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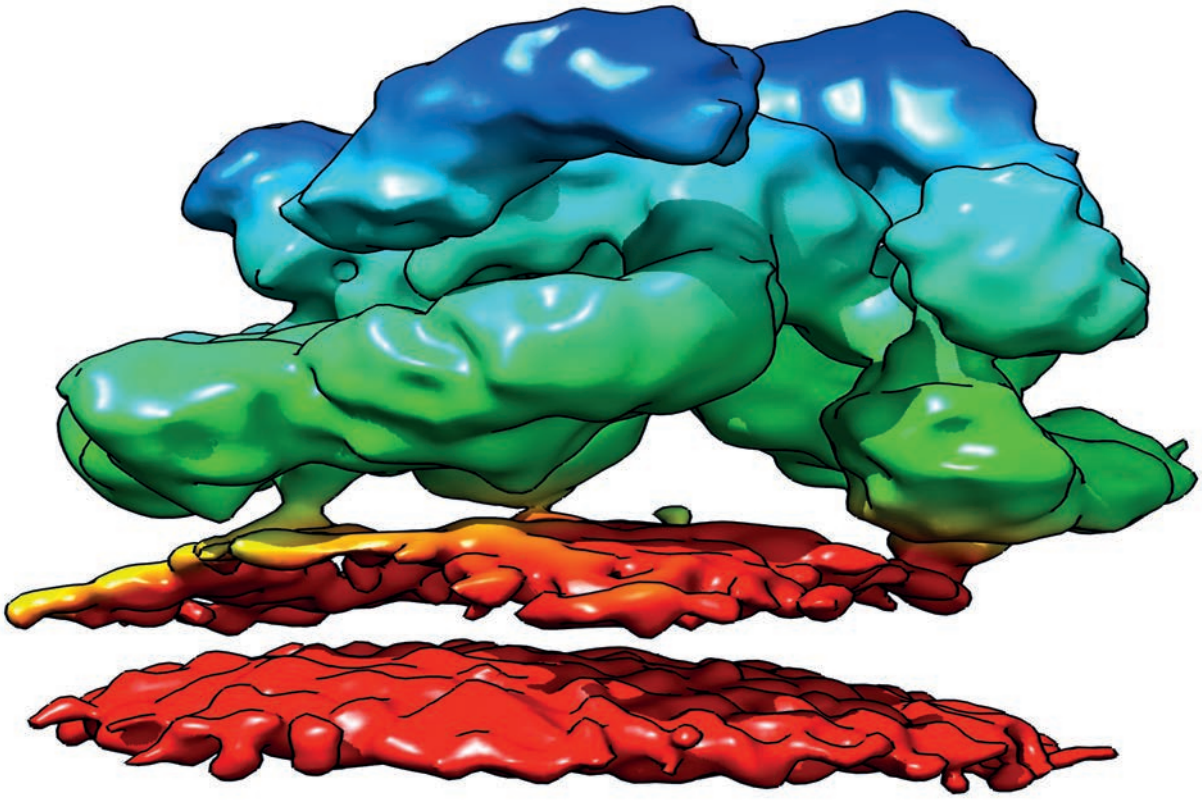
DER UNIVERSITÄT HEIDELBERG



RESEARCH AND EDUCATION IN MOLECULAR LIFE SCIENCES

HEIDELBERG UNIVERSITY BIOCHEMISTRY CENTER
REPORT 2014-2016

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Cover: Structural detail of the coat of a COPI vesicle
Photo: Portrait p. 38: DFG/Ausserhofer

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Welcome to the BZH!

Our primary pursuit at the Heidelberg University Biochemistry Center (BZH) is to bring together enthusiastic and innovative minds from both students, whose thoughts are not restricted by dogma, and from experienced scientists that have made major contributions to their fields. We strive to create a dynamic environment for intensive information exchange/discussions where technical limitations are challenged and collaborations are encouraged. At the BZH, researchers are provided with state-of-the-art technology platforms and minimized administrative duties. Since its foundation in 1997 the BZH has developed into a leading interdisciplinary research institute in the area of molecular life sciences. Currently, the BZH is home to 14 research groups, including 4 junior groups, housing in total about 200 scientific and nonscientific coworkers. Its scientists are associated with three different faculties - Biosciences, Chemistry and Earth Sciences, and the Medical Faculty - covering different research fields, but sharing one major common mission: reaching a detailed mechanistic and structural understanding of molecular machines.

The macromolecular machines studied at the BZH fulfill a broad range of functions, ranging from basic cellular processes, like protein translation that generate a constant supply of new proteins to superordinate processes at the organismal level, like vascular/neuronal links that mediate the proper innervation and blood supply to the entire organism. Overall, the macromolecular assemblies and analyzed processes include: chromosome segregation by kinetochores; mRNA expression and translocation; biogenesis of ribosomes; organization of the nuclear pore; membrane protein synthesis and insertion into membranes; modulation of protein activity by specific lipids; membrane trafficking, covering transport vesicle budding and fusion; unconventional protein secretion; protein evolution in biological networks; and unique redox processes in disease-causing Trypanosomes.

In order to study these molecular machines, the research groups employ/combine a broad variety of techniques allowing the dissection of large protein assemblies into individual components followed by the reconstitution of functional subcomplexes or of the entire machine, revealing the composition and mechanistic contributions of specific entities/building blocks. These methods include biochemistry, cell biology, lipidomics, proteomics, and structural approaches like electron microscopy, SAXS, and X-ray crystallography to obtain structural information at atomic resolutions. Remarkably, many state-of-the-art techniques have been established at the BZH in the form of facilities/platforms including protein mass spectrometry (Johannes Lechner), lipidomics (Britta Brügger), light/fluorescence microscopy (Carmen Ruiz de Almodóvar), electron microscopy (Dirk Flemming), and a protein crystallization platform (Irmgard Sinning and Jürgen Kopp). This advanced methodological infrastructure builds an excellent hub for individual and joint research projects. In the long run, the BZH would like to further develop/expand its structural expertise by establishing cryo-electron microscopy.

The BZH provides a competitive research environment, renown on a national and international level. Several research consortia funded by the DFG are led by BZH scientists. The research-training group GRK 1188 (Walter Nickel) “Quantitative analysis of dynamic processes in membrane transport and translocation” has successfully completed the maximum of two funding periods in September 2015. At the end of December 2015, the SFB 638 (Felix Wieland) has completed with a very positive evaluation its last

funding period after studying “dynamics of macromolecular assemblies in biosynthetic transport” for a total of 24 years (including the previous SFB 352). The SFB/TRR 83 (Thomas Söllner) analyzing the “molecular architecture and cellular functions of lipid/protein assemblies” bringing together scientists from Heidelberg, Bonn, and Dresden, is now in its second funding period. The new SFB/TRR 186 (Walter Nickel), that started its first funding period in July 2016, includes researchers from both Berlin and Heidelberg who investigate the “molecular switches in the spatio-temporal control of cellular signal transmission". Overall, 70% of the scientists receive extramural funding from various sources including an ERC grant (Carmen Ruiz de Almodóvar). Concomitantly, with the establishment of the SFB/TRR 186 a new junior research group, co-funded by the Heidelberg University, the DKFZ and EMBL for 5 years has been established at the BZH and the new group leader (Doris Höglinger) will start in spring 2017. The former junior group leader Tamás Fischer was recruited as an Associate Professor to the National Australia University in Canberra in July 2016. In 2014, a new professorship was established at the BZH with Britta Brügger becoming full professor (W3) for Biochemistry/Chemical Biology. In 2014, Irmi Sinning received the Leibniz prize for her contributions in structural biology and in 2016 she became a member of the Heidelberg Academy of Sciences and Humanities. In 2015, the Eduard Buchner prize was awarded to Ed Hurt and in the same year Britta Brügger received the Walter Shaw young investigator award in lipid research. Thus, thriving research at the BZH has become a seeding point for successful career development.

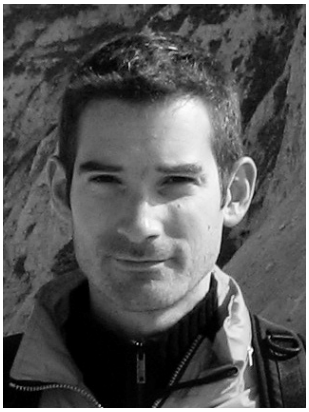
Research at the BZH is complemented by comprehensive teaching activities (coordinated by Cordula Harter) in three faculties. Annually, more than 1000 students receive theoretical training in Chemistry, Biology and Medicine and learn how to plan and conduct experiments in wet labs. The Bachelor Program in Biochemistry at the BZH, which started in 2012 is jointly offered by the Faculties of Chemistry and Biosciences and has been expanded by a consecutive Master program in 2015. Britta Brügger serves as Dean of Studies and Rainer Beck serves as the study coordinator of these programs. In addition, approximately 70 graduate students conduct their PhD thesis at the BZH, learning and applying state of the art technologies. The PhD students form an active community and in 2016 they have started to initiate an annual 2-3 day PhD student retreat organized by themselves, including the invitation of guest speakers, research group presentations, round table discussions, and social events at an external location. All graduate students participate in programs of HBIGS, an international graduate school supported by the German Excellence Initiative, or in the internal graduating program of the BZH.

All of the above activities would not be possible without highly motivated and enthusiastic coworkers providing a broad spectrum of services such as caretaker, dishwashing, technical assistance, teaching, administration, and core facilities all supporting the actual research. These colleagues generate a very productive, inspiring and pleasant working atmosphere on a daily basis, shaping the BZH and building the cornerstone to ensure and further improve excellence in teaching and research in the future.

I hope this brochure captures your attention and inspires your interest in our activities in research and teaching at the BZH.

Prof. Dr. Thomas Söllner

Director, BZH Heidelberg, 31.12.16



2000 - 2006	Ph.D. – BZH
2006 - 2007	PostDoc – BZH
2007 - 2008	Lab head – Novartis Pharma AG, Basel, Switzerland
2008 - 2013	PostDoc – Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland
since 2013	Junior Group Leader – Excellence Cluster “CellNetworks”, BZH

Julien Béthune

Interplays between endomembranes, RNA-binding proteins and cellular polarization

Goal

We are interested in the mechanisms of post-transcriptional gene silencing by miRNAs, and their interplays with endomembranes. This includes the development of new techniques for the identification of protein-protein interactions. We are also characterizing the function of COPI transport vesicles during the differentiation of pluripotent cells using CRISPR/Cas9-mediated genome editing.

Background

In the past decade, microRNAs (miRNAs) have emerged as key regulators of most cellular functions by potentially post-transcriptionally repressing at least 50% of expressed mRNAs. Not surprisingly, misregulation of miRNAs leads to diseases including cancer. Argonaute (Ago) proteins are the central players in miRNA-mediated silencing, using miRNA as guide to find their mRNA targets. Interestingly, Ago largely associates with endomembranes, notably with the endoplasmic reticulum. Data from different laboratories using various model systems suggest that membrane-associated Ago have specific functions that we try to characterize. Membrane-enclosed transport vesicles mediate protein and lipid transport within the secretory pathway. In fungi, co-transport of mRNAs and endomembranes was shown to regulate cell polarization. More generally, in higher eukaryotes, a functional role of the early secretory pathway, notably COPI vesicles, during polarization events such as neurogenesis has been suggested but is still hardly studied.

