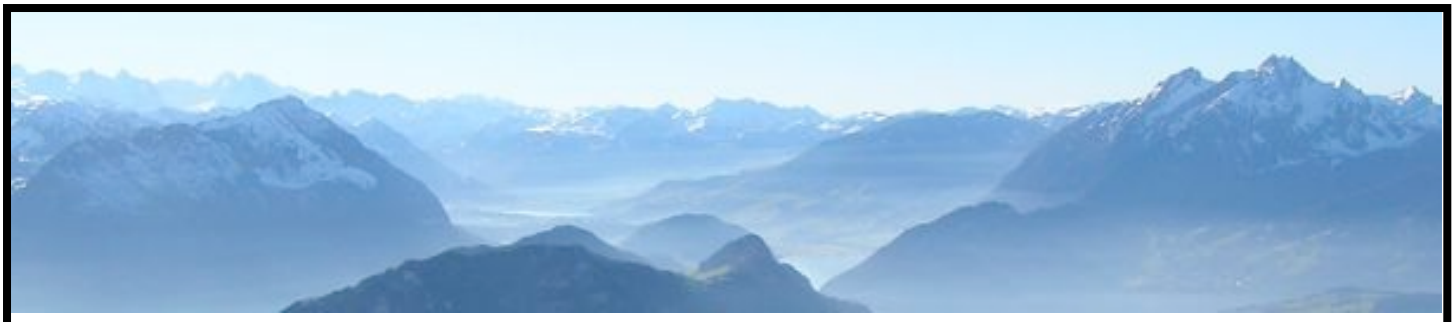


Biozentrum Postdoc Retreat

2011

August 18th-19th

Weggis, Switzerland

Biozentrum PostDoc Club

Conference Schedule

(Talks in Saphir room)

DAY 1: August 18th	
9h00	Registration opens
9h30	Coffee + Croissant
Session 1: Chaired by Sébastien Morin	
10h20	Welcome comments
10h40	*Keynote speaker:* R. Aebersold
11h50	*Postdoc talk:* Fisun Hamaratoglu
12h10	*Postdoc talk:* Jeroen Geurts
12h30	Lunch
Faust Poster Session	
13h15	Poster setup
13h30	Start
15h15	End
Social activities	
15h30	Meeting for activity
20h00	*KGF* Dinner
21h00	Get together !

Posters should be removed before the dinner on day 1.

Hotel checkout on day 2 should be completed by 10h00.

DAY 2: August 19th	
Session 2: Chaired by Neil Burton	
9h00	*Career talk:* T. Schulte
9h30	*Postdoc talk:* Misha Kudryashev
9h50	*Postdoc talk:* Jean Hausser
10h10	*Postdoc talk:* Mireia Coscolla Devis
10h30	Coffee break
Session 3: Chaired by Olga Timoshenko	
11h00	*Postdoc talk:* Lukas Cajanek
11h20	*Postdoc talk:* Stephan Duss
11h40	*Postdoc talk:* A. Papadimitropoulos
12h00	*Postdoc talk:* Prasad Kolla
12h30	Lunch
Session 4: Chaired by Oguz Kanca	
13h50	*Postdoc talk:* Ceylan Eken
14h10	*Postdoc talk:* Oguz Kanca
14h30	*Postdoc talk:* Ruben Deogracias
15h00	Coffee break / Group picture
Session 4: Chaired by Oguz Kanca	
15h30	*Postdoc talk:* Sébastien Morin
15h50	*The Postdoc Club*
16h10	*Keynote speaker:* E. P. Fischer
17h10	Closing remarks
18h05	Boat departs for Luzern...

Career Centre (with Thomas Schulte)

(Meetings in Malatelier room)

DAY 1: August 18th	
9h00—9h40	Sébastien Morin
9h40—10h20	Neil Burton
11h30—12h10	Anna Chirkova
12h10—12h30	Mireia Coscolla Devis
13h30—14h10	Jean Hausser
14h10—14h50	Ruben Deogracias
15h00—15h40	Stefan Jungblut
18h10—18h50	Olga Timoshenko
19h00—19h20	Caroline Peneff
19h20—20h00	Fatiha Boukhtouche

DAY 2: August 19th	
9h50—10h10	Amit Sundriyal
10h10—10h30	Jeroen Geurts
11h00—11h20	Oguz Kanca
11h20—11h40	Fisun Hamaratoglu
11h50—12h10	Raghavendran K. Shylini
12h10—12h30	Morteza Yazdani
13h30—13h50	Magdalena Baer
13h50—14h10	Adrien Vinet
14h20—14h40	Claudia Massa
14h40—15h00	Prasad Kolla
15h30—15h50	Misha Kudryashev
15h50—16h10	Stephan Duss

T. Schulte

Schulte Coaching

http://www.thomas-schulte-coaching.de

Presentation
Day 2 @ 9h00

PCC
Professional Certified Coach
International Coach Federation

Featured talks

I am a Postdoc--- Now What??

Prof Ruedi Aebersold — ETH Zürich and University of Zürich

A key career goal of many postdoctoral fellows is a career in science, preferably as an independent investigator in academia or group leader in the private sector. The road to achieving these goals is highly competitive. In contrast to popular belief this is not (just) an issue of luck - can I get a paper into Nature?- but to a large extent also the result of some planning.

In this presentation, we will discuss some key issues related to planning for a career in science. Specifically, we will discuss some current trends in life science research and how they might affect the profile of young scientists who are close to establishing their groups.

The idea of a genetic program

Prof Ernst Peter Fischer — Science historian

In the 1960s the idea of a genetic program was put forward in order to explain the action of genes. The term "program" offered the additional advantage that the public accepted it at the same time since they got hold of all the new computers and their programs. After a while nobody doubted the existence of programs and today we do all kind of reprogramming with stem cells and the like. But is the term genetic program really qualified or even sufficient to explain what happens in development? In the lecture it is argued that there is only a tiny bit of genetic programming going on in living cells. We need much better ideas in order to understand life and its mechanisms and causality. One possibility will be discussed, namely the idea of genetic creativity. It is "The Art of Genes" (Enrico Coen) that tells us "how organisms make themselves".

