators of autophagic activity.

We have established a novel approach that allows high-throughput screening for regulators of autophagic activity. Previously used methods to detect autophagic activity required high-resolution imaging techniques and only allowed detection of autophagosome formation. In contrast, our technique allows quantitative detection of both, formation and degradation of autophagosomes, using multi-parametric flow-cytometry. Using this novel approach, we have systematically tested known and newly identified regulators of autophagic activity. Furthermore, we have characterized these regulators with respect to their level of control upon the autophagic machinery.

Poster-9: Rapid Quantitative Western Blotting

Nina Jennewein, Marti Bernardo, Roland Eils, Nathan Brady and Anne Hamacher-Brady

Western Blotting is a powerful method in molecular biology to detect the presence of a protein of interest, and determine its relative expression levels between control and experimental conditions. We present a workflow to obtain quantitative, time-resolved measurements of activity of the BH3-only protein Bid.
MCF7 cells were transfected stably with an mCherry-Bid-GFP sensor and in response to a variety of apoptotic stimuli, endogenous Bid and Bid sensor activities were determined by Western blotting. Cell lysates were run using the Invitrogen NuPAGE precast gels, which allow for precise protein separation options, minimize gel-to-gel variability and allow the loading of up to 15 samples/gel. Blots were done using the Invitrogen iBlot transfer system to perform highly reproducible and complete protein transfer within 10'30" minutes. The LI-COR infrared imaging system was used to detect simultaneously two different antibodies (e.g. protein of interest and loading control) on one membrane. Image analysis of band intensities allowed for quantification of Bid sensor and endogenous Bid activities, allowing us to distinguish between transcriptional and post-transcriptional control on Bid signalling.
Thus, the combination of different technologies allows not only for significant savings in terms cost and time, but greater reproducibility between experiments which is necessary for data quantification and to perform statistical analysis.

Poster-10: Modelling the interaction and the genetic networks of NFkB-signalling pathways in hematopoietic and leukemia/lymphoma cells

Nupur Bhattacharya[1], Maiwen Caudron[3], Meinhard Hahn[2], Axel Szabowski[4], Hauke Busch[5], Peter Lichter[2], Stephan Stilgenbauer[6], Karsten Rippe[3], Daniel Mertens[1&2]

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Malignant cells from B-cell chronic lymphatic leukaemia (CLL) recruit non-malignant haematopoietic bystander cells that are essential for the pathophenotype of the diseases and for survival of the tumour clone in vivo. These specialized non-malignant cells are differentiated by CLL cells and their signaling is essential for the survival of CLL cells (Burger et al. Blood 2000). This process involves APRIL and BAFF signaling via NFkB (Nishio et al. Blood 2005), which has previously been shown to be required for survival of CLL cells . However, up to date it is largely unknown how the different signals are interconnected and integrated. We aim to identify rate-limiting factors that control NFkB activity in CLL cells and thus identify novel therapy targets to supplement existing regimen in CLL and HL. We could previously show that external signalling is essential for both CLL and results in prognostically significant phenotypic changes in the transcriptome ; . We currently i) apply quantitative fluorescence microscopy approaches to derive information on interaction affinity/kinetics and cellular location/mobility of signal transduction components and ii) detect phenotypic and iii) trancriptional changes occuring after stimulation with systematically varied ligand concentrations, all on primary CLL cells.

Poster-11: Identification and characterization of novel TNFα/NF-κB signaling pathway components

Marie Metzig, Dorothee Nickles, Michael Boutros

Signaling pathways are crucial for the regulation of cell proliferation and differentiation, as well as for mediating adequate immune responses. Aberrant signaling is involved in the pathogenesis of human diseases like cancer. Further dissection of important signaling pathways is central to gain additional insight into carcinogenesis and to develop new therapeutic approaches.
Nuclear factor κB (NF-κB) transcription factors are central coordinators of inflammatory responses. Constitutively active NF-κB signaling is present in different human cancers and there is increasing evidence that it plays an important role in carcinogenesis. The classical signaling pathway that leads to NF-κB activation is stimulated by tumor necrosis factor alpha (TNFα), a multifunctional cytokine that is produced by various cells.
Our project aims to identify and further characterize new regulators of TNFα/NF-κB signaling pathways. Building on large-scale data sets obtained through genome-wide RNA interference (RNAi) screens for human TNFα/NF-κB signaling, we identified approximately 50 potential new genes that might encode for so far unknown components of TNFα/NF-κB signaling. Our validation process includes different secondary retests to select the most interesting candidates. We will present screening and secondary cell-based validation approaches.

Poster-12: Meta-Analysis of Microarray Data on Spinal Cord Injured Mice

Sachin Kumar, Elisabeth Letellier, Ana Martin-Villalba

We have previously shown that neutralization of CD95L after SCI is able to reduce the number of cells undergoing apoptosis and consequently improve functional recovery. Immune cell infiltration is a hallmark of SCI. To address were CD95L signal comes from, we generated a mouse line deficient in CD95L in myeloid cells. These mice exhibited better functional recovery compared to littermate controls. Thus global inhibition of CD95L results in a similar clinical outcome as the genetic ablation of CD95L in myeloid cells. Thus, it raises the question whether the observed effect is solely due to myeloid cells bringing CD95L into the injured spinal cord?
To better understand the mechanisms of CD95L induced damage on SCI, we compared different mice microarray datasets: 1) genetic depletion of CD95L in the myeloid cell lineage 2) ubiquitous depletion of the CD95L 3) mice treated with a neutralizing agent to CD95 (APG103) as well as the respective control. Interestingly we found a ~60% overlap in the genes differentially regulated in the dataset of mice treated with APG103 compared to the mice in which the CD95L has only been depleted in the myeloid lineage, indicating the high influence of immune cells in the CD95L-induced pathogenesis after SCI.
Ubiquitous CD95L deleted mice have a lymphoproliferative disease. This genotype effect doesn't allow a simple comparison to the other datasets. To avoid this problem analyzed ubiquitous CD95L deleted mouse with the Linear Model and empirical Bayes Method for Assessing (LIMMA) differential expression. We compared two groups i.e. a) wild-type naïve vs. ubiquitous CD95L deleted naïve mice for determining the genotype effect and b) wild-type injured vs. ubiquitous CD95L deleted injured mice to analyse the injury effect. Using linear modeling approach 1301 genes were found in interaction and were significantly regulated upon injury. Although experimental conditions may be similar, each experiment and animal background will show a slightly different list of statistically significant genes. Thus, to analyse