Research Highlights

Novel proteomics approaches for the analysis of specific functional complexes

To identify additional regulating factors of the miRNA-mediated silencing pathway, we have performed BioID on the Ago2 protein. BioID is a proximity-dependent labeling technique that leads to the biotinylation of proximal proteins within live cells. Proteomics analysis revealed that, when compared to co-immunoprecipitation (co-IP) approaches, BioID identified numerous known interacting proteins that are required for miRNA-mediated silencing but were absent in co-IP. Like many other proteins, Ago2 is part of several protein complexes corresponding to distinct functional steps of the miRNA pathway. To address the general challenge of assigning novel identified protein-protein interactions to specific functional protein complexes, we have developed a split-BioID assay that allows the conditional bi-

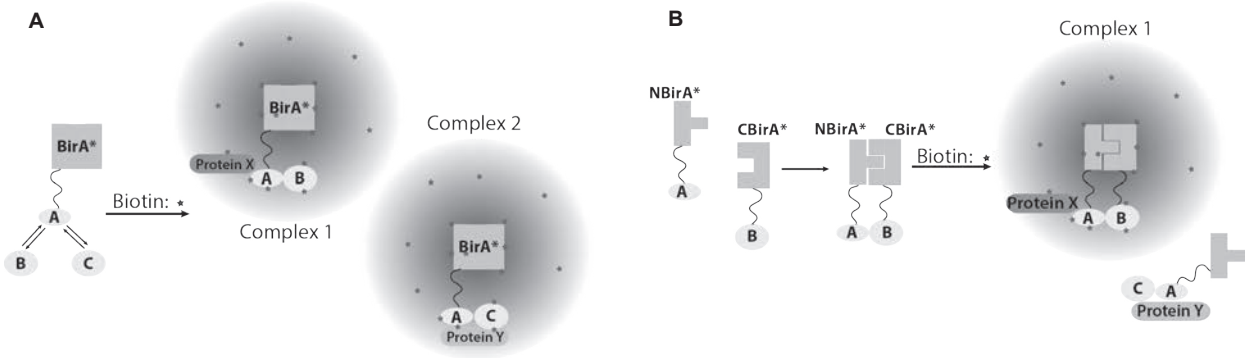


Figure 1: Split-BioID allows probing the proteome of spatiotemporally defined protein complexes, A: *Classical BioID applied to protein A that is part of two distinct complexes when interacting with protein B or C. Both protein complexes are labeled.* ***B:*** *Split-BioID is only activated upon interaction of proteins A and B, allowing conditional proteomics of complex 1.*

otinylation of proximal proteins upon the interactions of two known interacting partners (Fig. 1). With this assay we could successfully probe the proteomes of two functionally distinct protein complexes of which Ago2 is part of (Schopp et al., submitted). These combined approaches led to the identification of novel regulating factors of the miRNA pathway that we are currently characterizing.

Functional characterization of ER-associated Ago2

To understand the functional roles of membrane-associated Ago2, we make use of a mouse embryonic stem cell line in which a knockout of all four Ago proteins is inducible. Using a PiggyBac transposon-based system, we are generating rescue cell lines with various variant of Ago2 that are trapped to different endomembranes. The phenotypes of the rescue cell lines will then be analyzed and help us understanding why Ago2 associate with endomembranes.

Function of COPI-coated vesicles during stem cell differentiation

To investigate a proposed functional role of Golgi-derived vesicles during neurogenesis, we have used murine pluripotent cell lines as a model system for cellular differentiation. Using the CRISPR/Cas9 approach, we have knocked out the γ1-COP or γ2-COP coatomer paralog subunits. We found that removal of γ1-COP leads to defective embryoid bodies formation, which is the first step of differentiation. As a consequence, neurite extension is then later strongly impaired. These data are the first evidence for a paralog specific function of the COPI pathway (see report Wieland group) and suggests that COPIγ1 is critical during early steps of stem cell differentiation. We are currently characterizing the molecular mechanisms underlying this observation.

Selected Publications

Schopp, I., Amaya Ramirez, C., Kreibich, E., Skribbe, M., Wild, K., and Béthune, J. (2016). Split-BioID: a conditional proteomics approach to monitor the composition of spatiotemporally defined protein complexes. Submitted

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Julien Béthune

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1998	Ph.D. - University of Frankfurt, Germany
1998 - 2000	PostDoc - Memorial Sloan Kettering Cancer Center, New York, USA (Prof. James E. Rothman)
2000 - 2002	PostDoc - BZH (Prof. Felix Wieland)
2002 - 2014	Research Scientist - BZH (Prof. Felix Wieland)
Since 2014	Professor for Biochemistry / Chemical Biology, BZH

Britta Brügger

Roles of lipids in biological systems

Goal

We aim to elucidate roles of lipids in modulating activities of proteins, including enzymes, receptors and proteins involved in transport processes. To study protein-lipid interactions and to understand the functional consequences of these interactions we follow an interdisciplinary approach combining cell biology, chemical biology, biochemistry and lipidomics.

Background

Lipids serve multiple tasks within cells, ranging from structural to signalling functions. Hydrophobic by nature lipids usually appear within cells as integral parts of cellular membranes and storage organelles or associated with cytosolic carriers such as lipid transfer proteins.

As opposed to the classical view of membrane lipids being solely structural components to build a lipid mono- or bilayer and to serve as solvent for membrane proteins, we now appreciate that lipids fulfil multiple roles beyond these functions. This is reflected by the complexity of individual lipid species present in a membrane, with hundreds of different species from at least ten different lipid classes typically present in a mammalian membrane. Although lipids are continuously exchanged between subcellular organelles, mediated by vesicular carriers, transfer proteins or membrane contact sites, there must be regimens to ensure that each cellular compartment maintains its specific lipid profile. To achieve this, alterations in membrane lipid profiles must be sensed, evaluated and appropriate responses have to be initiated. Changes of membrane lipid compositions can be global, organelle-specific or restricted to a hotspot within a membrane, and can occur as an adaptation to environmental extrinsic or intrinsic signals, such as circadian rhythmic, cell cycle, signalling cascades, vesicle biogenesis and consumption, to name a few. To understand the consequences of lipid alterations within membranes we focus on elucidating structural and functional aspects of interactions of lipids with proteins within membranes. To achieve this, we follow an interdisciplinary approach, combining cell biology with chemical biology, biochemistry and lipidomics (Figure 1).

Our interest in protein-lipid interactions was raised by our observation that N-stearoyl-sphingomyelin is enriched in COPI vesicles, while the bulk of sphingomyelins is segregated. To understand the molecular basis of this enrichment, we employed functionalized lipids that can be used to identify interacting proteins within cells. Using this approach, we identified p24, one of the key

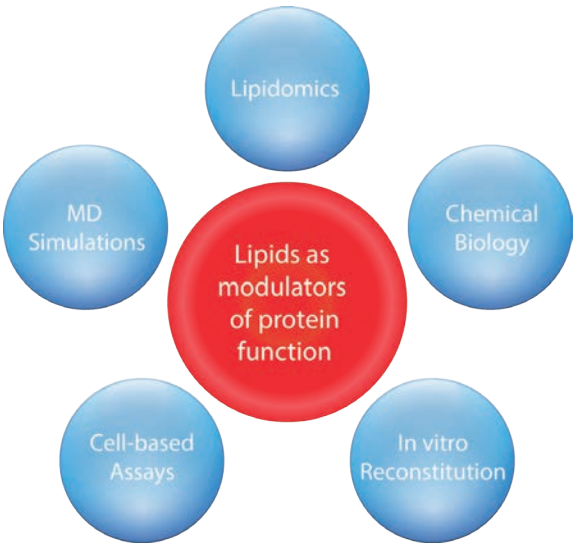


Fig. 1: Analysis of protein-lipid interactions within membranes. *To elucidate if and how lipids interact with proteins, we employ cellular, in vitro and in silico approaches.*

players in COPI vesicle biogenesis, to specifically interact with a sphingolipid. Combining different cellular and FRET-based in vitro reconstitution assays, we identified N-stearoyl-sphingomyelin as the preferred lipid interacting with p24. As a consequence of this specific protein-lipid interaction the formation of active p24 dimers is facilitated (Fig. 2).

Research Highlights

Protein-lipid interactions

Based on our initial studies on the presence of a sphingolipid-binding motif in single-spanning transmembrane proteins, we extended the analyses towards multi-spanning transmembrane proteins. Together with Gunnar von Heijne a motif-probability algorithm was established for large datasets to evaluate customized amino acid motifs in transmembrane helices. With this approach we identified and validated a number of proteins that contain the putative sphingolipid-binding motif in single- and multi-spanning transmembrane proteins. Among the candidate proteins, G-protein coupled receptors (GPCRs) were significantly enriched, suggesting a role of sphingolipids in modulating GPCR function. For one member of this group, a metabotropic glutamate receptor, in collaboration with Irmi Sinning we had shown a coupling of activity with localisation to cholesterol-enriched membranes. Based on these previous studies, we have now identified variants compromised in lipid binding. These variants are now investigated to understand structural and functional consequences of compromised lipid binding.

In case of EBOLA virus, we could unravel a role for a specific interaction of the viral membrane protein GP2 with cholesterol in conferring membrane activity, an important function both in viral entry and exit that might point towards novel therapeutic strategies for EBOLA infections.

To allow for an unbiased identification of sphingolipid-interacting proteins we synthesised a novel

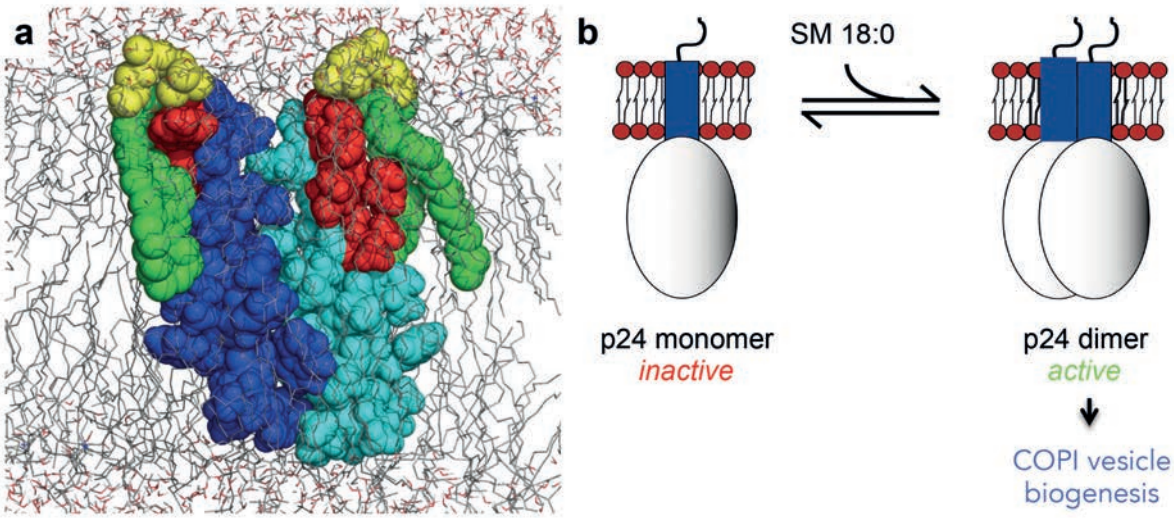


Fig. 2: A specific interaction of the membrane protein p24 with a single sphingomyelin species facilitates formation of p24 dimers. a, *A molecular dynamics simulation of a homodimer interacting with two molecules of N-stearoyl-sphingomyelin (Erik Lindahl, Stockholm).* ***b,*** *Interaction of N-stearoyl-sphingomyelin with p24 modulates its function by facilitating formation of dimers. Dimeric p24 serves as platform for the recruitment of cytosolic COPI coat components to the membrane, a first step in the formation of COPI vesicles.*

sphingolipid tool, a photoactivatable and clickable sphingosine (with Per Haberkant and Carsten Schultz, EMBL). This bifunctional sphingosine is metabolised in cells to bifunctional sphingolipids but its degradation via sphingosine-1-phosphate also generates bifunctional glycerolipids. To achieve specific labelling of only sphingolipids we employed a CRISP/Cas9 approach to knock-out the enzyme sphingosine-1-phosphate lyase in different cell lines, including fibroblast, T cells and lung epithelial cells. As additional tools, we also established the cellular synthesis of a clickable and photoactivatable phosphatidylcholine. Together with bifunctional cholesterol, we can now target the major membrane lipids.