The Biozentrum Postdoc Club

Magdalena Baer — Chair of the Biozentrum Postdoc Club

The Biozentrum PostDoc Club was originally initiated to facilitate the social and scientific interactions between PostDocs and serve as intermediate between PostDocs and the Institute for issues concerning their different needs and problems. Our goal is to support new and established PostDocs by providing useful information and tips on career development, funding as well as daily academic life. All PostDocs in the Biozentrum are now automatically members of the Club, and people willing to contribute to the Club's actions are encouraged to join the Executive Committee. 2011 is an important year for the Club as we organize our first retreat. In the future we would like to transform it into a joint meeting of PostDocs from all life science institutions in Basel. We believe it could be a first step in creating a kind of Basel PostDoc Network, an initiative not only promoting interactions but also broadening the offer on career development and training activities.

Oral + Poster postdoc presentations

(In order of presentation, with numbers also used for poster session)

(1)

Dpp signaling activity requires Pentagone to scale with tissue size in the Drosophila wing imaginal disc

Fisun Hamaratoglu, Aitana M. de Lachapelle, George Pyrowolakis, Sven Bergmann, Markus Affolter

The wing of the fruit fly, Drosophila melanogaster, with its simple, two-dimensional structure, is a model organ well suited for a systems biology approach. The wing arises from an epithelial sac referred to as the wing imaginal disc, which undergoes a phase of massive growth and concomitant patterning during larval stages. The Decapentaplegic (Dpp) morphogen plays a central role in wing formation with its ability to co-ordinately regulate patterning and growth. Here, we asked whether the Dpp signalling activity scales, i.e. expands proportionally with the growing wing imaginal disc. Using new methods for spatial and temporal quantification of Dpp activity and its scaling properties, we found that the Dpp response does adapt to the size of the growing tissue. Interestingly, scaling is not perfect at all positions in the field and the target gene domain scaling is ensured specifically where they define vein positions. We tested two models that can account for the observed scaling: the French flag and the expansion-repression models. We found that the target gene domains are not defined by a simple French flag decoding. However, analysis of mutants for pentagone, an important secreted feedback regulator of the pathway, indicate that Pentagone acts as an expander of the Dpp gradient during disc growth.

(2)

The VEGF and PDGF-BB signalling balance determines the switch between normal and aberrant angiogenesis

Jeroen Geurts, Elena Groppa, Roberto Giannì-Barrera, Michael Heberer, Andrea Banfi

The therapeutic potential of angiogenic growth factors, Vascular Endothelial Growth Factor (VEGF) in particular, for treatment of vascular diseases has not been realized. The major issue for VEGF delivery is the transition between normal capillary growth and formation of aberrant hemangioma-like structures, which takes place in an all-or-none fashion across a discrete microenvironmental threshold level. Importantly, normal and aberrant angiogenic phenotypes display differential mural cell recruitment, which is mediated through platelet-derived growth factor-BB (PDGF-BB) signalling. In this study, we sought to investigate the role of PDGF-BB signalling in VEGF-induced normal and aberrant angiogenesis using gain- and loss-of-function approaches.

Interestingly, PDGF-BB gain-of-function at high VEGF level completely prevented growth of aberrant structures and yielded capillary networks instead. Conversely, PDGF-BB loss-of-function promoted growth of aberrant structures at low, otherwise safe, VEGF levels. Immunostaining demonstrated that PDGF-BB co-expression promoted early recruitment of NG2+/SMA- pericytes, which consequently associated with neovessels during remodelling. Abrogating endogenous PDGF-BB signalling at low VEGF levels did not affect the formation of mother vessels or their morphology in the activation stage. However, recruitment of NG2-positive pericytes to the new vascular structures was drastically reduced. Instead, many structures associated with smooth muscle cells, which suggests that their recruitment might be PDGF-BB independent.

Together, these data strongly indicate that the threshold between normal and aberrant angiogenesis is not an intrinsic property of VEGF dose alone, but rather depends on the balance between angiogenic stimulation by VEGF and vascular maturation by PDGF-BB-mediated pericyte recruitment.

(3)

Structure of large protein complexes in situ by cryo electron tomography and image processing

Misha Kudryashev, Daniel Castano-Diez, Henning Stahlberg

Cryo electron tomography combines a potential of high resolution 3D imaging of isolated molecules and even entire small cells with close to native preservation of the material excluding chemical fixation or staining. Resulting resolution varies from 6 to 2 nanometers dependent on the type of sample. Multiple copies of macromolecular complexes may be computationally extracted and statistically processed increasing the resolution and providing information about heterogeneity of the object. Due to minimal contrast in the images non-trivial image processing strategies are required for this. I will give an overview of the technique and will present structural details of large macromolecular complexes in situ: bacterial flagellar motor and bacterial type III secretion system as well as purified respiratory supercomplexes.

(4)

Identification of microRNA target genes: a question of time-scales

Jean Hausser, Erik van Nimwegen, Mihaela Zavolan

Gene expression is controlled at several levels. While transcription of messenger RNAs (mRNAs) from DNA is controlled by a complex network of transcription factors, mRNA stability and protein translation are regulated by an ensemble of proteins and non-coding RNAs. Among the later, a class of short, non-coding RNAs called microRNAs (miRNAs) has been shown to repress gene expression. MiRNAs recognize specific elements in the 3' Untranslated Transcribed Regions (UTR) of mRNAs. Upon binding such elements, miRNAs repress the translation and increase the decay rate of the target mRNA. There are hundreds of known miRNAs, and determining what genes are repressed by these miRNAs is crucial to understanding gene regulation in general. This question can be addressed experimentally by measuring which mRNAs and proteins are down-regulated following the over-expression of a miRNA.

Our bioinformatics analysis showed that properties such as the U content of the target mRNA, and the structural accessibility and position of binding sites within the 3'UTR characterize functional miRNA binding sites. However, the same properties systematically failed to characterize miRNA binding sites that lead to down-regulated protein levels upon miRNA over-expression. This is very surprising because changes in protein levels are expected to be the main read-out of miRNA-mediated gene silencing.