different experimental datasets a statistically-based meta-analytic approach for microarray analysis systematically combines results from the different datasets to provide a single estimate of the degree of differential expression for each gene. This approach provides a more precise view of genes that are of significant interest, while simultaneously allowing analysis of the differences between each experiment and animal background.
We apply GeneMeta package from Bioconductor (http://bioconductor.org) for statistically-based meta-analytic approach of our three datasets. We found 612 statistically significant genes showing common pattern of gene regulation as we previously observed in the dataset from the CD95L $^{f/f;LysMcre}$ mice as well as the APG103 treated mice dataset. Out of 612 genes, 60% genes were common with the CD95L $^{f/f;LysMcre}$ mice dataset and 70% genes were common with the APG103 treated mice dataset, clearly demonstrating that the immune system is playing a major role at the time point of our analysis. Using Principal Component Analysis (PCA), treated group (i.e. CD95L $^{f/f;LysMcre}$; ubiquitous depletion of the CD95L and therapeutic neutralization of CD95L with APG103) were cluster nicely which confirms the major role of the immune system shown previously by using the Meta-analyses.

Poster-13: The role of the nuclear receptor tailless in glioblastoma formation

H.-K. Liu and G. Schütz

The nuclear receptor tailless (Tlx) is an orphan member of the family of nuclear receptors. The gene is expressed in the ventricular zone of the developing brain and eye as well as in adult neural stem cells. Genetic analysis in the mouse has indicated the crucial role of Tlx in the generation and maintenance of neural stem cells in the adult subventricular zone. Overexpression of the gene leads to increased neurogenesis in the adult brain and to spontaneous development of glioma-like lesions in old mice. Overexpression of Tlx in the absence of p53 leads to rapid formation of glioblastoma-like lesions.
In the SB Cancer proposal we wish to study the consequences of transformation of neural stem cells to brain tumor stem cells with regard to gene expression profiling. By comparing the gene expression profiles of normal neural stem cells and brain tumor stem cells derived by Tlx overexpression in the brain we hope to identify crucial regulatory pathways for the transformation from normal neural stem cells to brain tumor stem cells. Tlx serves as a transcriptional repressor and extensive expression profile analysis by ChIP/SEQ experiments will help to understand the molecular pathways directly controlled by Tlx. This analysis will allow the identification of the direct target genes for Tlx in neural stem cells and brain tumor stem cells. Little is known of the early events in the development of glioblastoma. Gene profile analysis of the developing tumors at different stages of the initiation and progression will be performed to systematically visualize the multiple pathways involved in the development of these tumors. We hope that the genetic alterations taking place at the early stages in tumor development will provide a time-resolved analysis of gene expression in the transition from neural stem cells to cancer stem cells.

Poster-14: Stochastic modeling of T-cell signal transduction

Domenico Prellino[1], A.Brümmer[1], O.Acuto[2] and T.Höfer[1]

1Modeling of Biological Systems (B086), DKFZ; 2Dunn School of Pathology, University of Oxford

Tyrosine phosphorylation of T-cell antigen receptor (TCR) is an early and obligatory event in TCR activation by antigens. Phosphorylation of immunoreceptor tyrosine-based activation motif (ITAM) provides the structural basis for interaction of the Src-family kinase ZAP70 with the TCR. ZAP70 contains two tandemly arranged Src-homology 2 (SH2) domains that associate with the phosporylated ITAMs before ZAP70 phosphorylation by Lck and the subsequent activation of several proteins important for the signal cascade. Experiments have suggested that ITAM-ZAP70 system can sense
the stability of the antigen-TCR complex and thereby mediate the distinction between foreign and self-antigens. However, the molecular mechanisms of this discrimination are unclear. Here we present the first results from a "double-ITAM" model for early signaling in TCR. We take into account five time ordered states for a single cell system: first and second phosphorylation of each ITAM, ZAP70 recruitment, ZAP70 phosphorylation by Lck and ZAP70 phosphorylation by a ZAP70 bound to the other ITAM. By performing Mathematica and C++ stochastic simulations we attempt to quantify the percentage of ITAM phosphorylation, ZAP70 binding and double ZAP70
phosphorylation events as a function of the quality of the antigen and we also
analyse the kinetics of the same events.

Poster-15: Modeling JAK2-STAT6 pathway in PMBL and CD19+ B cells from healthy donors

Valentina Raia, Marcel Schilling, Armin Pscherer, Stefan Joos, Peter Lichter, Jens Timmer, Ursula Klingmüller

JAK/STAT signaling pathway is frequently constitutively activated in primary mediastinal B-cell lymphoma (PMBL) and classical Hodgkin Lymphoma (cHL). In our study we focused on the PMBL-derived cell line MedB-1, which has multiple copies of JAK2 gene, constitutive phosphorylation of STAT6 protein and complete inactivation of JAK2 inhibitor SOCS1. To construct a mathematical model of the pathway, we determined the number of molecules/cell of the major proteins involved, we investigated the kinetics of the proteins after IL13-stimulation and we revealed protein-protein interactions by co-immunoprecipitation (co-IP). The proteins of major interest are the subunits of IL13 receptor (IL13Ralpha1, IL4Ralpha), JAK2, SHP1 (a JAK2-phosphatase) and STAT6. As a control we used CD19+ B cells from healthy donors (controls). Performing time-course experiments in MedB-1 cells followed by immunoprecipitation and quantitative immunoblotting analysis, we detected an induction of phosphorylation of IL4Ralpha, STAT6, SHP1 and JAK2 after IL13-stimulation. In MedB-1 cells JAK2 activation was sustained in time compared to controls. The co-IP studies in MedB-1 cells revealed interaction of IL13Ralpha1 with IL4Ralpha and SHP-1, while JAK2 showed interaction with SHP1 and STAT6 but not with IL4Ralpha and IL13Ralpha1. The determination of number of molecules/cell allowed us to calculate SHP1/JAK2, SHP1/STAT6 and STAT6/JAK2 ratios in MedB-1 cells and controls. The obtained values confirm

various potential sources of deregulation affecting the JAK/STAT pathway in MedB-1 cells.
The collected data create a very powerful basis for the understanding of the dynamics of JAK2/STAT6 pathway in both MedB-1 cells and controls and will soon allow us to construct a mathematical model of the pathway. As a future step, we plan to investigate the same pathway in other PMBL and cHL cell lines and eventually in patients. The final goal is the establishment of mathematical modeling tools predicting which molecules are most suitable to be targeted in PMBL and cHL diseases.