One important and experimentally challenging tool is the mass spectrometric analysis of protein-lipid crosslink products. Here we have succeeded in establishing a method to identify crosslinked molecules via MALDI-TOF. In addition, we have established a protocol for mass spectrometry-reporter based identification of clicked lipid species and of crosslinked protein-lipid adducts by nano-electrospray ionisation mass spectrometry.

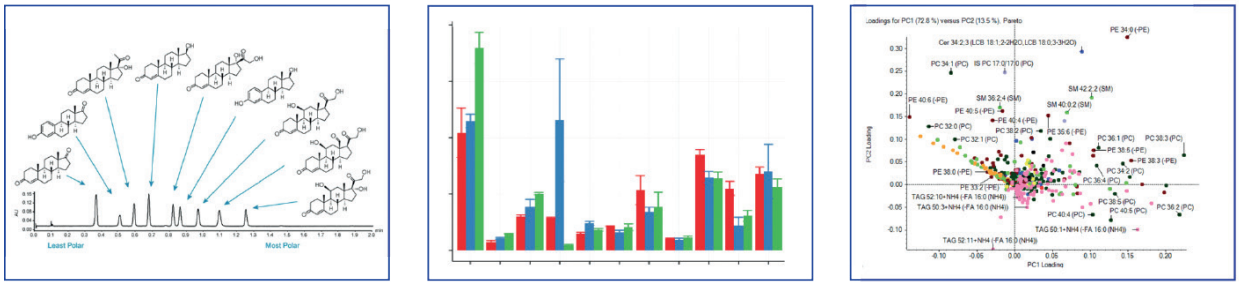
To extend the experimental approach for the identification of protein-sphingolipid interactions to those specific for a given sphingolipid species, we have generated by CRIPR/Cas9 a set of ceramide synthase knockout cell lines. Lipidomics analysis of these cells showed that we successfully manipulated the sphingolipid species profiles of these cells. A SILAC proteomics approach in combination with labelling of cells with bifunctional sphingolipids identified a set of proteins interacting with very-long chain sphingolipids. We now focus on validation and functional characterization of a subset of these proteins.

Lipidomics

The second focus of our group is lipidomics, with the aim to define lipid classes including their molecular species at a quantitative level. Lipidomics has experienced rapid progress in recent years, mainly because of continuous technical advances, now providing quantitative lipid analysis with an unprecedented level of sensitivity and precision. The growing category 'lipid' includes a broad diversity of chemical structures with a wide range of physical-chemical properties. Reflecting this diversity, we apply different methods and strategies to quantify lipids (see also the report on the Lipidomics platform). In order to facilitate and accelerate data evaluation we have developed an R-based application for data analysis, including visualisation, exploration and statistical analyses of big data sets (Fig. 3).

With support of the cluster of excellence CellNetworks Heidelberg we have expanded the lipidomics platform, now also including ion mobility as well as supercritical fluid chromatography.

In the last three years, we have extended our analysis towards minor species, including lipid second messengers such as sphingosine and sphingosine1-phosphate and complex glycosphingolipids such as gangliosides. As



Separation & Identification | Evaluation & Quantification | Exploration & Mapping

Fig. 3: A lipidomics workflow from mass spectrometry to data evaluation. *Following extractions with organic solvents to recover lipids from biological samples, mass spectrometric analysis is performed. Data sets are then analysed, explored and subjected to statistical interpretations.*

for the separation of structural isomers, we do have promising preliminary data using a novel differential ion mobility setup. We are currently exploring this approach for the analysis of phosphoinositide isomers.

Besides our own work and collaborations on Heidelberg Campus, we continued to perform analyses for external national and international collaborators.

In one of these collaborations we investigated alterations in the cardiolipin homeostasis in Barth Syndrome, a cardiomyopathy caused by loss of tafazzin (Peter Rehling, Göttingen). Tafazzin is a mitochondrial acyltransferase, which is required for remodelling-based maturation of cardiolipin species. In a mouse model severe structural changes of respiratory chain supercomplexes at a pre-onset stage of the disease were found. In addition, we could show that the enzymatic block in cardiolipin maturation in mice led to a shift in cardiolipin species distributions and an increase of the cardiolipin precursor monolysocardiolipin, with both effects being strictly tissue-dependent. However, the molecular mechanisms linking defects in respiratory chain complexes to specific cardiolipin species are not yet understood and subject of on-going analyses.

With work for the group of Yves Rouille (Pasteur Institute, Lille) we expanded our analyses of viral lipid envelopes to bovine viral diarrhea virus (flaviviridae family) that is characterised by a lipid composition, which is significantly distinct from the site of virus budding, the endoplasmic reticulum. These data suggest extensive lipid sorting prior to/during budding of progeny viral particles.

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Awards and Honors

2015 Walter A. Shaw
Young Investigator Award
in Lipid Research

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1988	Ph.D. - Heidelberg University, Germany
1989 - 1991	PostDoc - Princeton University and Rockefeller Research Laboratory, New York, USA (Prof. James E. Rothman)
1992 - 1998	Group Leader and habilitation - Ludwig-Maximilians-Universität München, Germany (Prof. Walter Neupert)
1998 - 2000	Professor - Ludwig-Maximilians-Universität München, Germany
since 2000	Full Professor - BZH
2010 - 2013	Director - BZH

Michael Brunner

Molecular Clocks

Goal

Circadian clocks are timekeeping devices that measure time on a molecular level and coordinate the temporal organization of global gene expression. The endogenous cell-autonomous pacemakers are synchronized via various signal transduction pathways with the exogenous geophysical 24 h day/night cycle. The molecular mechanisms underlying these phenomena are in the focus of our research.

Background

Circadian clocks are cell-autonomous oscillatory systems that modulate rhythmic expression of a large number of genes. In eukaryotes these clocks are based on networks of interconnected transcriptional, translational and posttranslational feedback loops. Circadian clocks are synchronized with the exogenous day by environmental cues such as light and temperature. In the absence of entraining cues clock-specific oscillations persist with an intriguingly precise period that corresponds to an endogenous robust self-sustained subjective day-night rhythm of approximately 24 h.

Research Highlights

Dawn- and dusk-phased circadian transcription rhythms in *Neurospora crassa* coordinate anabolic and catabolic functions

The transcription factor White Collar Complex (WCC) is at the core of the circadian clock of the filamentous fungus *Neurospora crassa*. WCC activates directly and indirectly activates transcription of clock-controlled genes (*ccgs*). Amongst the genes directly controlled by the WCC is the clock gene *frequency* (*frq*). FRQ is a circadian co-repressor that inhibits its own synthesis in a negative feedback loop by regulating the activity and abundance of the WCC in rhythmic fashion. Several hundred genes were found to be under the direct control of the WCC. Amongst these rhythmic genes are about 30 genes encoding transcription regulators (e.g. the transcriptional repressor CSP1), which themselves control the expression of subsets of genes. This way, the *Neurospora* circadian clock modulates transcription of ~10% of the genome, which in turn results in oscillations of physiology and metabolism.

The vast majority of these transcript rhythms are generated by dawn and dusk specific transcription. Analysis of the clock-controlled transcriptome of *Neurospora crassa* together with temporal profiles of elongating RNA polymerase II indicates that transcription contributes to the rhythmic

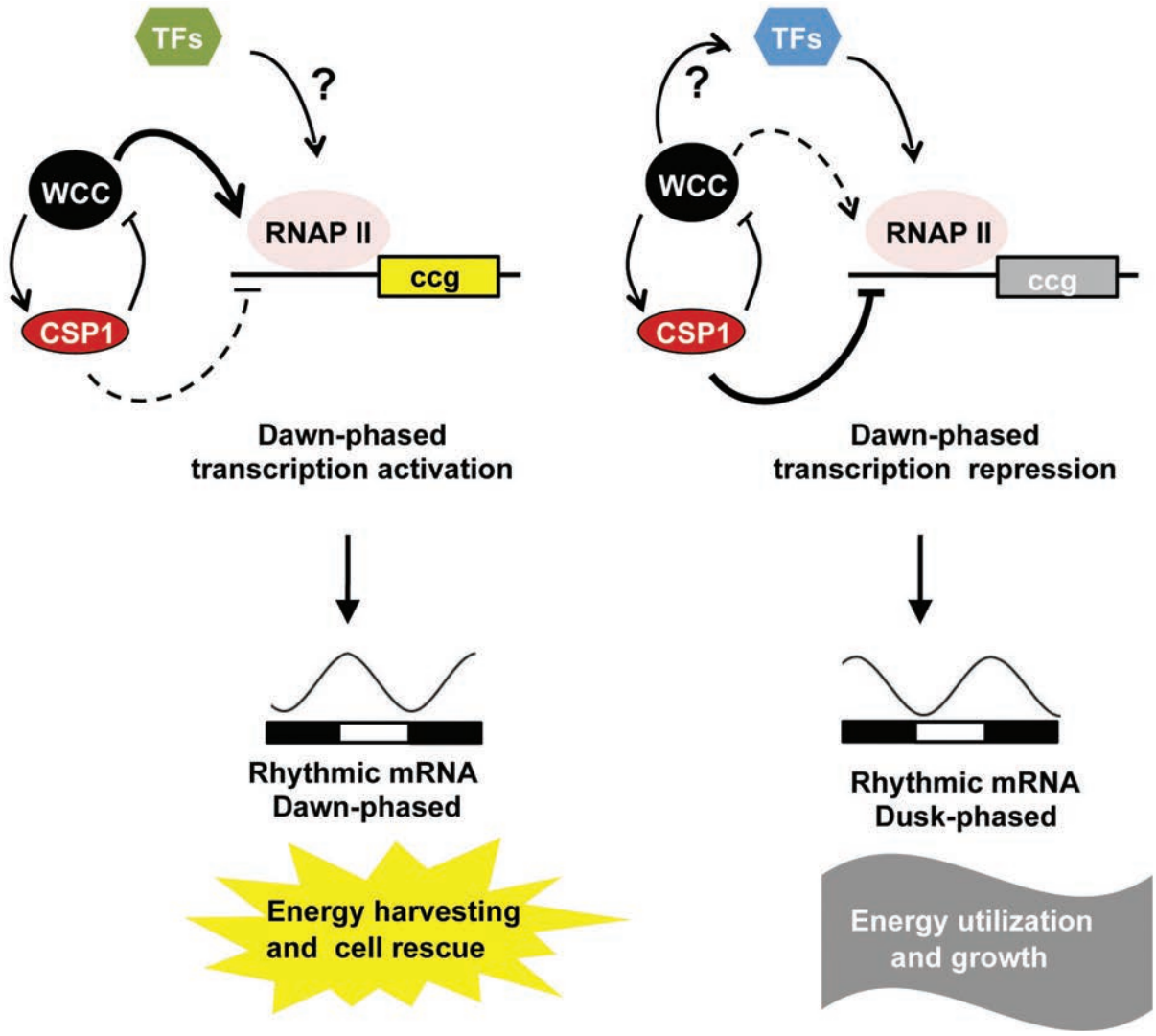

Fig. 1: Model of dawn- and dusk-phased transcription regulation. *The WCC, the core transcription factor of Genes activated by the WCC are transcribed predominantly during subjective late night to early morning where the activity of WCC is high. The activity of other transcription factors (TFs) including CSP1 appear to modulate phase and amplitude of dawn-phased genes. Dawn-specific genes are mainly involved in energy harvesting and cell rescue. The phases of genes repressed by CSP1 are clustered around dusk. Yet, unidentified TFs must be involved in rhythmic activation of these genes since phase and amplitude, but not the rhythmic expression of tested genes, were affected in Δcsp1. Our data suggest that expression of such TFs could be under the control of WCC. Dusk-phased genes are involved in energy utilization and growth, indicating a temporal distinction of cellular pathways and functions coordinated by the circadian clock of* Neurospora.

expression of the vast majority of ccgs. The ccgs accumulate in two main clusters with peak transcription and expression levels either at dawn or dusk. Dawn-phased genes are predominantly involved in catabolic and dusk-phased genes in anabolic processes, indicating a clock-controlled temporal separation of the physiology of *Neurospora*. Genes whose expression is strongly dependent on the core circadian activator WCC fall mainly into the dawn-phased cluster while rhythmic genes regulated by the glucose-dependent repressor CSP1 fall predominantly into the dusk-phased cluster. Surprisingly, the number of rhythmic transcripts increases about twofold in the absence of CSP1, indicating that rhythmic expression of many genes is attenuated by the activity of CSP1.