Through quantitative and probabilistic modeling, we shed light on this unexplained and crucial aspect of datasets characterizing the action of miRNAs genome-wide. We found that protein decay rates are a major confounding factor in experiments aimed at identifying miRNA targets by means of quantitative proteomics. This finding has implications for the development of miRNA target prediction tools that build on sets of functional and non-functional miRNA binding sites. In addition, it suggests ways of improving the design and analysis of experiments aimed at identifying miRNA targets by means of quantitative proteomics.

(5)

Impact of small insertions, deletions and copy number variation in the genome of Mycobacterium tuberculosis

Mireia Coscollá, Richard Copin, Joel Ernst, Sébastien Gagneux

The impact of strain variation for human disease has been well established for a number of bacterial pathogens like Escherichia coli, Neisseria menigitidis, Haemophylus influenzae, Bordetella and Streptococcus species. If we are to understand the relationships between genotype and phenotype in Mycobacterim tuberculosis complex (MTBC), a proper understanding of the genetic diversity is needed.

MTBC comprises group of a bacteria with low DNA sequence diversity compared to other pathogenic bacteria. However, long sequence polymorphisms have demonstrated a biogeographic population structure for MTBC. However, other sources of genetic like short insertions deletions (indels) and copy number variation (CNV), have not been explored before and could also be important sources of genetic diversity in MTBC. For example, small indels have been already demonstrated a source of genetic diversity and source of clinically important phenotypes, like immune evasion, in other pathogens.

We analyzed 177 whole genome sequences representive of the global diversity of MTBC. We used BWA and samtools to map the sequencing reads to the reconstructed ancestor genome of MTBC. We used a a phylogenetic framework to study the abundance of single nucleotide polymorphism (SNPs), small indels and copy number variations (CNVs) in different gene categories. Results of these analyses will be presented

A wider knowledge of variability of MTBC will allow a better understanding of associations between strain-specific genotypes and relevant clinical phenotypes.

(6)

Impact mechanisms of Wnt signaling: from embryonic stem cells to dopaminergic neurons

Lukas Cajanek, Vitezslav Bryja, Ernest Arenas

Wnts are glycolipoproteins regulating many aspects of embryo development. Wnts have been also found to regulate various stages of development of ventral midbrain (VM) dopaminergic (DA) neurons, a neuronal population which progressive degeneration is a main cause of Parkinson's disease. Wnt ligands act as morphogens and activate several downstream pathways. Perhaps the best defined is the Wnt/β-catenin pathway, also referred to as the canonical Wnt signaling pathway. Other branches of Wnt signaling are collectively referred to as non-canonical and comprise a growing list of pathways including the Wnt/Planar Cell Polarity (PCP) and Wnt/Ca2+ pathways as two of the most intensively studied.

Embryonic stem cells (ESCs) possess several unique features that make them ideal tools for studying development, and for advancing therapies for devastating diseases. We used mouse ESCs (mESCs) lacking components of Wnt/β-catenin signaling pathway to deciphers its role in mESC differentiation into DA neurons. We provide evidence that abrogation of Wnt/β-catenin signaling increases neuroectodermal differentiation and the number of mESC-derived DA neurons.

Non-canonical Wnt signaling promotes DA neurons differentiation and maturation. However, mechanism mediating these effects have not been characterized. Thus, we focused on mechanisms of signaling of Wnt5a, a prototypical non-canonical Wnt ligand, and its functional aspects. We show by analyses of Wnt5a -/- mice embryos the importance of Wnt5a for proper VM morphogenesis and DA neuron differentiation.

We further demonstrate that Wnt5a effects on DA differentiation are mediated via small GTPase Rac1, which is a downstream effector of Wnt5a signaling in DA cells. Subsequently, we examined molecular aspects of the Wnt5a/Rac signaling in closer detail. We found β-arrestin, casein kinase 1, and Tiam1 as novel regulators of Wnt5a-mediated Rac1 activation.

(7)

Human breast epithelial and mesenchymal precursors assemble a functional stem cell niche

Stephan Duss, Heike Brinkhaus, Daniel Frey, Dirk Schaefer, Patrick Ringenbach, Sylvie Thiebault, Mohamed Bentires-Alj

Stem cell/niche interactions are essential for tissue homeostasis by controlling stem cell quiescence and activation. The cellular compartments necessary for self-renewal and quiescence of breast stem/progenitor cells remain speculative. Recent evidence implies that aberrant stem cell/niche interactions are involved in tumor development and progression but the underlying signaling circuitries remain ill defined.

In order to model mammary stem cell/niche interactions, we asked whether primary epithelial stem/progenitor cells and mesenchymal stem cells can assemble a functional entity that maintains the differentiation potential of stem/progenitor cells.

We established methods for the isolation, tagging and propagation of different cell types from the normal and neoplastic human breast. Ex vivo co-culture of breast primary epithelial and mesenchymal precursors allowed long term propagation of mammary epithelial stem/progenitor cells. We therefore conclude that epithelial and mesenchymal precursors are sufficient to assemble a functional breast stem cell niche.

Our tools should allow the identification of signaling networks involved in normal and cancer stem cell/niche interactions and may identify novel therapeutic targets.

(8)

Bioreactor-based engineered models for basic research and clinical translation

Adam Papadimitropoulos, Ivan Martin

The use of bone marrow- or adipose tissue-derived mesenchymal stromal cells (MSC) for treatment of genetic or immunologic pathologies, as well as in the emerging field of regenerative medicine, typically requires cell expansion by sequential passages in monolayer (2D) cultures. The process generally leads to large cell numbers, but is known to be associated with a progressive decrease in early progenitor properties of MSC. In this study, we propose a novel paradigm for the efficient expansion of human MSC within porous, three-dimensional (3D) scaffolds under direct alternate perfusion, which totally bypasses the 2D culture step. The resulting engineered tissues can be either dissociated to recover the expanded MSC for use in cellular therapy, since reproducibly exhibited higher clonogenic properties and more efficient multilineage differentiation capacity as compared to 2D grown cells, or employed directly for clinical uses due to their reproducibly osteogenicity, as assessed by direct ectopic implantation of the 3D scaffold-MSC constructs. Moreover, the MSC-based engineered tissues can be loaded with additional cell types (i.e. osteoclatic progenitors, hematopoietic, endothelial) for the development of advanced 3D in vitro co-culture models to study physiological interactions among several cell types.