Poster-16: The balance between BH3-only pro-apoptotic proteins and cell death rescue converging at mitochondria in the human MCF7 breast cancer cell

Yara Reis, Moritz Herrmann, Roland Eils, Nathan Brady and Anne Hamacher-Brady

Mitochondrial dysfunction is implicated as a causative factor in many diseases, including neurodegenerative diseases (e.g. Alzheimer or Parkinson disease), ischemia/reperfusion and cancer. BH3-only proteins are pro-apoptotic members of the Bcl-2 family and are known to trigger mitochondrial membrane permeability, leading to the release of pro-apoptotic intermembrane space proteins (e.g. cytochrome c and AIF) into the cytosol and consequent activation of caspase-mediated cell death. BH3 only proteins can act either by inhibiting anti-apoptotic Bcl2/-xL proteins or by promoting directly mitochondrial dysfunction- as it is the case of tBid and Bnip3.
We have applied high resolution fluorescence microscopy, and multi-parametric flow cytometry in order to investigate the role of tBid and Bnip3 in the process of mitochondria clearance, also known as mitophagy or mitoptosis. Our results demonstrate that both tBid and Bnip3 trigger mitochondrial autophagy, as evidenced by their co-localization with fluorescent proteins (FP)-tagged targeted to autophagosomes, late endosomes, and mitochondria in live cells. Using both computational and experimental methodologies, we are testing the hypothesis that mitochondrial clearance is cytoprotective, allowing the cell to increase its threshold to mitochondrial-mediated apoptosis.

Poster-17: Image-based detection of crosstalk between pro-death and pro-survival receptor signaling

Daniela Richter, Roland Eils and Nathan Brady

Image-based detection of crosstalk between pro-death and pro-survival receptor signaling
Endocytosis of plasma membrane receptors and their subsequent trafficking via endosomes play an important role in controlling signal specificity, strength and duration. In response to death stimuli, both pro- and anti-death pathways are differentially activated in order to determine cell fate. Here we investigate the relationship between pro-survival Epidermal Growth Factor Receptor (EGFR) and pro-apoptotic TNF-Related Apoptosis-Inducing Ligand Receptor (TRAILR) during programmed cell death.
We use an imaging-based approach to detect the crosstalk between the pro- and anti-apoptotic pathways. EGFR-GFP and truncated TRAILR-GFP/YFP fusion proteins are used to detect the process of internalization and subcellular localization in response to a stimulus by high-resolution 3D timelapse fluorescence imaging. Endosomal trafficking is visualized as with fluorescent protein (FP)-tagged endosomal markers for early, late and recycling endosomes. Subcellular compartments are labeled with specific organellar-targeted FP-fusion proteins (e.g. golgi, endoplasmic reticulum, mitochondria). High-content automated image analysis is used to quantify the subcellular localization of EGFR-GFP following stimulation of the cell with either TNF or TRAIL, and similarly the response of TRAIL receptors and decoy to EGF treatment. The goal of our approach is to develop an objective high-throughput approach to correlate the spatial response of survival and death receptor trafficking with survival and death phenotypes.

Poster-18: Identification of the RAGE-dependent Gene Regulatory Network in Inflammation-associated Carcinogenesis

Astrid Riehl, Christoffer Gebhardt, Julia Németh, Moritz Durchdewald, Daniel Haag, Hauke Busch, Benedikt Brors, Meinhard Hahn, Bernd Arnold, Angelika Bierhaus, Peter Nawroth, Peter Angel and Jochen Hess

Chronic inflammation and cancer have long been linked together, however, complex and numerous interactions between the inflammatory microenvironment and tumor cells have made it difficult to unravel underlying molecular principles.
In the well-established DMBA/TPA-induced skin carcinogenesis mouse model, inflammatory mediators produced by epithelial and inflammatory cells provide soluble factors that cause sustained tissue activation during tumor promotion. Mice deficient for the Receptor of Advanced Glycation Endproducts (RAGE) exhibit significantly impaired tumor development compared to wildtype controls. Furthermore, RAGE deficient mice are resistant to TPA-induced epidermal hyperplasia and show a reduced inflammatory response upon TPA or DMBA/TPA treatment. This suggests RAGE function for signal amplification, sustained inflammation, and tumor promotion.
In order to elucidate the RAGE-dependent gene regulatory network, we performed global gene expression profiling of DMBA/TPA-induced papilloma as well as of a kinetic series of TPA-treated mouse skin samples. We could already identify promising gene clusters of differentially expressed genes. Complementing our *in vivo* analysis, we established immortalized mouse keratinocyte cell lines of RAGE deficient and control mice. A highly time-resolved kinetic of these keratinocyte cell lines responding to respective RAGE ligands will now be evaluated via global gene expression analysis as well. Using a combination of functional genomics and systems biology modelling on the different sets of microarray data, we aim to construct the topology and dynamics of the RAGE-regulated genetic network. Interconnection of the data of *in vivo* and *in vitro* array studies will provide further insight into the participation of the different cellular compartments – keratinocytes and inflammatory microenvironment – involved in the DMBA/TPA inflammation-associated carcinogenesis model. The identification of key nodes within this RAGE-regulated gene regulatory network might then prove suitability for translational cancer research.