Transcription induced inactivation of promoters/transcriptional memory

mRNA transcripts are often present at only a few copies per cell and many genes are transcribed in bursts, with brief periods of high activity interspersed by long periods of inactivity. Burst size, i.e. the number of transcripts per burst, and burst frequency, i.e. the number of transcriptional bursts per time unit, are gene-specific and appear to depend on the promoter architecture.

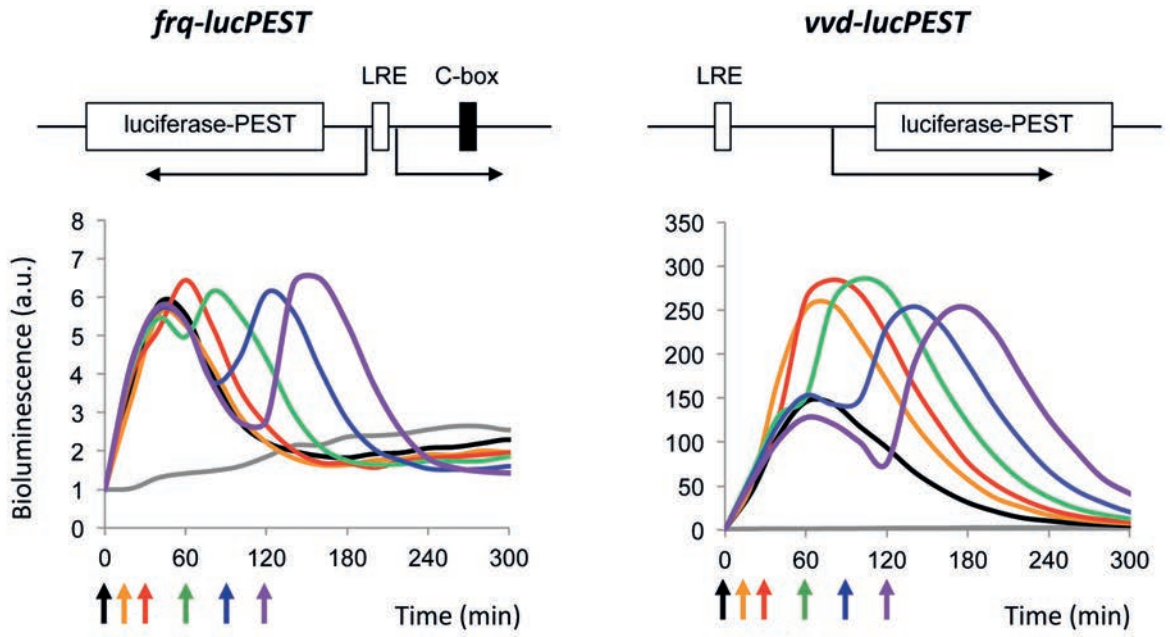

Fig. 2: The frq promoter is refractory towards restimulation. *Activation frq and vvd promoters by a single LP at t=0 min (black arrow, black curve) or restimulation by a 2nd challenging LP after 15, 30, 60, 90, and 120 min (colored arrows and curves). The data indicate that frq is refractory towards restimulation for ca. 45 min, while the vvd promoter is not under the same conditions.*

We use the natural light-inducible gene expression system based on the transcription activator and blue-light photoreceptor White Collar Complex (WCC) of *Neurospora*. The system allows repetitive stimulation of transcription within a short period of time. Activation of WCC by a single short light pulse (LP) triggers a synchronized wave of transcription at a large number of promoters. We have observed burst sizes between one and more than 50 transcripts per burst followed by periods of inactivity in the range of hours.

Challenging the *frequency* (*frq*) promoter with consecutive light pulses revealed that the promoter supports transcription of ~1 mRNA molecule and then becomes refractory towards further activation for about 45 min. This negative transcriptional memory is dependent on slow chromatin remodelling of the core promoter.

Coordination of the human circadian clock and the cell cycle

The circadian clock and the cell cycle are major cellular systems that organize global physiology in temporal fashion. The circadian

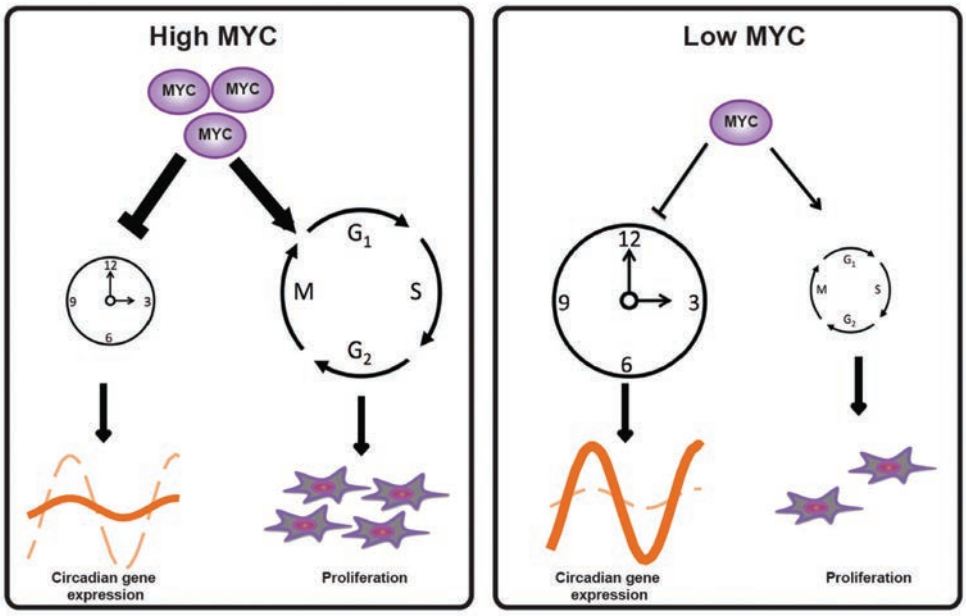

Fig. 3: Model of the co-ordinating function of MYC.
Left panel: high levels of MYC suppress the circadian clock by MIZ1-dependent down-regulation of BMAL1/CLOCK (see text), which results in low amplitude expression rhythms of clock-controlled genes. On the other hand, high MYC levels support cell growth and proliferation (for example, by inhibition of the cyclin dependent kinase inhibitor genes p15 *and* p21*). Right panel: at low levels of MYC, the circadian clock is not inhibited and supports high amplitude expression rhythms of clock-controlled genes. Low levels of MYC do not support cell growth and proliferation.*

clock of mammals is constituted by the core transcription factor BMAL1/CLOCK, which rhythmically activates expression of clock genes including *CRYs*, *PERs*, *REV-ERBs*, and *RORs*. CRYs and PERs are inhibitors of CLOCK/BMAL whereas REV-ERBs are repressors that control in coordination with ROR activators expression of *BMAL1* and *CLOCK*. The D-box-specific transcription factors E4BP, DBP, TEF and, HLF additionally contribute to the regulation of specific clock genes.

Disruption or misalignment of circadian rhythms in humans has been associated with numerous pathological conditions including cancer. *MYC* is an oncogene, which is severely deregulated in different cancers and, amplification of MYC often correlates with tumor aggression and poor prognosis. MYC is a transcription factor that supports cell growth and proliferation (cell cycle progression) by regulating transcription of up to 15% of the transcriptome.

We found that MYC is a key regulator that coordinates the circadian clock with cell growth. Overexpression of *MYC* attenuates the clock and conversely promotes cell proliferation while downregulation of *MYC* strengthens the clock and reduces proliferation. Inhibition of the circadian clock is crucially dependent on the formation of repressive complexes of MYC with MIZ1 and subsequent downregulation of the core clock genes *BMAL1* (*ARNTL*), *CLOCK* and *NPAS2*. In addition, MYC has the potential to activate expression of the circadian repressor REV-ERBα, which down-regulates *BMAL1*. *BMAL1* expression levels correlate inversely with *MYC* levels in 102 human lymphomas.

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2006 - 2010	PostDoc -National Cancer Institute, NIH, MD, USA
2010 - 2016	Junior Group Leader - BZH
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Tamás Fischer

Epigenetics and Genomic Stability

Goal

To understand the role of chromatin and RNA surveillance mechanisms in genome organization and in the maintenance of genomic stability.

Background

Recent results have revealed another layer of complexity in the eukaryotic transcriptome. While previous expression analyses focused mostly on mRNA expression levels, new transcriptome profiling techniques provide unbiased, genome-wide information about transcription activity. These studies have revealed that genomic regions previously thought to be silent, such as heterochromatic regions, intergenic regions and antisense portions of the genome, are actually transcriptionally active and produce a significant amount of non-protein-coding RNA (ncRNA) transcripts. Although a growing number of examples show that some of these ncRNAs can affect key nuclear events, the majority of these transcripts, so-called cryptic unstable transcripts (CUTs) are rapidly degraded by the RNA surveillance machinery. The general function of this widespread, pervasive transcription is not understood, but its level is strictly controlled by the chromatin structure. Defects in this repressive chromatin structure or impairment of the RNA surveillance system can lead to strong accumulation of cryptic transcripts and to genomic instability.

The main focus of the research in our laboratory is:

(i) to understand the role of chromatin in the repression of pervasive transcription;

(ii) to reveal the molecular mechanisms responsible for the recognition and degradation of CUTs;

(iii) to understand the link between ncRNA accumulation and genomic instability and how it contributes to cancer development.

Research Highlights

We recently identified the multi-subunit MTREC complex in *S. pombe* that specifically binds to CUTs and unspliced pre-mRNAs and targets them to the nuclear exosome for degradation (Zhou *et al*., 2015). Phylogenetic analysis of the subunits shows high similarity to subunits of the human Nuclear EXosome Targeting (NEXT) complex. Deletion or mutation of *S. pombe* MTREC complex subunits leads to strong accumulation of CUTs and unspliced or mis-spliced pre-mRNAs. MTREC complex physically interacts with the nuclear exosome, and with various RNA binding and processing complexes, including the cap-

binding, spliceosome, cleavage and poly-adenylation complexes. Our future goal is to further characterize the MTREC complex *in vitro* and *in vivo*, and to uncover the molecular mechanism by which this large, multi- subdomain complex is recruited to CUTs and aberrant mRNAs and targets them to the exosome (Figure 1).

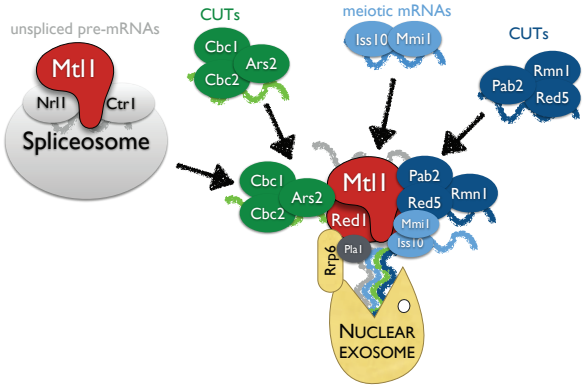

Figure 1. Proposed model for the role of MTREC complex in RNA surveillance. *Submodules of the MTREC complex, together with the Mtl1–Ctr1–Nrl1 complex, are recruited to different subsets of CUTs, meiotic mRNAs or unspliced pre-mRNA transcripts and deliver these RNAs to the MTREC complex. The RNAs are polyadenylated by the canonical poly(A) polymerase, Pla1. The RNA-loaded MTREC complex can dock to the nuclear exosome through the Red1–Rrp6 interaction. The helicase activity of the Mtl1 subunit then feeds the MTREC-bound RNAs into the exosome channel.*

Although excessive pervasive transcription can cause genomic instability, pervasive transcription in general might also have positive effects for the cell. We recently discovered that pervasive transcription and the resulting RNA-DNA hybrids are essential for the efficient repair of DNA double-strand breaks (DSBs). RNA-DNA hybrids are generated by RNA polymerase activation around DSB sites, and stabilization or destabilization of RNA-DNA hybrids strongly affects the length of strand resection during HR-mediated DSB repair (Ohle *et al.*, 2016). These observations strongly suggest that RNA polymerase transcription might have a direct role in strand resection, probably by opening the chromatin structure around the DSB site. This is a very exciting and novel development in the field of DNA-repair, and our mid-to long-term research goals will focus on the molecular mechanism behind these findings both in the fission yeast *S. pombe* and in mammalian cells (Figure 2).