In summary, the multifaceted use of bioreactor-based models is relevant not only for the streamlining and standardization of MSC expansion and graft manufacturing, thereby facilitating the translation of bone tissue engineering strategies into the clinic, but also to support the development of multi-cell co-culture models in a controlled 3D environment, as paradigm for engineered stem cell niches.

(9)

Quantitative proteomic (iTRAQ) analysis of 1st trimester maternal plasma samples in pregnancies at risk for preeclampsia

Varaprasad Kolla , Paul Jenö, Suzette Moes, Olav Lapaire, Irene Hoesli, Sinuhe Hahn

A current major obstacle is that no reliable screening markers exist to detect pregnancies at risk for preeclampsia. Quantitative proteomic analysis employing isobaric labelling (iTRAQ) has been suggested to be suitable for the detection of potential plasma biomarkers, a feature we recently verified in analysis of pregnancies with Down syndrome foetuses. We have now examined whether this approach could yield biomarkers to screen pregnancies at risk for preeclampsia. In our study, we used maternal plasma samples obtained at 12 weeks of gestation, six from women who subsequently developed preeclampsia and six with uncomplicated deliveries.

In our analysis, we observed elevations in 10 proteins in the preeclampsia study group when compared to the healthy control group. These proteins included clusterin, fibrinogen, fibronectin, angiotensinogen, increased levels of which are known to be associated with preeclampsia. An elevation in the immune-modulatory molecule, galectin 3 binding protein, was also noted. Our pilot study, therefore, indicates that quantitative proteomic iTRAQ analysis could be a useful tool for the detection of new preeclampsia screening markers.

(10)

PMN-Ectosomes as immunomodulators

Ceylan Eken, Salima Sadallah, Juerg A. Schifferli

Activation of human polymorphonuclear neutrophils leads to the release of vesicles by budding from the cell surface. These vesicles, called ectosomes (PMN-Ect), expose phosphatidylserine in the outer membrane leaflet, a feature they share with apoptotic cells. Of particular interest, PMN-Ect down-modulate human monocyte-derived macrophage and dendritic cell activation occurring via TLR-2 and TLR-4, and induce the release of TGF-β1 by these cells.

In our recent studies, we demonstrated that exposure of human macrophages to PMN-Ect activated directly 3 independent pathways: a PS-mediated activation of the Mer receptor tyrosine kinase pathway, an immediate Ca2+ flux and a rapid release of stored TGF-β1. Interestingly, the effects of PMN-Ect pre-exposure were lasting for 24 hours with the macrophages being less receptive to TLR-2 activation and TGF-β1 stores remaining low.

In sum, PMNs, known to be central in inflammation, release potent anti-inflammatory and immunosuppressive effectors in the form of ectosomes at the earliest stage of inflammation, already provide a drive to its resolution.

(11)

Everything you always wanted to know about wing development but were afraid to ask

Oguz Kanca, Emmanuel Caussinus, Markus Affolter

Wing disc of Drosophila melanogaster is a powerful and well established system to study organogenesis. Throughout the larval life a group of about 30 cells divide and acquire different gene expression signatures to form mature wing disc, consisting of about 50000 cells that correspond to the blueprint of adult wing.

Lack of live imaging and in vitro culture system, together with the complexity of growth patterns and the vast growth rate of the disc make it difficult to reach a complete understanding of wing disc development. In order to provide data about the growth and patterning of wing disc, we developed a method to mark cells through expression of randomly chosen, genetically inheritable fluorescent proteins. The color that is chosen by a cell will be inherited by that cells progeny, thus will provide the fate map of that region. By controlling the time and space of color choice the method can be applied to give in depth analysis of cell division patterns without the need for live imaging. Cell division patterns can be used to infer information about clonality of the disc regions, disc growth characteristics and gene expression dynamics.

(12)

Increasing BDNF levels in the brain using a blood brain barrier-permeable GPCR agonist

Rubén Deogracias, Morteza Yazdani, Martijn Dekkers, Mihai S. Ionescu, Kaspar Vogt, Yves Barde

BDNF plays an important role for normal brain function and is associated with a number of disorders, including Rett Syndrome, an autism-spectrum disorder caused by mutations in the MECP2 gene. As it was previously shown that increasing BDNF levels by genetic manipulation improves the symptoms of MeCP2 mutant mice, we explored the possibility of modulating BDNF expression in the brain using compounds already tested for safety in humans. We used FTY720, a S1P-1 receptor agonist recently approved as the first oral treatment of multiple sclerosis which is able to activate MAPK pathway. While both neurons and astrocytes express the S1P-1 receptor, BDNF levels are increased exclusively in neurons upon FTY720 addition. FTY720 increases spontaneous electrical activity in neuronal cultures, resulting in activation of the ERK1/2 and CREB pathways. In addition, FTY720 prevents NMDA-induced neuronal death through a BDNF-dependent mechanism. Administration of FTY720 in MeCP2 mutant mice leads to a significant increase in BDNF levels in the hippocampus and the striatum and extends their lifespan. Our data suggest then that the modulation of the GPCR S1P-1 may be a useful way to increase endogenous levels of BDNF in the CNS.

(13)

NMR for dummies: What can it do for you ?

Sébastien Morin

In the Biozentrum, two structural biology research groups use Nuclear Magnetic Resonance (NMR) as their main research tool for protein studies. NMR is indeed a very powerful tool for structural biologists. However, it is often seen as a black box for outsiders. In this talk, the basics of solution NMR will be presented, as well as a few examples on the different uses of NMR for protein studies, such as structure determination, quantification of dynamics, and characterisation of interaction surfaces and affinities.