Poster-19: Decoding of Calcium Oscillations by Phosphorylation Cycles: Analytic results

Carlos Salazar, Antonio Politi, Thomas Hoefer

Experimental studies have demonstrated that Ca^{2+}-regulated proteins are sensitive to the frequency of Ca^{2+} oscillations, and several mathematical models for specific proteins have provided insight into the mechanisms involved. Because of the large number of Ca^{2+}-regulated proteins in signal transduction,

metabolism and gene expression, it is desirable to establish in general terms which molecular properties shape the response to oscillatory Ca^{2+} signals. Here we address this question by analyzing in detail a model of a prototypical Ca^{2+}-decoding module, consisting of a target protein whose activity is controlled by a Ca^{2+}-activated kinase and the counteracting phosphatase. We show that this module can decode the frequency of Ca^{2+} oscillations, at constant average Ca^{2+} signal, provided that the Ca^{2+} spikes are narrow and the oscillation frequency is sufficiently low – of the order of the phosphatase rate constant or below. Moreover, Ca^{2+} oscillations activate the target more efficiently than a constant signal when Ca^{2+} is bound cooperatively and with low affinity. Thus, the rate constants and the Ca^{2+} affinities of the target-modifying enzymes can be tuned in such a way that the module responds optimally to Ca^{2+} spikes of a certain amplitude and frequency. Frequency sensitivity is further enhanced when the limited duration of the external stimulus driving Ca^{2+} signaling is accounted for. Thus our study identifies molecular parameters that may be involved in establishing the specificity of cellular responses downstream of Ca^{2+} oscillations.

Poster-20: Direct Activation of PI3K through CD95

Ignacio Sancho-Martinez, Ana Martin-Villalba

PI3K activation upon CD95 stimulation has been found in Glioma cells. Here we unravel a novel CD95Ligand/CD95 non-apoptotic pathway. In Glioma cells triggering of CD95 fails to induce a functional death-inducing-signaling-complex (DISC). In this scenario, binding of CD95Ligand to CD95 recruits the p85 subunit of phosphatidylinositol 3-kinase to CD95. p85 binds to CD95 by interaction of both SH2 domains to the previously described YXXL motif present in the Death-Domain of CD95. Yes, a non-receptor tyrosine kinase belonging to the Src family has been found to co-precipitate in the CD95/PI3K complex thus forming the so called PI3K-Activation-Complex (PAC). Importantly, Yes knockdown completely abolished PI3K activation upon CD95 stimulation also allowing some degree of DISC formation. Furthermore, we study cell-decisions by comparing two different Glioma cell lines with different sensitivities to CD95-induced apoptosis. In this case, a cell line resistant to CD95-induced apoptosis (T98G) and a highly sensitive cell line (LN18) where compared in their ability to activate PI3K. Both cell lines showed efficient PAC formation and PI3K activation. As LN18 cells have an intact PTEN whereas T98G cells carry a mutated PTEN, we studied the effect of wtPTEN overexpression in T98G. PTEN overexpression did not sensitize cells to CD95-induced apoptosis. Along this line, we have started to study stoichiometric differences in both cell lines. Interestingly, T98G cells showed significantly higher levels of Yes than LN18 but they do not differ in the levels of FADD. Our aim is to fully characterize the differences between both cell lines at the molecular level, information that might be used to modulate the CD95 output signal.

Poster-21: Negative and Positive Feedback Loops Regulating PI3K/Akt Signaling

Bin She[1], Lu Wang[2,4], Marcel Schilling,[1]Carlos Salazar[2], Seong-Hwan Rho[3], Jens Timmer[3], Thomas Hoefer[2], and Ursula Klingmueller[1]

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The hematopoietic cytokine erythropoietin (Epo) triggers upon binding to its receptor (EpoR) the PI3K/Akt signaling pathway that is essential for erythroid cell proliferation and differentiation.
We analyzed by quantitative immunoblotting the stoichiometry and activation kinetic of pathway components and developed a mathematical model describing the properties of the pathway. The pathway can be divided into two parts:
Signaling through PI3K can be activated through direct binding to the phosphorylated EpoR (pEpoR) at position Y479 or indirectly through the adaptor protein Gab1 in primary cells at the colony forming unit-erythroid stage (CFU-E) or Gab2 in the lymphoid pro-B cell line expressing the EpoR (BaF3-EpoR). To study the two mechanisms of PI3K activation we selectively activated one of the pathways in BaF3 cells by using specific EpoR mutants. The results indicate that both pathways cooperate and form a positive regulatory loop to amplify the Akt activation. In order to better understand the contribution of the different pathways, we are applying our mathematical model and performing prediction on the reaction flux through a different pathway.
Termination of signaling can be mediated by direct dephosphorylation of Akt and by dephosphorylation of PIP3 by two lipid phosphatases the SH2 domain containing inositol-5-phosphatase (SHIP1) and the phosphatase and tensin homolog (PTEN). To gain further insight, we perturbed the system by overexpression of SHIP1 and PTEN by retroviral transduction. Applying data-based mathematical model and control analysis revealed that SHIP1 and PTEN have different roles in control of signal termination.
Based on our mathematical models, we propose that at early stage, PI3K signaling generates a transient self-amplifying loop in a PIP3- and Gab-dependent manner and at later stage, sustained inhibitors of signaling, are activated, such as the lipid phosphatases PTEN and SHIP1.

Poster-22: Systems biology approach to understand the control of Bcl-2 signaling on calcium homeostasis

Simon Turschner, Juliane Claus, Marko Müller, Nina Jennewein, Anne Hamacher-Brady, Roland Eils and Nathan Brady

Apoptotic decisions are made via interactions between pro- and anti-apoptotic Bcl-2 family members located (i) at the mitochondria and (ii) at the ER. In the mitochondria apoptotic processes involve mitochondrial outer membrane permeabilization, while at the ER pro- and anti-apoptotic Bcl-2 family members affect Ca^{2+} homeostasis. Ca^{2+} release from the ER to the cytosol is controlled by inositol 1,4,5-triphosphate receptor (IP3R)–mediated Ca^{2+} leak and sarco-endoplasmic reticulum calcium ATPase (SERCA) pumping action. The interactions of Bcl-X_L and Bcl-2 with IP3R as well as the interaction of Bcl-2 with

SERCA function to suppress apoptotic release of Ca^2 and are antagonized by pro-apoptotic Bax.
Mathematical modeling provides an important approach to analyze the complexity of signaling pathway interactions and crosstalk. Here we implemented two kinetic models based on ODEs to describe (1) the interactions between Bcl-2 family members at the mitochondria and (2) the relationship between SERCA and IP3R in maintaining ER calcium homeostasis. Sensitivity analysis of the steady states was performed on both models. To reveal key reactions and proteins most sensitive to parameter perturbations, PCA (principal component analysis) was applied to the resulting sensitivity matrices. Current work is aimed at understanding crosstalk between Bcl-2 and calcium models.