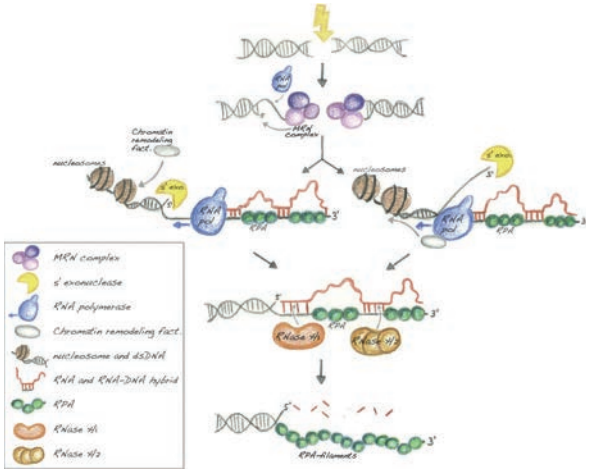

Figure 2. RNA-DNA Hybrids and RNase H Activity Are Required for Efficient DSB Repair. *Suggested model for the HR-mediated DSB repair pathway. Following the appearance of a DSB, the MRN complex is recruited to the broken DNA ends and, with the help of Sae2, initiates 5´ end resection. Pol II is recruited to the 3´ ssDNA overhangs and jump-starts transcription, without the requirement of PIC assembly or the recruitment of additional regulatory proteins. The nascent RNA transcripts are prone to re-hybridize with the ssDNA template strand and form RNA-DNA hybrids, which directly compete with the ssDNA-binding RPA complex. Subsequent, long-range resection of the 5´ strand is performed by the 5´-3´ exonuclease Exo1 or the Dna2-Sgs1/BLM complex. Additional chromatin remodelling activities likely facilitate the progression of nucleases through the chromatin environment. Pol II transcription either follows the strand resection process (left) or actively drives strand resection by opening the chromatin and the DNA helix ahead of the nucleases (right). RNA-DNA hybrids might play a role in controlling the speed and the length of the strand resection process by stalling or terminating Pol II transcription. RNA-DNA hybrids need to be degraded by RNase H enzymes in order to achieve full RPA loading on the ssDNA overhangs and to complete the DNA repair process.*

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1986 - 1994	Group Leader - European Molecular Biology Laboratory (EMBL) Heidelberg, Germany, Cell Biology
1990	Habilitation in Biochemistry - University of Regensburg, Germany
since 1995	Full Professor - BZH
2003 - 2005	Director - BZH

Ed Hurt

Deciphering nuclear pore complex and and ribosome biogenesis

Goal

We want to find out how the nuclear pore complexes and ribosomal subunits are assembled in the cell. To unravel the underlying mechanisms, we perform biochemical, structural and genetic studies in the model organisms *Saccharomyces cerevisiae* and *Chaetomium thermophilum*, which gave mechanistic insight in how these huge macromolecular assemblies are built up from smaller subcomplexes and how these modules are functionally linked together.

Background

Nucleo-cytoplasmic transport occurs through the nuclear pore complexes (NPCs), which are huge assemblies embedded into the nuclear envelope. Each NPC, whose size is ca. 60 MDa in yeast and 120 MDa in human, is built up by multiple copies (8, 16, 32) of ~30 different nucleoporins (also called Nups), which in most cases are conserved during evolution. The overall NPC structure shows eightfold symmetry with a number of distinct substructures, which were later on also identified as biochemical entities. Prominent structural building blocks are the inner ring complex, which anchors the nuclear and cytoplasmic ring, from which peripheral elements, the nuclear basket and cytoplasmic filaments, emanate. Within the center of the NPC is the transport channel, which is filled by a network of phenylalanine-glycine (FG) rich repeat sequences found in a number of the nucleoporins that constitute the permeability barrier of the NPC. FG repeat domains can transiently interact with shuttling transport receptors, which enables them to pass through the NPC either alone or with bound cargoes. Most nucleoporins are organized in stable subcomplexes, the building blocks of the NPC. For many years, my lab has been studying these NPC modules in yeast, with a focus on the conserved Y-shaped Nup84 complex, the Nsp1-Nup49-Nup57 channel complex and the Nup82-Nup159-Nsp1 complex at the cytoplasmic filaments.

The second major interest of the lab concerns the synthesis of the ribosomal 60S and 40S subunits, which are composed of four ribosomal RNAs (18S, 25S, 5.8S and 5S) and about 80 ribosomal proteins. Eukaryotic ribosome biogenesis is embedded into a complicated pathway of processing, modification and maturation steps, in which a myriad of non-ribosomal factors (ca. 200) form transient interactions with the pre-ribosomal particles, thereby driving ribosome synthesis. The earliest intermediate identified in this path is the nucleolar 90S pre-ribosome, which later gives

raise to pre-60S and pre-40S particles. Pre-60S particles require extensive nuclear maturation prior to export to the cytoplasm, whilst in contrast the simpler pre-40S particles are rapidly exported to the cytoplasm, where maturation is completed.

Research Highlights

In the past years, we have performed structure-function analyses of the NPC in *Saccharomyces cerevisiae* and *Chaetomium thermophilum* by combining, respectively, the excellent genetic and thermophile properties of these two model organisms. With the help of *Chaetomium thermophilum*, we were able to reconstitute and characterize the inner ring complex (IRC) composed of Nup192-Nic96-Nup170-Nup53 and show that another nucleoporin Nup145N can serve as a linker nucleoporin to connect it to other NPC modules. Specifically, the mostly unstructured Nup145N contains several distinct short linear motifs that enable this protein to bind specifically to the two large structural Nups of the IRC, Nup192 and Nup170. At the same time, the Nup145N C-terminal domain can interact with the Nup82-Nup159-Nsp1 complex, thus connecting the IRC with the Nup82 complex at the cytoplasmic face of the NPC (Figure 1).

Furthermore, we could clarify how the IRC can be physically joined to the Nsp1-Nup49-Nup57 channel complex via a short linear motif in Nic96 (Fischer et al., 2015). Finally, we were able to reconstitute an extensive supercomplex of the NPC consisting of three major modules – IRC, Nsp1 channel complex and Nup82 complex - containing a total of eleven Nups. In summary, all these studies gave insight into how distinct subcomplexes of the NPC are interconnected to form higher-order assemblies, which shed new light on how the NPC can be built up from smaller building blocks, flexibly joined by short linear motifs provided by linker Nups.

In order to obtain protein complexes of increased stability isolated directly from *Chaetomium thermophilum*, we developed a transformation procedure including an endogenous resistance marker system suitable for the thermophile (Kellner et al., 2016). Using this method, we were able to generate stable transformants expressing various nuclear pore complex proteins or ribosome biogenesis factors fused to a ProtA-TEV-Flag tandem affinity-tag for the purification of native thermostable nucleoporin supercomplexes or pre-ribosomal particles (see below).

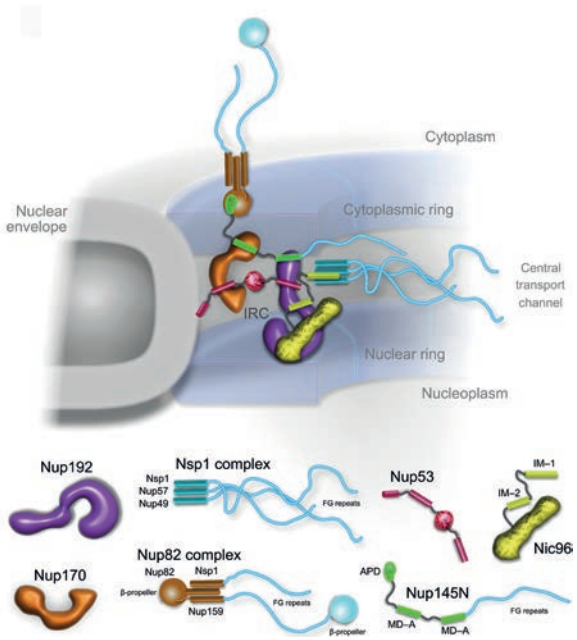

Figure 1: Reconstitution of the inner pore ring module (IRC) with outer ring Nup82 complex and channel Nsp1 complex.

In our research investigating the mechanism of ribosome assembly, we were able to demonstrate how the huge dynein-like ATPase Rea1 and its cofactors Rsa4 and Ytm1 are essential for the assembly for the large subunit. A combination of genetic and biochemical methods allowed us to identify Nsa2 as direct binding partner of Rsa4. Structural approaches including cryo-EM of the Arx1 and Rix1-Rea1 particles (in collaboration with the Beckmann lab at the Gene Center in Munich), crystallisation of Rsa4 and the Rsa4-Nsa2 complex (in collaboration with I. Sinning at the BZH) and NMR analysis of Nsa2 domains (in collaboration with E. Barbar, Oregon State University, Corvallis) enabled us to understand the molecular details of the Rsa4 - Nsa2

interaction (Baßler et al., 2014). Altogether, these data revealed how the Rea1 ATPase creates a mechano-chemical force that can be transmitted via Rsa4 and Nsa2 towards the nascent rRNA of the PTC, inducing rRNA relocation, as recently revealed by cryo-EM analysis of the Rix1-Rea1 pre-60S particle (Barrio-Garcia et al., 2016). By performing cryo-EM analysis on two similar particles that were mutated on either Rix1 or Rea1, a mechanistic model for the transition between these two pre-60S intermediates could be suggested.

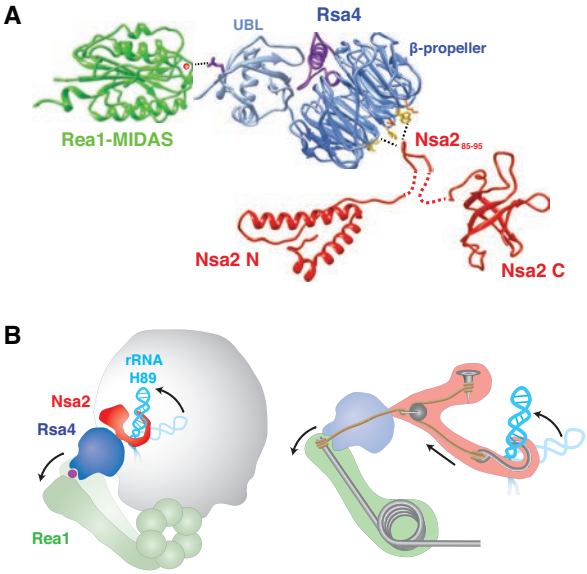

Figure 2. Ribosome assembly factor network revealing how the Rea1 AAA ATPase can remodel the pre-60S subunit during ribosome assembly *(adapted from Baßler et al., 2014).*

The transport of the pre-ribosomal subunits from the nucleus to the cytoplasm is a critical step during eukaryotic ribosome biogenesis, making this cascade-like process as a whole apparently irreversible. To migrate through the hydrophobic FG repeat network of the central transport channel of the nuclear pore complex, the huge hydrophilic pre-60S subunit requires several export factors on its surface. In the past year, we could clarify the mechanism how the RNA export receptor Mex67-Mtr2 can be timely recruited to the pre-60S subunit at the ribosome P0 stalk (Sarkar et al., 2016), which on the mature 60S subunit constitutes a landing platform for the translational GTPases. Initially, a structural homologue of P0 called Mrt4 binds to the nascent ribosome stalk inside the nucleus, thereby blocking not only P0 but also Mex67-Mtr2 binding at this site. Later on Yvh1 releases Mrt4 from the pre-ribosome, creating a time window for Mex67-Mtr2 association at this temporary site, which in the cytoplasm eventually becomes occupied by the ribosomal P0 stalk protein. Thus, a spatio-temporal mark on the ribosomal P stalk accurately regulates timed recruitment of nuclear export receptor to the nascent 60S subunit.