Poster only postdoc presentations

(Numbers used for poster session)

(14)

Deglycosylation of host glycoproteins by C. canimorsus

Francesco Renzi, Pablo Manfredi, Manuela Mally, Loïc Sauteur, Paul Jenoe, Guy R. Cornelis

Capnocytophaga canimorsus is a Gram-negative bacterium which lives as a commensal in dogs oral cavity. Infection upon scratches or bites can cause fatal septicaemia in humans. C. canimorsus is able to feed on cultured mammalian cells, including phagocytes, by harvesting glycan moieties from host cells. Analysis of the genome of C. canimorsus 5, a human strain isolated from a fatal septicaemia, revealed the presence of 13 different putative polysaccharide utilization loci (PULs). Systematic knockout of the 13 PULs revealed that 6 PULs are involved in growth during cell culture infections with most dramatic effect observed for PUL5. Here, we show that C. canimorsus 5 has the capacity to deglycosylate N-linked glycoproteins as human IgG and fetuin and we analyze the mechanism. We show that deglycosylation is achieved by a large complex spanning the outer membrane and consisting of the Gpd proteins and sialidase SiaC. GpdD-G-E-F are surface-exposed outer membrane lipoproteins. GpdD-E-F contribute to the binding of glycoproteins at the bacterial surface while GpdG is a endo-beta-N-acetulglucosaminidase cleaving the N-linked oligosaccharide after the first GlcNAc residue. GpdC, resembling a TonB-dependent OM transporter is presumed to import the oligosaccharide into the periplasm after its cleavage. SiaC is an outer membrane-anchored lipoprotein oriented towards the periplasm. It interacts with GpdC and removes the terminal sialic acid residue of the oligosaccharide. Finally, degradation of the oligosaccharide proceeds sequentially from the desialylated non reducing end by periplasmic exoglycosidases. This work represents the first characterization of a bacterial complex specialized in deglycosylation of N-linked host glycoproteins.

(15)

Translational fidelity as a potential target for new antimicrobials delivery

Anna Chirkova, Dirk Bumann

Infectious diseases represent an incessant threat for the human population worldwide. Fast evolution and extremely adaptive metabolism of bacterial pathogens keep the pace of the race between new antimicrobials development and antibiotic-resistance evolvement.

To address the extremely high demand for novel strategies in antimicrobials development we will re-evaluate known molecular structures involved in protein biosynthesis as potentially new targets. The mechanisms of translational antibiotics action are mostly attributed to the protein biosynthesis block, while their translation accuracy related effects have not been fully exploited.

We believe that manipulation with translational accuracy can have implications in intracellular pathogen propagation during infectious disease. It has demonstrated that levels of erroneous translation rise upon nutrition limitation and have an impact on bacterial growth rate and sensitivity to the innate immune effectors mechanisms. Targeting translational accuracy in Salmonella which is nutrition-limited residing within their vacuole inside a host cell can be a powerful approach to control the infection.

Our aim is to define a quantitative rational of how the protein synthesis accuracy in the intracellular pathogen Salmonella can be compromised by to the extent that invokes pathogen virulence loss.

(16)

Targeting host metabolism to modulate Shigella infection

Olga Timoshenko, Nikki Freed, David Kentner, Petra Tienz, Cécile Arrieumerlou, Dirk Bumann

Intracellular pathogens use resources of their host cell to grow while the host suffers from this metabolic burden. Understanding this complex interaction network will facilitate development of new antimicrobials. Our goal is to provide quantitative system-level description of the battle between host and pathogen for metabolic resources and to identify target/target combinations to combat the infection. Our model system is infection of human cells with bacterial pathogen Shigella flexneri.

Using an array of methods (computer modeling, metabolomics, proteomics, enzyme activity assays) the BattleX project investigates the metabolic state of host cell during Shigella infection. Based on data on host enzymes abundance and activity as well as metabolite profiles and turnover data we selected 74 human enzymes for perturbation using RNA interference. As a readout system we measured Shigella proliferation using sensitive and high throughput methods such as flow cytometry, luminescence assay and time-lapse fluorescent microscopy. Our data showed that overall Shigella growth was rather robust to individual host cell perturbations. However, some of these perturbations redirected metabolite fluxes in the host cell and had a detectable effect on the host-Shigella interface.

We combine these experimental data with quantitative modeling to infer potential combination approaches targeting multiple human enzymes and/or Shigella enzymes that might be more effective to block Shigella growth in infected human host cells. The entry of the human

(17)

Dynamic regulation of alternative splicing by neuronal activity and molecular diversity of synaptic receptors

Harald Witte, Takatoshi Iijima, Karen Wu, Peter Scheiffele

Synapses are highly organized cellular junctions between nerve cells, which underlie transmission and processing of signals in neuronal networks. Transsynaptic cell adhesion molecules, like presynaptic neurexins and their postsynaptic binding partners, the neuroligins, bridge the synaptic cleft between the pre- to postsynaptic sides and play an important role in synaptogenesis. Neurexins and neuroligins are encoded by multiple genes and undergo extensive alternative pre-mRNA splicing, generating in case of the neurexins more than 3’000 potential variants. Importantly, neurexin alternative splicing is assumed to underlie an adhesive code for selective protein interactions at synapses as specific isoforms are characterized by different affinities for neuroligins and other binding partners. The mechanism behind the control of splice isoform choice and the actual extent to which neurexin isoforms are generated on the protein level, however, are not understood.

Here, we investigated the alternative splice choice of neurexins at site 4, which regulates interaction of neurexins with neuroligins and other ligands. We found that depolarization of cultured neurons rapidly and reversibly alters alternative exon inclusion at site 4. This process requires calcium influx via L-type voltage gated calcium cannels (L-VGCC) and calmodulin-dependent protein kinase IV (CaMKIV) activity. Downstream of CaMKIV we identified the KH-domain RNA-binding protein SAM68 as regulator of exon skipping at site 4. In splice reporter assays, SAM68 is sufficient to drive skipping of exon20. Consistently, SAM68 knockout mice exhibit reduced skipping and, thereby, a reduction of neurexin variants lacking exon 20 at site 4. Importantly, depolarization-induced alternative splicing changes in neurexins are abolished in SAM68 knockout neurons. Thus, SAM68 is a key mediator for calcium-dependent regulation for alternative splicing of neurexins and might contribute to the neural activity-dependent assembly and synaptic remodeling in the nervous system.