B: CoReNe

Poster-23: Generative models and statistics of hypergraphs in biology

F. Blöchl, C. Riehle, S. Dietmann, F.J. Theis

Over the last few years complex networks science has brought plenty of insight into the large-scale organization of various biological systems. However, it becomes more and more evident that in many cases simple graphs are insufficient to integrate all relevant information and more complex structures are needed. For instance, many important networks display a natural bipartite structure and are therefore more appropriately modeled by hypergraphs, a structure in which two types of nodes exist with links only between different kinds. This situation is for example found in protein-complex networks, localization informations (gene or protein in tissue, species, etc.), functional categories or microRNA-mRNA interactions. For hypergraphs, extensions of most tools from classical network analysis have been developed, but also new characteristics emerge. However, the study of generative models to explain the observed properties is still in its infancy.
We analyze the characteristics of three different examples: the protein-complex network - the benchmark in this field -, a gene-tissue graph and the human microRNA-mRNA interactions. The last two graphs have the speciality of containing thousands of nodes of the one type, but only up to 200 of the other kind. In our three hypergraphs the degree-distribution of one node type is a power-law, while the other is almost scale-free, Poisson or different. In the tissue data, we additionally find high hyper-clustering-coefficients. This hints at a modular structure of gene expression, probably related to protein complexes.
We also compare four different generative models to reproduce our data. By means of node numbers, degree statistics, hyper-clustering-coefficients and overlap we show that the gene-tissue graph can be modeled using a hyper-preferential-attachment model. For the microRNA-mRNA network a generative model is under development. Here we have to go one step further, and use a tripartite approach, also taking into account microRNA location on the genome.

Poster-24: Algorithmic Verification of Bistability

Christian Breindl, Frank Allgöwer

Many cellular signaling networks are able to operate in two stable modes. Transitions between these modes can be triggered by external stimuli, whereas without stimulation, the system remains in its current state. This behavior can mathematically be described by a model which is able to exhibit (at least) two distinct stable steady states.
Another approach that captures the variability of the biological system even better is to establish a model that possesses two distinct forward-invariant regions in the state space.
In order to prove that a given model indeed exhibits the bistability property, we present an algorithm that uses a set of simple rules to find certain types of forward-invariant regions. If two such sets can be found, the desired bistability property can be verified for the model. However, if no such sets can be detected, no conclusion can be drawn.

Poster-25: Calculation of kinetic constants for bistable biochemical networks

Giovani Gomez Estrada, Fabian Theis

We propose an optimisation procedure for the calculation of kinetic constants that produce bistable effects in biochemical reaction networks. Bistability is a key biological capability of several networks of interest, e.g. apoptosis and MAPK cascades: that is, for a given set of concentrations, a network can exhibit not only a single but several stable output values, which are usually correlated to major phenotypes.
Bistable phenotypes are often observed in biological networks, but the uncertainty of kinetic data makes modelling difficult and hampers further quantitative analyses. The problem thus lies in the estimation of a vector of kinetic constants such that the biochemical network accepts two stable states, possibly with strong switch-like behaviour. To do so, we employ state-of-the-art optimisation methods using a combination of local and global minimisation, in which a design vector of kinetic constants is optimised in such a way that two states are observed for a single output variable. This constrained optimisation process is subject to upper and lower bounds conditions in the kinetic constants, and its loss function is calculated over the range of outputs.
The proposed procedure is indeed able to calculate these kinetic constants such that two visibly different states are seen. Moreover, the proposed approach improves in about 30% other solutions found in the literature. A notorious advantage of our procedure compared to others is the flexibility to set constraints on kinetic constants, which in turn narrows the search space. The optimisation procedure is however sensitive to initial conditions, so more parameter studies need to be done. So far, our results are discussed in the context of complex enzyme-driven reaction networks modelled with mass action kinetics. Finally, we show initial findings and possible extensions to multi-stability in reaction networks.

Poster-26: A first look at apoptotic signalling pathways of human HEK293 cells exposed to nanoparticles

Furong Tian, Giovani Gomez Estrada

The industrial use of nanoparticles is delivering novel products of enhanced strength and lightness, but nevertheless these particles might possess serious health threats. It is not quite clear how and as to what extend these health threats appear, since different tissues respond differently to different doses of nanoparticles. To find out possible mechanisms of action, we analysed the intrinsic

and extrinsic apoptotic signalling pathways of HEK293 cells exposed to single wall carbon nanotubes (SWCNT).
We focus on whether the main response is related to death receptors or mitochondrial disruption. Transmembrane protein FasL was found down-regulated, which might indicate the DISC, Death Inducing Signalling Complex, is not formed. Moreover, pro-apoptotic Bcl-2 members, Bax and Bak, were found to be up-regulated, and inhibited the p53 signalling pathway. Interestingly, pro-survival genes like FGFR2, BAG1 and BMP2 were found over-expressed.
Our results are three fold. Firstly, these preliminary data show the intrinsic pathway plays the leading role for this apoptotic response. The DISC is probably not formed due to the size of nanoparticles, which might avoid this immune surveillance system. Secondly, the presence of MMP9 also suggests MOMP, mitochondria outer membrane permeabilization, takes place and ROS was produced. Finally, there are indications of strong crosstalk between pro-apoptotic and pro-survival signals, which could count for the radically different behaviour under different SWCNT dosage. We are developing a model based on mass action kinetics to study Bcl-2 family proteins and MOMP. The current model does not yet include the couple between pro-apoptotic and pro-survival signals, but by doing so we expect to provide insights for the next set of experiments.