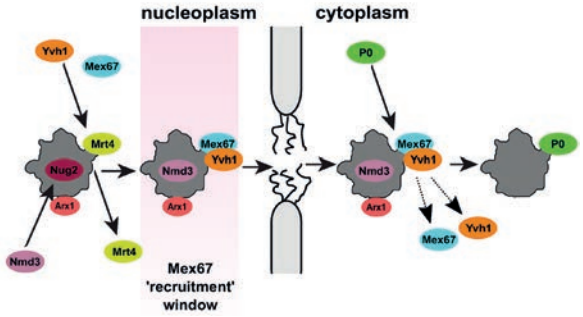

Figure 3. Model of the Mex67-Mtr2 recruitment ot the pre-60S particle *(adapted from Sarkar et al., 2016).*

Despite the advances in our compositional understanding of pre-ribosomal particles, only little structural data were available for the earliest pre-ribosomes. The 90S pre-ribosome composed of ~70 assembly factors and several snoRNAs is the earliest intermediate formed during eukaryotic ribosome synthesis. In collaboration with the Beckmann lab, we could solve the cryo-EM structure of the *Chaetomium thermophilum* 90S pre-ribosome at subnanometer resolution, which revealed how the network of biogenesis factors surrounds the nascent pre-rRNA. One of these key modules, the U3 snoRNP, is strategically positioned at the center of the particle to orchestrate pre-rRNA folding and processing (Kornprobst et al., 2016). Thus, by exploiting *Chaetomium thermophilum* we could gain unforeseen structural insight into how the 90S pre-ribosome facilitates co-transcriptional folding of the nascent pre-rRNA, which occurs in a mold-like scaffold, reminiscent of how polypeptides use chaperone chambers for their protein folding.

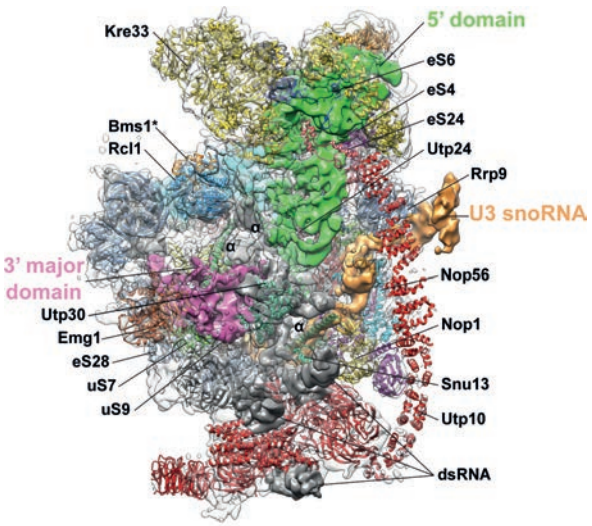

Figure 4. Cryo-EM structure of the 90S pre-ribosome *(adapted from Kornprobst et al., 2016).*

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Monika Gaik, Dirk Flemming, Alexander von Appen, Panagiotis Kastritis, Philipp Stelter, Khanh Huy Bui, Alessandro Ori, Jochen Bassler, Jessica Fischer, Elisar Barbar, Martin Beck and Ed Hurt: Structural basis for assembly and function Nup82 complex in the nuclear pore scaffold
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Markus Kornprobst, Martin Turk, Nikola Kellner, Jing-Dong Cheng, Dirk Flemming, Martin Koš, Isabelle Koš, Matthias Thoms, Otto Berninghausen, Roland Beckmann and Ed Hurt: Architecture of the 90S pre-ribosome: a structural view on the birth of the eukaryotic ribosome
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Nat. Struct. Mol. Biol. 10.1038/nsmb.3312 (2016).

Awards and Honors

2015	Eduard Buchner Prize
2011	Reinhart Koselleck Project
2007	Feldberg Prize
2001	Gottfried Wilhelm Leibniz Prize
2007	Member of ACADEMIA EUROPAEA
2005	Member of LEOPOLDINA
1994	Member of EMBO

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2002 - 2008	PostDoc - Wellcome Trust Centre for Cell Biology, University of Edinburgh, Great Britain
2008 - 2015	Junior Group Leader - Excellence Cluster “CellNetworks”, BZH
since 2016	Junior Group Leader - BZH

Martin Koš

Ribosomal RNA processing and modification

Goal

Our aim is to extend our understanding of molecular mechanisms underlying ribosome biogenesis and its regulation in response to environmental stresses and during senescence. We focus on the roles of RNA modifications and functions of specialized ribosomes.

Background

Ribosome biogenesis is a major energy consuming process in all organisms that is tightly regulated with cell growth. This highly conserved process begins with transcription of a large ribosomal RNA (rRNA) precursor that is subsequently covalently modified and processed into mature 18S, 5.8S and 25S rRNAs. The process of ribosome maturation is very complex and highly dynamic. At least 180 non-ribosomal proteins and 70 small nucleolar RNAs (snoRNAs) have been implicated in ribosome biogenesis in yeast. A dysregulation of ribosome biogenesis is observed in cancer and multiple human diseases. Recently, existence of differently modified so called specialized ribosomes within cells was revealed, however, their function remains unknown.

Research Highlights

Role of rRNA modifications

In addition to the complex structure, rRNAs are also extensively modified by methylation and pseudouridylation at approximately 100 sites. The role of these modifications in either biogenesis or function of mature ribosomes remains largely unclear. We analyzed the role of a cluster of rRNA methylations located near the inter-subunit bridge B3 in 25S rRNA. We identified Rcm1 as an enzyme required for highly conserved cytosine-5 methylation of C2278 in the 25S rRNA (Gigova *et al.* 2014). We showed that methylation at two sites, C2278 and G2288 is required for stability of the mature 60S ribosomal subunit. Ribosomes lacking these two methylations exhibited changes in the structure of 25S rRNA and the loss of several ribosomal proteins (Figure 1.)

Oligomerization of TGS1 is required for an efficient trimethylation of snRNAs and snoRNAs

Trimethylguanosine Synthase catalyses the transfer of two methyl groups to the m7G cap of snRNAs, snoRNAs and telomerase RNA TLC1 to form a 2,2,7-trimethylguanosine cap. While *in vitro* studies indicate that Tgs1 functions as a monomer and the dimethylation of m7G caps is

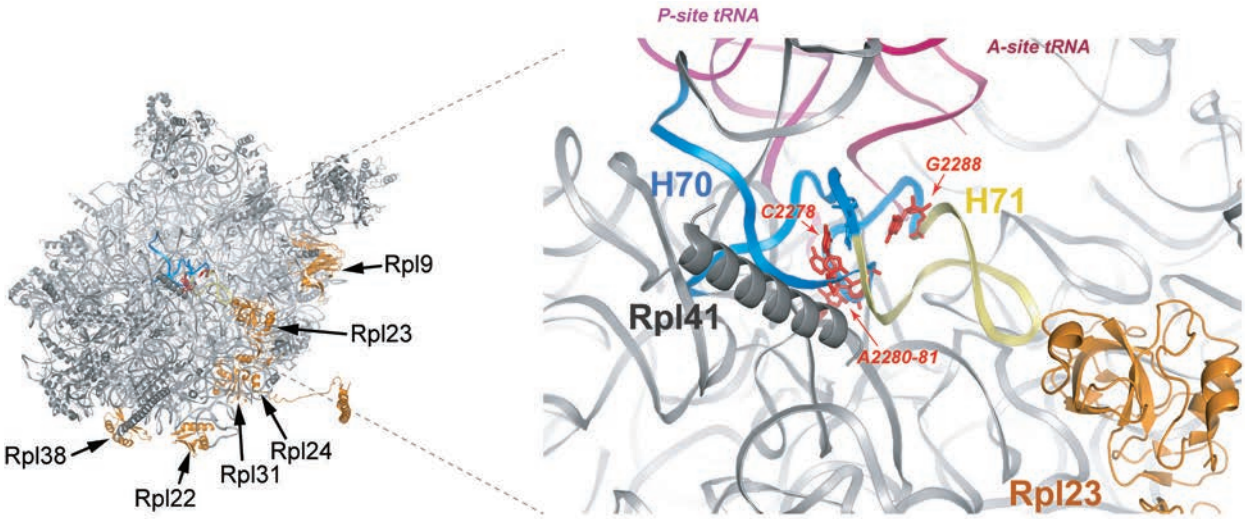

Fig. 1.Figure 1. Proteins affected in ribosomes lacking methylations of nucleotides C2278 and G2288.
Left: *crystal structure of the 60S ribosomal subunit. Proteins that are lost from ribosomes lacking rRNA methylation are highlighted in orange.* ***Right:*** *Detailed view of the domain IV of 25S rRNA. The methylated nucleotides are in red, the helix H71, which participates in the intersubunit bridge B3, is highlighted in yellow. From Gigova et al. 2014.*

not a processive reaction, partially methylated sn(o)RNAs are typically not detected in living cells. We found that both yeast and human Tgs1p possess a conserved self-association domain located at the **N-terminus. A disruption of Tgs1 self-association led to a strong reduction of sn(o)RNA trimethylation (Boon *et al*., 2015). We speculate, that TGS1 forms dimers *in vivo* that are capable to efficiently dimethylate the caps of the targeted RNAs without the need to dissociate from the substrate.

Regulation of alternative rRNA processing pathways by TORC1 in stress response

We observed that the processing of pre-rRNA abruptly changes when yeast cells are exposed to an environmental stress or upon depletion of nutrients (Kos I *et al*., submitted). We found that this switch in pre-rRNA processing is regulated by the Tor complex 1 (TORC1) and the casein kinase 2 (CK2). Importantly, Sch9 (the yeast homologue of S6 kinase), considered to be the major downstream effector of TORC1 regulating ribosome biogenesis, is dispensable indicating that an unidentified branch of TORC1 signalling controls ribosome biogenesis at the post-transcriptional level.

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Luise Krauth-Siegel

The unique trypanothione-based redox metabolism of trypanosomatids

Goal

Aim of our work is to unravel the role of the unusual dithiol trypanothione in trypanosomatids and to contribute to the development of new antiparasitic drugs by identifying target molecules and the analysis of parasite specific enzyme inhibitors.

Background

Trypanosomatids, the causative agents of various tropical diseases, possess a redox metabolism that is based on the bis(glutathionyl)spermidine-conjugate trypanothione. The dithiol is the donor of reducing equivalents for a wide variety of essential pathways (Fig. 1).

Research Highlights

Trypanothione reductase (TR) as a drug target molecule

TR is an attractive drug target molecule (Leroux and Krauth-Siegel 2016). However, its large active site allowing multiple possible ligand orientations renders a structure-based inhibitor design challenging. In a joint effort of several groups, biological testing, kinetic and mutational studies, and virtual docking simulations revealed a new series of small-molecule inhibitors (Persch et al. 2014). The co-crystal structures showed that the ligands interact with the hydrophobic wall of the so-called "mepacrine binding site", but, remarkably, the binding conformation of the inhibitors varied for TR from *Trypanosoma brucei* and *T. cruzi*. The study gives new insight into the molecular recognition of nonpeptidic small-molecule ligands by TR and provides the basis for an ongoing lead optimization.