(18)

Analysis of coronin function in Dictyostelium discoideum

Adrien Vinet, Thomas Fiedler, Romain Froquet, Pierre Cosson, Jean Pieters

The coronin protein family is characterized by a central WD repeat domain linked to a C-terminal coiled coil region. For most of the coronin molecules, the exact in vivo function remains unknown. In mammals, coronin 1, the most conserved coronin isoform, was recently shown to modulate Ca2+ signalling, thereby regulating the Ca2+/calcineurin pathway. Whereas mammalian cells express up to seven isoforms, the lower eukaryote Dictyostelium discoideum expresses a single coronin protein.

In this work, we analyzed a role for coronin in the unicellular amoeba Dictyostelium discoideum, which is a well known model for many essential biological processes. This haploid organism is especially useful for the study of immune cell functions, including chemotaxis and phagocytosis. Under conditions of starvation, Dictyostelium cells aggregate by secreting cAMP, and form a motile slug, which ultimately forms a fruiting body allowing spore dispersal. Our experiments showed that coronin-deficient Dictyostelium cells are defective in chemotaxis toward a cAMP gradient. As a consequence, the subsequent stages of the multicellular formation, i.e. aggregation and development in fruiting bodies, were also affected. Nevertheless, folic acid chemotaxis was not impaired. Furthermore, a phagocytosis defect was observed but turned out to be dependent on the particle type. These results lead us to hypothesize that Dictyostelium coronin is also involved in specific signaling pathways. Interestingly, we observed a hyper-phosphorylation on tyrosine-53 of actin in coronin-deficient cells, which could explain at least part of the observed phenotypes.

The co-expression of several coronin isoforms in mammalian cells may prevent a uniquivocal elucidation of their function; Definition of the function of the single coronin molecule in Dictyostelium may therefore help to better understand the role of the coronin proteins in the eukaryotic kingdom.

(19)

Origin of glia in the Drosophila central brain

Gudrun Viktorin, Nadia Riebli, Anna Popkova, Angela Giangrande, Heinrich Reichert

The neural stem cells that give rise to the neural lineages of the postembryonic brain can generate their progeny directly or through transit amplifying intermediate progenitor cells (IPs). The IP-producing neural stem cells in Drosophila are called type II neuroblasts, and their neural progeny innervate the central complex, an integrative brain center involved in locomotion control. Here we use genetic lineage tracing and clonal analysis to show that the IPs of these type II neuroblast lineages give rise to glial cells as well as neurons during postembryonic brain development. Our data indicate that two main types of IP lineages are generated, namely mixed neuronal/glial lineages and neuronal lineages. Genetic loss-of-function and gain-of-function experiments show that the gcm gene is necessary and sufficient for gliogenesis in these lineages. The IP-derived glial cells, like the IP-derived neuronal cells, make major contributions to the central complex. In postembryonic development, these IP-derived glial cells surround the entire developing central complex neuropile, and once the major compartments of the central complex are formed, they also delimit each of these compartments. During this process, the number of these glial cells in the central complex is increased markedly through local proliferation based on glial cell mitosis. Taken together, these findings uncover a novel and complex form of neurogliogenesis in Drosophila involving transit amplifying intermediate progenitors. Moreover, they indicate that type II neuroblasts are remarkably multipotent neural stem cells that can generate both the neuronal and the glial progeny that make major contributions to one and the same complex brain structure.

(20)

Assessment of targeted mass spectrometric workflows for absolute quantification of low abundant human proteins

Manuel Bauer, Erich A. Nigg, Alexander Schmidt

Recent advances in liquid chromatography (LC)- mass spectrometry (MS) have considerably improved protein quantification accuracy and sample throughput, making it an indispensible tool in system biology studies. Especially, the implementation of targeted MS methods, namely selected reaction monitoring (SRM), allows consistent screening of preselected protein sets with high sensitivity across large number of samples. Due to their non-scanning properties, triple stage quadrupole (QQQ) instruments are usually employed for SRM experiments, however, other MS-instruments can also be successfully used to perform SRM-like analysis.

Here we compared the performance of conventional data-dependent and different directed and targeted MS approaches on two different instruments, namely a Thermo LTQ Orbitrap Velos and a Thermo TSQ Vantage (QQQ). We assessed the limits of quantification (LOQ) and detection (LOD) for each method by analyzing a dilution series of 20 human, heavy labeled reference peptides covering six orders of magnitude in peptide concentration alone or spiked into a digested human cell lysate.

Interestingly, all approaches were capable of quantifying the selected peptides over the whole peptide concentration range when analyzing the peptides alone. However, the dynamic detection range was considerably reduced in the spike-in experiment that represents a more realistic proteome sample. Here, the LOD and LOQ of the targeted approaches were 10 (3) times lower than that of conventional (directed) MS strategies, underlining the improved ability of these approaches in quantifying low abundant protein species. Differences in dynamic range, sensitivity and throughput of the different targeted approached were apparent and will be discussed in more detail together with their applicability for systems biology studies.

With these methods we will be able to assess biological questions involving proteins being expressed at very low levels, like components of the centriole duplication cycle, which were not accessible by previous LC-MS methods.

(21)

Binding avidity and its Implications for transport through the nuclear pore complex

Larisa E. Kapinos, Rafael Schoch, Roderick Y. Lim

Nuclear pore complexes (NPCs) regulate the selective exchange of macromolecular cargoes across the nuclear envelope. Access is limited to cargo-carrying transport receptors (e.g. importinß or impß), which interact with several Phe-Gly (FG) repeat rich domains (i.e. FG-domains) that are end-tethered in and around the NPC. The collective behavior of the FG-domains remains ambiguous. In particular, it is unclear how the FG-domains interact with the transport receptors to optimize NPC cargo translocation. It is known that: (i) each FG-domain is natively unfolded/intrinsically unstructured with multiple FG-repeat motifs, and (ii) each transport receptor has several FG-binding pockets.

The purpose of this work is to understand (1) how FG-domain structure is dependent on surface density and (2) how this can affect receptor-FG binding interactions and consequently a transport through the nuclear pore. By Surface Plasmon Resonance (SPR) experiments we show that impß exhibits different binding affinities to different end-tethered FG-domains (Nup62, Nup98, Nup153 and Nup214). Importantly, the dissociation constant (KD) behaves non-monotonically as a function of the surface grafting density and suggests the existence of an optimal surface density, which maximizes the binding (avidity effect). Using a special approach in our SPR experiments we correlate the binding affinity with the conformational changes of the FG-domain-layer upon impß binding. Our results strongly indicate that the number and localization of each FG-domain in the NPC can play an important role in optimizing nucleocytoplasmic transport efficiency.