Poster-27: The Odefy CellNetAnalyzer plugin: continuous models using Hill Cubes

Jan Krumsiek, D. Wittmann, S. Klamt, F.J. Theis

We present Odefy, a plugin for the CellNetAnalyzer MATLAB package which allows to convert any boolean regulatory network into a continuous ODE model. For this we use a novel modeling technique called Hill Cubes, which is based on multivariate polynomial interpolation. The user can quickly simulate regulatory networks and visualize the simulation results or save the ODE model to the file system. All parameters of the dynamical system can easily be accessed using the graphical user interface or additional helper MATLAB functions.

Poster-28: Identifying and modeling a microRNA influenced regulatory system in stem cell development

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The differentiation of embryonic stem (ES) cells during gastrulation into the cell types of the three germ layers is controlled by multiple interacting gene regulatory mechanisms. In addition to transcription factor (TF) driven regulations, there is strong evidence that microRNAs play an important role during stem cell maintainance and differentiation [Hornstein, Nat Genet, 2006]. The identification of microRNA-controlled gene regulation using expression data remains difficult, mainly for two reasons: (i) there is still a lack of microRNA expression data with adequate time resolution, and (ii) the inhibitory effect of microRNAs on translation stays invisible on the mRNA level. Here we study gene regulation under the influence of microRNAs controlling differentiation of ES cells into endoderm and mesoderm lineages. We uncover microRNA-driven gene regulation by measuring the indirect influence of an intronic microRNA on a microRNA-TF-target-gene motif.
Starting from two known TFs regulating endoderm development, Foxa2 and Sox17 [Matsuura, Stem Cell, 2006], we identify several potential intronic microRNAs targeting the corresponding transcripts using a weighted combination of the available microRNA-target-site prediction tools. To quantify microRNA expression we take the host-gene expression of the embedded intronic microRNAs as proxy [Tsang, Molecular Cell, 2007]. From the expression data of the TF targets, we identify a qualitative regulatory motif that stabilizes the differentiation into mesoderm lineage consisting of the mesoderm specific host gene Mest and the embedded mmu-mir-335 and the TFs. Based on this motif we develop a hill-function based quantitative model to study the dynamical behavior of the system.
Using microarray expression data of differentiating ES cells, we identify a regulatory system including an intronic microRNA that stabilizes differentiation during development. Through dynamical modeling we identify a bistable system that can switch between mesoderm and endoderm state starting from an undifferentiated cell. This sheds light into the potential cellular mechanisms driving ES cell differentiation.

Poster-29: Conservation in regulatory microRNA networks

Carsten Marr, Andre Aberer, Dominik Lutter, Daniel Schmidl, Fabian Theis

The discovery of microRNAs adds a new layer of complexity to the intricate network of gene regulation. Genome-wide inhibition of mRNA translation mediated by the binding of microRNAs in the transcripts' 3' UTRs can be abstracted as a bipartite graph. While the conservation of microRNAs and target transcripts has been reviewed recently [Chen and Rajewsky, Nat. Rev. Genet. 8, 2007], little is known about network properties of bipartite graphs of different species.
Here we compare the regulatory microRNA – mRNA interactions of seven species as predicted by the recently published 'Probability of Interaction by Target Accessability' (PITA) algorithm [Kertesz et al., Nat. Genet. 39, 2007]. In order to make inter-species comparisons possible, we introduce a concept to generate networks from the algorithm's predictions. To this end, we compare PITA predictions of species-specific transcripts with randomized sequences. We analyze the global properties of the seven networks and put them in a phylogenetic perspective. From the comparison of deviations in local network motifs, we discuss the conservation of motifs and their impact on functional properties, like species-specific diseases.
By combining bioinformatic tools for microRNA target predictions with concepts from graph theory, we reveal functional aspects of conserved regulatory transcript interactions.

Poster-30: A delay stochastic process with applications in molecular biology

R. Schlicht

Regulatory networks that generate oscillations often involve delays. In addition, if molecules appear in small numbers, these processes are subject to stochastic effects. We here present a non-Markovian stochastic model that provides a precise mathematical description of these phenomena. The explicit construction of the model is well suited for both theoretical analysis and direct simulation. We can derive exact analytical expressions for the reaction rates.
We discuss how to distinguish oscillation from mere noise, and show that the stochastic model can explain the oscillations that

drive the formation of somites in the vertebrate embryo, an important process in developmental biology.

Poster-31: Systems biology of the dopaminergic neurons: Deciphering the role of micro RNAs and ncRNAs

Dietrich Trümbach, Dominik Lutter, Martin Sturm, Jens Hansen, Fabian J. Theis, Wolfgang Wurst, Nilima Prakash

There is considerable evidence that ncRNAs play a key role in genetic network regulation underlying neural differentiation, dendrite formation, synaptic plasticity and memory formation. More than 1000 different ncRNAs (miRNA and siRNA) species have been predicted in silico in the mammalian genome. Each of these ncRNA might regulate up to 300 different RNAs. Furthermore, translation of each mRNA might be regulated by multiple ncRNAs. Thus, ncRNAs add an additional level of complexity on gene network regulation on a qualitative and quantitative level in time (at different stages during development and adulthood) and space (in the cellular context).
Based on candidate genes involved in the development of ventral midbrain neuronal population and disease pathways we have identified 158 different miRNAs in silico which are potentially participate in these processes. Out of these 158 miRNAs we got a final ranked list of 33 miRNAs by comparing the target site predictions in the following databases and software programs: TargetScan, PicTar, miRBase and TargetSpy (MIPS). Currently, 30 miRNAs of the ranked list undergo in vivo validation by LNA based In Situ Hybridisation.