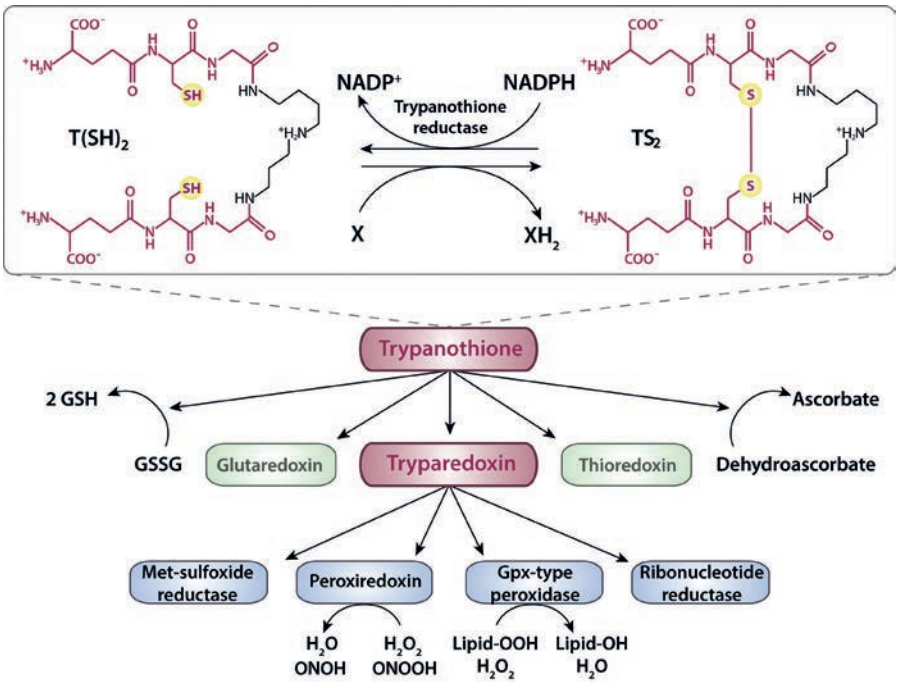

Fig. 1: The trypanothione metabolism.

Hydroperoxide detoxifying peroxidases play completely different roles in the infectious bloodstream and procyclic insect stage

African trypanosomes express three virtually identical non-selenium glutathione peroxidase-type enzymes which preferably detoxify lipid-derived hydroperoxides. Bloodstream *Trypanosoma brucei* lacking the mitochondrial isoenzyme display only a weak and transient proliferation defect and *in vivo* studies in mice confirmed the negligible role of the protein. In contrast, parasites that lack the cytosolic peroxidases undergo extremely fast lipid peroxidation and cell lysis. The phenotype, which can be completely rescued by supplementing the medium with the α-tocopherol derivative Trolox, is due to damage of the parasite lysosome (Fig. 2; Hiller et al. 2014).

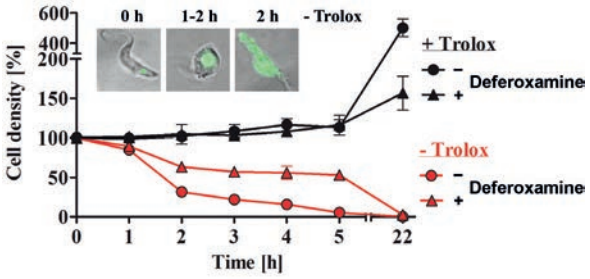

Fig. 2 Bloodstream T. brucei that lack the cytosolic peroxidases undergo lysosomal disintegration and cell lysis. *In the presence of Trolox, the deficient parasites fed with Alexa Fluor-conjugated dextran display a discrete lysosomal staining (0 h) and proliferate like wildtype cells. Yet upon withdrawal of the antioxidant, the signal becomes progressively spread over the whole cell body (1-2 h) and the parasite die. The lethal phenotype is attenuated in the presence of the iron-chelator deferoxamin.*

T. brucei acquire iron by endocytosis of host transferrin. Supplementing the medium with iron or transferrin induces, whereas the iron chelator deferoxamine and apo-transferrin attenuate lysis of the Px I-II-deficient cells. These data demonstrate that in the infectious form of African trypanosomes, the lysosome is the primary site of oxidative damage and cytosolic trypanothione/ tryparedoxin-dependent peroxidases protect the organelle from iron-induced membrane peroxidation.

In the insect form of *T. brucei* selective deletion of the genes revealed that parasites that lack either the cytosolic or mitochondrial peroxidase proliferate nearly as wild type cells whereas the knockout of the complete genomic locus is lethal (Schaffroth et al. 2016). The parasites lose their mitochondrial membrane potential and lyse. The cellular damage is prevented by Trolox, ubiquinone derivatives, and deferoxamine. In glucose-rich medium, cell death is attenuated suggesting that oxidants generated by the respiratory chain contribute to the lethal phenotype. Depending on the developmental stage, the lysosome or the mitochondrion is the origin of iron-mediated oxidative membrane damages. Strikingly, in the insect stage either the cytosolic or the mitochondrial form of the peroxidases is required and sufficient to protect the mitochondrion.

Selected Publications 2014 - 2016

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Johannes Lechner

Kinetochore and Mitosis

Goal

To understand the function of regulatory proteins at the kinetochore.

Background

Reliable chromosome segregation depends on the correct attachment of kinetochores (KT) to KT-microtubules (kMTs). In budding yeast, chromosomes detach during S-phase. Subsequently the unattached KTs (uaKTs) are captured by nuclear random microtubules (nrMTs) in prometaphase. While they are unattached, KTs activate the spindle assembly checkpoint (SAC) and thus prevent cells from entering anaphase. Crucial for SAC activation is that in the absence of KT-microtubule interaction, the KT protein Spc105 becomes accessible to phosphorylation by the KT-localized protein kinase Mps1. This leads to the assembly of the SAC proteins at the KT and consequential metaphase arrest. Interestingly, uaKTs not only assemble SAC proteins but also sequester the CLASP, Stu1. CLASPs contain TOGL domains that provide microtubule rescue function. Stu1 has two N-terminal TOGL domains (TOGL1 and 2). Only TOGL2, provides direct rescue activity whereas TOGL1 serves as a KT-binding domain. In metaphase, Stu1 has at least two roles. First, it stabilizes interpolar MTs (ipMTs) via a direct interaction that involves the TOGL2 domain and a basic serine-rich unstructured region (ML). Secondly, it localizes to the KTs via TOGL1 and ML and stabilizes kMTs. Consequently, Stu1 is essential for the formation of a metaphase spindle. In the following we were intrigued to reveal the regulatory mechanism of Stu1 sequestering at uaKTs and particularly what benefit it provides for the cell.

Research Highlights

Mechanism and regulation of Stu1 sequestering.
We revealed that besides Stu1, also Slk19, another protein important for spindle stability, is accumulating at uaKTs. Slk19 is required for Stu1 sequestering and vice versa. However, either protein can localize to uaKTs in the absence of the other in basal (low detectable) amounts. Furthermore, we showed that Stu1 sequestering and localization requires Spc105, the activity of Mps1, and the presence of the six Mps1 consensus sites in Spc105 whose phosphorylation initiate SAC protein assembly. In addition, if Mps1 is ec-

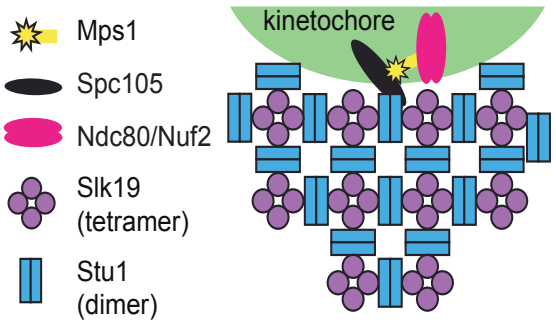

Figure 1. *Model depicting the sequestering of Stu1 at unattached kinetochores*

topically localized close to Spc105, SAC activation (as published by others) and Stu1 sequestering become independent of KT detachment. Thus taken together, the signaling mechanism that translates KT detachment into SAC protein assembly and Stu1 sequestering is similar (if not identical). Our current model (Figure 1) thus assumes that the Mps1-dependent phosphorylation of Spc105 promotes the binding of Stu1 to uaKTs. This may cause a conformational change in Stu1 that initiates the co-polymerization of Stu1 dimers and Slk19 tetramers via propagated conformational changes.

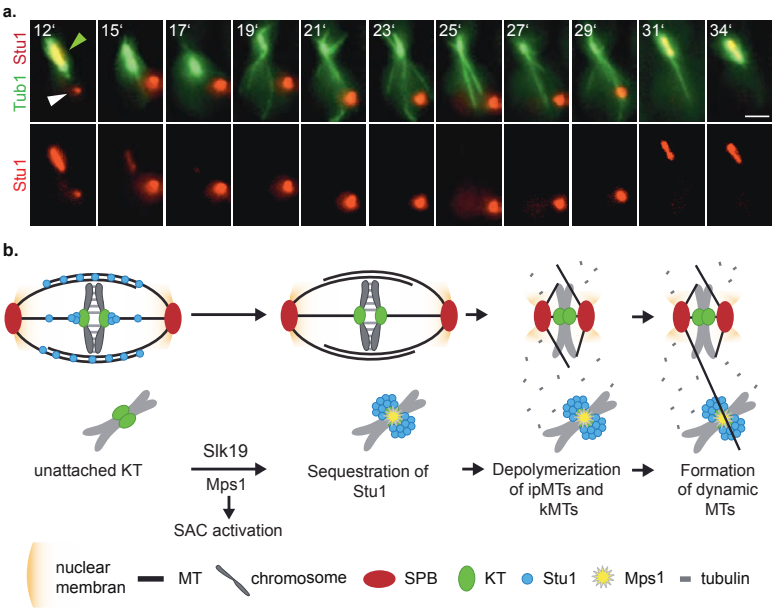

Figure 2. (a) *Time lapse micrographs showing the sequestering of Stu1 at an uaKT (white arrowhead) while Stu1 is withdrawn from the spindle (green arrowhead). With time the spindle collapses and random MTs appear. After capturing of the uaKT (29 s), the effects are reversed. Bar, 2µm.* ***(b)*** *Proposed model how sequestering of Stu1 enhances the formation of nrMTs and thus promotes capturing of uaKTs.*

We found that a putatively unstructured region of Stu1, the C-terminal loop (CL) is essential for sequestering but (in contrast to the KT-binding domain TOGL1) not for the basal localization of Stu1 to uaKTs. Therefore, we suggest that the CL domain plays a major role in Stu1-Slk19 interaction and/or in allowing the necessary conformational changes.

The role of Stu1 sequestering for KT capturing.

In order to observe Stu1 sequestering with time in the presence of an intact metaphase spindle, uaKTs were produced by KT inactivation and reactivation. This revealed that while Stu1 is accumulating at an uaKT it is withdrawn from the spindle MTs and attached KTs (Figure 2a) resulting in the collapse of the spindle. In correlation with this, random dynamic MTs appeared that were revealed to be predominately nuclear. Upon capturing of the KT, the sequestered Stu1 was transported back to the spindle region and the metaphase spindle was re-established. Quantification (in respect to MT number and length) showed that cells exhibited about 20 times more nrMTs occurrence if uaKTs were present than if they were not. This was primarily a consequence of spindle destabilization since the depletion of Stu1 had a similar effect on the formation of nrMTs as the sequestering of Stu1 at uaKTs. On the other hand, *stu1ΔCL* cells that are defective in sequestering and leave the spindle uncompromised showed no enhanced nrMT formation. The occurrence of dynamic MTs that span the nucleus randomly appears ideal for capturing uaKTs. Indeed, we found that the capturing efficiency of *stu1ΔCL* cells was severely compromised in comparison to wildtype cells or Stu1-depleted cells. We thus propose the model shown in Figure 2b. uaKTs withdraw Stu1 from the mitotic spindle and thus destabilize ipMT and kMTs. The freed tubulin is then used to form dynamic MTs that considerably increase the chance for capturing. uaKTs thus drive their own capturing and to do so, they use the same signaling strategy that they use to prolong the time for it (via SAC activation).