(22)

Salmonella heterogeneity in host tissue

Neil Burton, Alexander Schmidt, Kathrin Schemmer, Dirk Bumann

Salmonella in vivo virulence has mostly been studied by bulk analysis of infected tissues. However, our histochemical and immunohistochemical analysis revealed that Salmonella reside in distinct host environments dominated by markedly different cell types and host metabolic activities. To determine the impact of such distinct microenvironments on local Salmonella subpopulations, we have used Salmonella indicator strains, which produce GFP under the control of specific stress responsive promoters, to analyze bacterial heterogeneity within infected host tissue. Using FACS analysis, we observed particularly high variability in GFP production from a nitric oxide (NO) responsive promoter across the population of infecting salmonella. Using confocal analysis of tissue sections from the same mice, we have found that bright green bacteria localize largely to specific tissue regions which stain densely for the host enzyme inducible nitric oxide synthase (iNOS). We have been able to sort and analyze the proteomes of these subpopulations of infecting microorganisms. Three enzymes implicated in NO detoxification and NO damage repair were present in large excess in the bright green salmonella, consistent with expectations.

(23)

Interactions of the GPCR CCR5 with HIV, chemokines, and inhibitors

Sébastien Morin, Lydia Nisius, Fabian Kebbel, Hans Juergen Sass, Stephan Grzesiek

The entry of the human immunodeficiency virus 1 (HIV-1) into host cells usually requires the sequential interaction of the viral envelope glycoprotein 120 (gp120) with the host-cell factor CD4 and with either CCR5 (CC chemokine receptor 5) or CXCR4, both G-protein coupled receptors (GPCR). This leads to the fusion of viral and host cell membranes. The normal physiological role of CCR5, however, is the regulation of immune-cell trafficking upon activation by its endogenous ligands: macrophage inflammatory protein 1a (MIP-1a), MIP-1b and RANTES (Regulated on Activation, Normal T-cell Expressed and Secreted). Since both, viral gp120 and the chemokines bind to the extracellular parts of the receptor, binding of a natural ligand, e.g. RANTES, obstructs the interaction of CCR5 with the viral protein, thereby hindering HIV infection. This makes RANTES and other chemokines potential lead structures for novel anti-HIV agents.

Here, we present advances on the study of the interaction of CCR5 with HIV gp120, RANTES variants, and the small-molecule inhibitor maraviroc. Surface plasmon resonance (SPR), as well as nuclear magnetic resonance (NMR), and electron microscopy (EM) were used. Results are discussed in the context of in vivo and in vitro data, as well as with the help of a CCR5 3D homology model based on the recently-solved crystal structure of CXCR4.

Structural and mechanistic data on the interaction partners of CCR5 have the potential to contribute to the rational design of future anti-HIV agents.

(24)

Automated and Real-Time Monitoring of Cell Migration, Cell Invasion and Cell Scattering Using the xCELLigenceSystem from Roche Applied Science

Roche Diagnostics (Schweiz) AG

In this poster we will discuss the application of xCELLigencesystem for dynamic monitoring of cell migration and cell invasion. The xCELLigencesystem from Roche Applied Science (RAS) utilises non-invasive microelectronic readout to monitor cellular status in real-time and under label-free conditions. RAS recently introduced the Cell Invasion and Migration (CIM) device, which is a modified Boyden Chamber device, integrated with gold micro-electrodes and can be used to monitor the directional movement of cells in response to chemotactic factors.

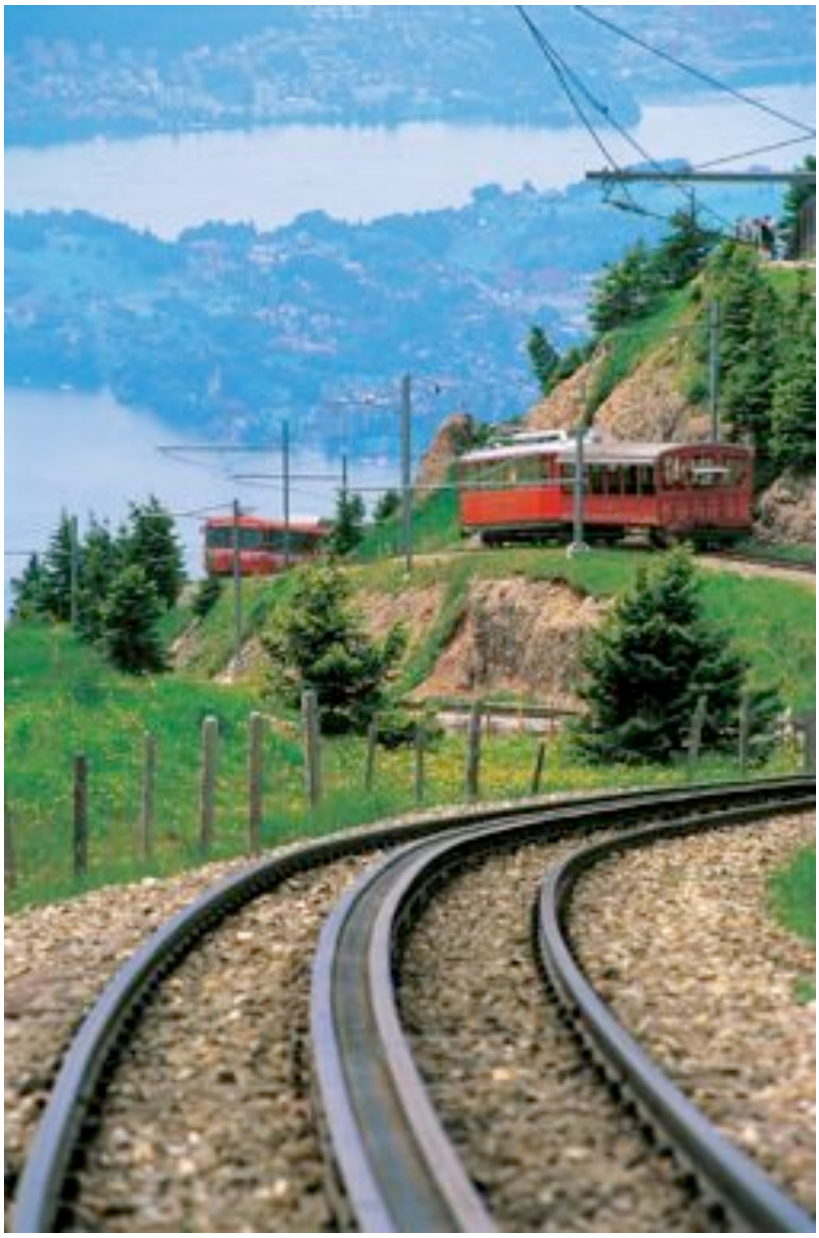
We will demonstrate the utility of this device for migration and invasion of various cancer cell lines as well as migration of endothelial cells. Furthermore, we will also show dose-response inhibition of migration using small molecule inhibitors of tyrosine kinases and siRNAs targeting key signalling proteins involved in migration and invasion. Finally, we will also demonstrate how the xCELLigencesystem can be used to assess cell scattering in the context of epithelial-mesenchymal transition (EMT).