Poster-32: Intuitive Modeling of Dynamic Systems with Petri Nets and Fuzzy Logic

Lukas Windhager, Ralf Zimmer

ODE based models of biological systems allow exact modeling of their dynamics. However, they require laborious efforts for detailed parameter estimation and often lack comprehensibility, especially for users without mathematical background. Other approaches, like Boolean networks, are successfully applied for qualitative analysis of systems but often cannot capture complex dynamics.
We propose a new approach to overcome such limitations by combining the graphical representation provided by Petri nets with the modeling of dynamics by powerful yet intuitive fuzzy logic based systems. The mathematical functions and formulations typically used to describe or quantify dynamic changes of systems are replaced by if-then rules, which are both easy to read and formulate. Precise values of kinetic constants or concentrations are substituted by more natural fuzzy representations of entities.
The main innovation of our *PNFL* (Petri Nets with Fuzzy Logic) approach is the use of elements from fuzzy logic theory to describe biological systems: Fuzzy sets describe arbitrary entities or properties of a system; Fuzzy logic systems define the dynamics of biological processes and dependencies between entities. Petri nets are used as a scaffold for the fuzzy logic based definitions of biological entities and processes.
Our new modeling approach *PNFL* allows a semi-quantitative modeling of biological systems such as signal transduction pathways or metabolic processes. A prototype system has been successfully applied during different developmental stages to several small test systems, like typical network motifs (e.g. feed-forward loops, switches) and several oscillator models (Higgins-Selkov, minimal mitotic, coupled oscillators). In addition, a larger model of the EGF signal transduction pathway was evaluated by replacing mass action kinetics with fuzzy logic systems.

Poster-33: From Discrete to Continuous Modeling

Dominik M. Wittmann, Jan Krumsiek, Florian Blöchl, Steffen Klamt and Fabian J. Theis

The understanding of regulatory networks is a core objective in systems biology. Experimental investigation of these networks, e.g. by gene knock-outs, mostly yields qualitative data, which allow the construction of boolean models. While often able to capture the essential behaviour of a network, these models can never reproduce detailed time courses of concentration levels. Therefore an obvious question is how a discrete model can be made continuous.
Here we present a canonical way of transforming boolean models into continuous ODE models by using multivariate polynomial interpolation. The use of this interpolation technique allows to accurately transform any kind of logic operation into a system of ODE's. Our approach is standardized and can easily be applied to large networks. We also present first results about the differences and similarities between the discrete and the continuous model.
We test our method on a logical model of T-cell activation.The interactions in this network operate on different time scales. In the boolean model this is taken into account by differentiating between an early event and a mid-time event scenario, where slow interactions appear only in the latter. In the continuous ODE model we do not have to change the network topology but can simply adjust the speed of single interactions by a finetuning of parameters. A set of biologically plausible parameters can be given such that our model shows a realistic activation of T-cells.

The Human Brain Model

Poster-34: Identification of correlated neuronal groups by an accretion based data mining algorithm

D. Berger, G. Gerstein, M. Diesmann, C. Borgelt, S. Gruen

Assemblies of synchronously active neurons were suggested as the key mechanism for cortical information processing (Hebb, 1949). Testing this hypothesis requires to observe large groups of neurons simultaneously, which is possible now due to recent advancements in electrophysiology. However, tools for analyzing such massively parallel data are lagging behind. Mere pairwise tests are not enough to detect assembly activity and to identify assembly members – tests for higher-order correlations are needed. However, a combinatorial explosion prevents us from applying existing methods for this task to massively parallel spike data.

We are currently exploring a new method, which combines the accretion method (Gerstein, 1978) with data mining approaches, in particular frequent itemset mining (FIM). Synchrony is detected by the accretion approach: pairs of spike trains are tested for significant correlation and then reduced to new point processes containing only synchronized spikes. These processes are in turn correlated with single neuron spike trains and so on, until the maximal order of correlation is found. Ideas from FIM algorithms help to search the space of all neuron subsets efficiently.

However, FIM algorithms usually rely on a minimum support criterion to prune the search, since it guarantees soundness. In our framework, this criterion is not useful, since higher-order correlation does not necessarily imply a frequent occurrence of spike patterns. We rather want to know whether spike patterns occur significantly more often than expected given the firing rates. By exploiting properties of the chi-square measure, we designed a FIM related algorithm that processes large data sets efficiently. Using simulations of massively parallel spike data we show that our algorithm is well suited for detecting groups of neurons exhibiting higher-order correlations, and that detecting various assemblies within one data set is possible and independent of the number of assemblies individual neurons are participating in.

Poster-35: Quantitative analysis of the dynamics of adult hippocampal neurogenesis in mice

Susanne Lezius, Imke Kirste, Laurenz Wiskott, Gerd Kempermann

Adult hippocampal neurogenesis refers to the development of new granule cell neurons from precursor cells in the adult dentate gyrus. A kinetic model of this development has been established before. Therein the process of neurogenesis is composed of a sequence of different cell types. However, the exact dynamics of neuronal development in the dentate gyrus are unknown.

To quantify the development we collected time-series like data. After injection of BrdU the brains of 74 mice were analyzed at twelve different time points. The absolute number of BrdU-positive cells as well as the relative numbers of BrdU-positive cells of the respective types including the postmitotic cells were determined. These cell counts are consistent with the kinetic model. Moreover, we analyzed the absolute numbers of Nestin-GFP-positive, Doublecortin-positive, and Calretinin-positive cells. These cell counts show no time-dependence, which leads to the idea of a constant distribution of cells over the different cell types on a short timescale.

Based on the data a mathematical model containing the different developmental stages was established. Here we used the idea of the Leslie matrix which is a discrete and age-structured model of population growth and can be adapted to cell populations. Our model includes the effect of label dilution and confirms the number of three or four divisions until BrdU is not detectable anymore. From this model differentiation probabilities can be derived by fitting the parameters to the data. Furthermore, it enables us to monitor the temporal progress of the development. Another conclusion from the mathematical analysis of these data is that type-1 stem cells contribute to adult neurogenesis intermittedly.

The modeling is an ongoing process. Currently we use our previous results and include all knowledge about the process to achieve more biological reality.

Poster-36: Derivation of temporal precision of spike synchronization by spike dithering

Antonio. Pazienti, P. E. Maldonado, M. Diesmann, S. Grün

The only way to identify information processing in biological neuronal networks is to simultaneously record from many neurons at a time. Correlation analysis of such parallel spike data has demonstrated existence of significant correlated spiking activity on a fine temporal scale (ms precision), occurring at behaviorally relevant points in time. Maldonado et al (submitted) found by employing the unitary event (UE) analysis method (Grün et al, 2002b) that neurons in monkey primary visual cortex synchronize their spiking activity during free viewing of natural scenes. Significant spike synchrony occurs dynamically and short lasting in relation to fixation onset while the firing rates are still increasing as a response to visual input. However, it was suspected that the occurrence of spike synchrony is a false positive by-product of the changes of the firing rates.