Activities on site

During the spare time following the poster session (day 1), we propose going to Mount Rigi where a splendid view of the area surrounding Weggis can be admired. We propose taking a boat and then a train up to the top of Mount Rigi, and then walk down to the cable car which will bring us back to Weggis. On top, it is possible to walk around and/or have a drink or meal. The meeting point with one of the retreat organizers will be in the hotel lobby and departure will be at 15h30 on day 1. The activity is not covered by the registration fees and should cost 20-30 CHF (providing you own a Halbtax card).

Other activities are possible such as walking along the lake shore, taking a boat, or going to Luzern and enjoy the city and its museums.

The train to Mount Rigi



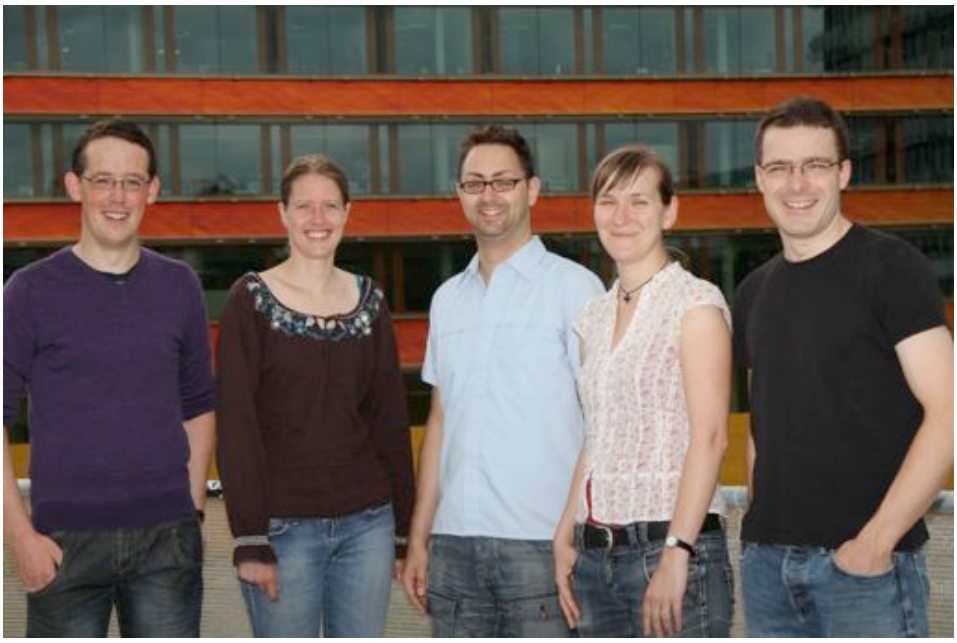
Getting involved !

The postdoc retreat and other activities organized by the Biozentrum Postdoc Club would not be possible without a team of dedicated volunteers.

If you would like to get involved in any activity, please don't hesitate to contact us (biozentrumpostdocclub@gmail.com) !

Biozentrum PostDoc Club

The Postdoc Club Committee



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sgAHE	Swiss Society for Anatomy, Histology, and Embryology
BIO	Swiss Society for Biochemistry / Section Biophysics
PHA	Swiss Society for Pharmacology and Toxicology (SSPT)
PHY	Swiss Physiological Society
SGO-SSO	Swiss Society for Oncology
ZMG	Swiss Society for Cell Biology, Molecular Biology and Genetics
STSBC	Società Ticinese delle Scienze Biomediche e Chimiche (STSBC)

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SGM	Swiss Society for Microbiology
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SGPf	Schweiz. Gesellschaft für Pflanzenphyiologie
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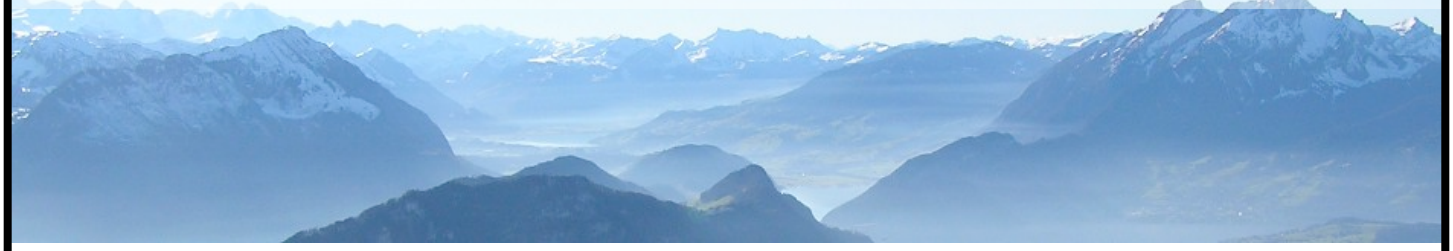

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