Despite the fact that the UE analysis is correcting for the impact of firing rates, we performed additional controls, e.g. by spike dithering (Date el al, 1998). Such random displacement of individual spikes within a small time interval indeed lead to the decrease of excess spike synchrony, however, the speed of decay appeared slow. Here, we present our theoretical studies on the impact of dithering on the detection of spike synchrony. In particular we analyze the speed of the decay of coincidences as functions of 1) different coincidence detection methods (disjunct binning and multiple shifts), 2) the allowed coincidence width, 3) the temporal jitter of the coincidences, and 4) physiological parameters such as firing rates and coincidence rate (Pazienti et al, 2007; Pazienti et al, 2008). We provide closed form analytic expressions for the probability of coincidence detection in the different scenarios, which are also validated by simulations. Decay of synchrony can only be observed if excess synchrony is present. Thus we were able to reproduce the experimental findings. Moreover, we show that dithering allows us to determine the temporal precision of synchronized spike events.

Poster-37: Linking synaptic plasticity to system-level learning in the framework of temporal-difference learning.

Wiebke Potjans, Abigail Morrison, Markus Diesmann

Synaptic plasticity is thought to underly learning. However the exact relation between changes in synaptic efficacy and system-level learning remains unclear. One theory often considered in this context is reinforcement learning. There is considerable evidence from behavioral and neurophysiological studies that learning in animals is based on reinforcement learning, in particular, on a variant known as temporal-difference (TD) learning. However, it is unclear how TD learning could be implemented in the brain. The major obstacle to overcome is that the classical TD learning algorithm is formulated in discrete-time steps, whereas the brain interacts by means of pulses in continuous time. Previous models have therefore either focussed on non-TD reinforcement learning models or on non-spiking TD models.

We present a spiking neuronal network model implementing TD learning based on biologically plausible plasticity rules. We show the equivalence between the discrete-time algorithm and the neuronal formulation by deriving a quantitative mapping from the discrete-time TD parameters to the parameters of synaptic plasticity.

The neuronal network is able to solve a non-trivial grid-world task with sparse rewards with similar speed as its discrete-time counterpart and attains the same equilibrium performance. We show that the derived mapping has a high degree of accuracy and the learning behavior is robust for a wide range of synaptic parameters. Furthermore, learning remains robust if we decrease the time the agent stays in one state until a limiting time span of 200 ms is reached. The synaptic plasticity rules underlying the learning process represent one possible implementation of TD learning. Alternative synaptic plasticity rules implementing TD learning in the actor-critic architecture are discussed.

See also

Potjans, W., Morrison A, & Diesmann, M. A spiking neural network model of an actor-critic learning agent. Neural Computation (in press).

Poster-38: The research networks of the Helmholtz Alliance on Systems Biology

Jan Eufinger

The **Helmholtz Alliance on Systems Biology** was initiated in 2007 as a centrally funded initiative comprising a network of several Helmholtz-centers, Universities and further external partners. The Helmholtz Association, the largest research organization in Germany, has allocated funds of about 24 million Euros until the year 2011, with the participating Helmholtz centers investing an almost equal sum, to further develop the existing capacities in the field of systems biology. The alliance is headed by Prof. Dr. Roland Eils, head of the Theoretical Bioinformatics Division at the DKFZ.

Scientific focus of the alliance is the elucidation of complex disease mechanisms using a highly connected and interdisciplinary approach to provide a better understanding of how complex diseases such as cancer or diseases of the cardiovascular and nervous systems develop. The aim of the alliance is to use systemic approaches to promote the understanding of the causes of complex diseases and to develop new strategies for treating them.

Research in the alliances focuses on a wide range of specific topics. Focuses include:

- *Signal transmission processes in cancer cells - The molecular bases of neurodegenerative and cardiovascular diseases - The influence of toxins on cell metabolism - The role of non-coding RNA in regulatory networks - Neuronal structure and the modeling of function in the brain*

The alliance will provide training opportunities for young scientists and technology platforms for all participating partners. It includes the following Helmholtz Centers in addition to universities and other partners:

www.helmholtz.de/systemsbiology

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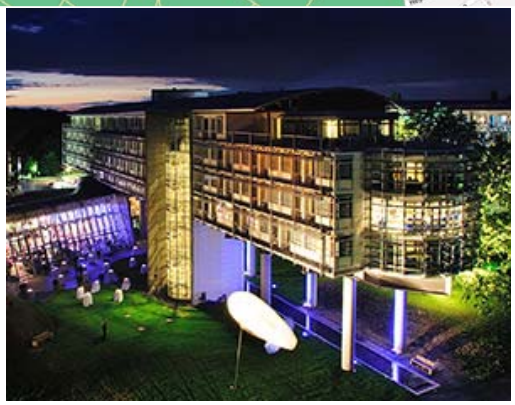
Venue Details:



Kongresshotel Potsdam am Templiner See

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Am Luftschiffhafen 1 14471 Potsdam
Phone: 0331 907-0

Venue Room: Kongresssaal

Public Transport:

Intercity (IC), Regionalexpress (RE) or S-Bahn reach **Potsdam-Hauptbahnhof**.

Public Transport in Potsdam:
Tickets needed from Potsdam HBF: Tariff area Potsdam A-B (1,70 €))
Tram: From **Potsdam-Hauptbahnhof** take **Tram-Line 91 or X98** until **Final Stop "Bahnhof Pirschheide"**. On the tram-platform walk on in direction of travel, passing the undercrossing you will reach the hotel.
Bus: From Potsdam Hauptbahnhof take **Bus Line 631** towards „**Werder**", get off at **„Luftschiffhafen"** and follow the hotel signs on the left from bridge. Or you take **Bus Line 695** until station **„Bahnhof Pirschheide"**. On the tram-platform walk on in direction of travel, passing the undercrossing you will reach the hotel.
Regional Train: Take the regional train to **„Potsdam-Pirschheide",** 5 minutes walk to the Congress Hotel Potsdam on Lake Templin from the railway station „Pirschheide".