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Walter Nickel

Unconventional Protein Secretion

Goal

To reveal the molecular components and mechanisms involved in unconventional secretion of Fibroblast Growth Factor 2 (FGF2) from tumour cells, to elucidate the relevance of the FGF2 secretion system for other unconventionally secreted proteins such as HIV-Tat and Interleukin 1β and to identify small molecule inhibitors of these processes to develop novel classes of anti-cancer drugs targeting tumour cell survival.

Background

The vast majority of extracellular proteins is secreted through the classical ER/Golgi-dependent secretory pathway. However, numerous extracellular proteins have been identified that do not carry signal peptides and whose export from cells is not blocked by Brefeldin A, an inhibitor of ER-to-Golgi trafficking. Among this group of extracellular proteins are mediators of physiological processes with biomedical relevance such as angiogenesis and inflammation, among others. For example, FGF2 is of critical importance for processes such as embryonic development, tissue regeneration, wound repair and hematopoiesis. Beyond its function in normal cell growth and differentiation, FGF2 plays critical roles under pathophysiological conditions. This is particularly evident in the context of cancer with FGF2 being a major mediator of tumour-induced angiogenesis. In addition, FGF2 acts as a survival factor that inhibits tumour cell apoptosis by an autocrine secretion-signalling loop. This process is believed to represent a frequent cause of tumour cell resistance against anti-cancer therapies.

The molecular machinery mediating FGF2 transport into the extracellular space has been elucidated through a combination of genome-wide RNAi screening approaches and biochemical reconstitution experiments. It consists of four *trans*-acting factors, ATP1A1, $PI(4,5)P_2$, Tec kinase and heparan sulfate proteoglycans, all of which are physically associated with the plasma membrane (Fig.1).

Key steps in unconventional secretion of FGF2 are sequential interactions with ATP1A1, Tec kinase and the phosphoinositide $PI(4,5)P_2$ at the inner leaflet, $PI(4,5)P_2$ triggered oligomerization and membrane insertion as well as extracellular trapping of FGF2 mediated by cell surface heparan sulfate proteoglycans. Key intermediates in this process are membrane-inserted FGF2 oligomers that form membrane pores, a process that is regulated by Tec kinase dependent tyrosine phosphorylation of FGF2. FGF2 remains folded

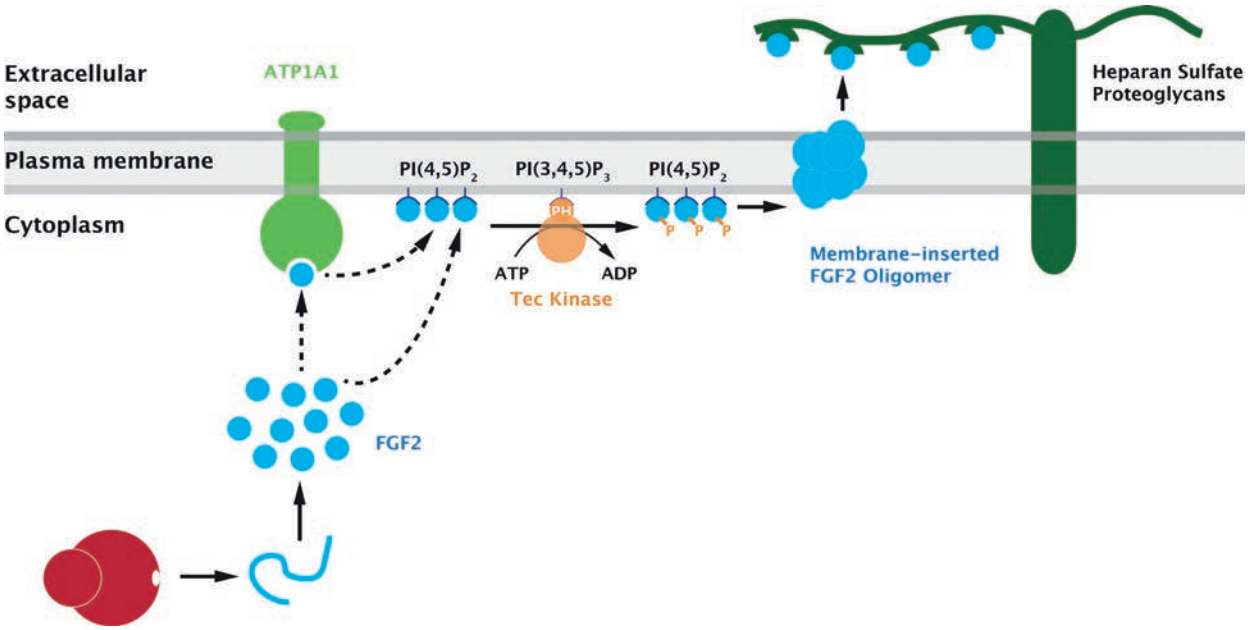

Fig. 1: Molecular components and mechanisms involved in unconventional secretion of FGF2 from tumour cells (La Venuta et al, 2015)

through all steps of this pathway, a property that is linked to specific requirements such as binding to $PI(4,5)P_2$, the formation of an oligomeric structure as part of a toroidal membrane pore and binding to heparan sulfates on cell surfaces. These steps are reflected by requirements for *cis*-elements in FGF2 that mediated binding to $PI(4,5)P_2$ (K127/R128/K133), binding to heparan sulfates (K133) and tyrosine phosphorylation (Y81). In conclusion, FGF2 is secreted through direct translocation across the plasma membrane, a novel type of protein translocation across membrane where the cargo molecule (FGF2) forms its own translocation intermediate through oligomerization and membrane insertion.

Research Highlights

A recent discovery has been the identification of a new *cis*-element required for FGF2 secretion from cells. It is composed of two cysteine residues (C77/C95) on the molecular surface of FGF2 that are absent from all FGF family members carrying signal peptides for ER/Golgi-dependent secretion, suggesting a specific role in unconventional secretion of FGF2. These cysteines form intermolecular disulfide bridges and are critical for FGF2 oligomerization and membrane insertion. Intriguingly, the four *cis*-elements in FGF2 required for unconventional secretion (K127/R128/K133 for binding to $PI(4,5)P_2$, K133 for binding to heparan sulfates, Y81 as the target of Tec kinase and C77/C95 required for oligomerization and membrane insertion) are transplantable. Following removal of the signal peptide of FGF4, a structural relative of FGF2, the hybrid protein was redirected into the unconventional secretory pathway of FGF2 resulting in its exposure on cell surfaces. These findings establish the core machinery mediating FGF2 secretion with four *trans*-acting factors and four *cis*-elements.

The relevance of the core features of FGF2 secretion for other unconventionally secreted proteins was elucidated through studies on HIV-Tat and Interleukin 1β secretion. On the one hand, we found that HIV-Tat is capable of forming membrane pores in a $PI(4,5)P_2$ dependent manner. Along with studies from other laboratories that demonstrated $PI(4,5)P_2$ dependent secretion of HIV-Tat from T cells, these findings strongly suggest that FGF2 and HIV-Tat share a common

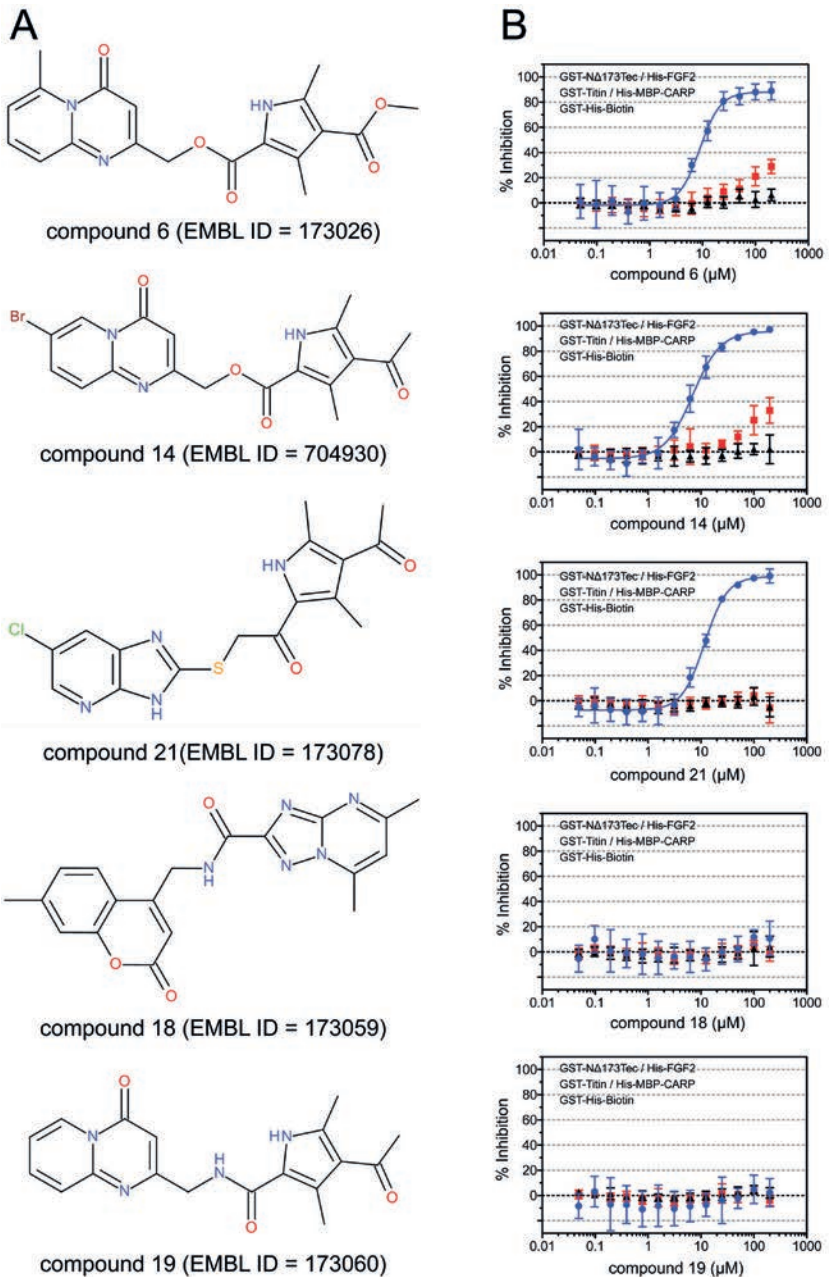

Figure 2: Identification of small molecule protein-protein interaction inhibitors of the Tec/FGF2 complex (La Venuta et al, 2016).

(A) *Chemical strcutures of active compounds (cpds 6, 14 and 21) and inactive derivatives (cpds 18 and 19).*

(B) *IC50 profiles of the compounds shown in panel A as determined with the AlphaScreen protein-protein interaction assay.*

mechanism of unconventional secretion. Furthermore, in collaboration with Pablo Pellegrin (Murcia, ES) and David Brough (Manchester, UK), we could demonstrate that inflammasome-dependent secretion of Interleukin 1β depends on $PI(4,5)P_2$ dependent membrane pore formation at the level of the plasma membrane. These findings imply that at least three major examples of unconventionally secreted proteins rely on a similar mechanism of transport into the extracellular space with FGF2 being the prototype cargo molecule.

Finally, another recent breakthrough was the development of a first generation of small molecule inhibitors targeting unconventional secretion of FGF2. While we address this aim in several ways, a first success has been the identification of compounds that prevent the interaction between FGF2 and Tec kinase (Fig. 2 and Fig.3). These compounds inhibit Tec kinase dependent tyrosine phosphorylation of FGF2 both in vitro and in cells. Intriguingly, the first generation of these inhibitors block FGF2 secretion from cells with an IC50 in the low micromolar range making them excellent lead compounds for drug development targeting tumour cell survival.

In conclusion, in recent years, the molecular mechanism and the molecular components required for unconventional secretion of FGF2 have been elucidated in great detail. Future challenges will be to fully reconstitute this process with purified components, to obtain structural insight into membrane-inserted FGF2 oligomers as the key intermediates of this process, to analyse the spatio-temporal coordination of this process in living cells and to develop drug-like inhibitors that prevent autocrine FGF2 signalling in the context of tumour cell survival strategies.

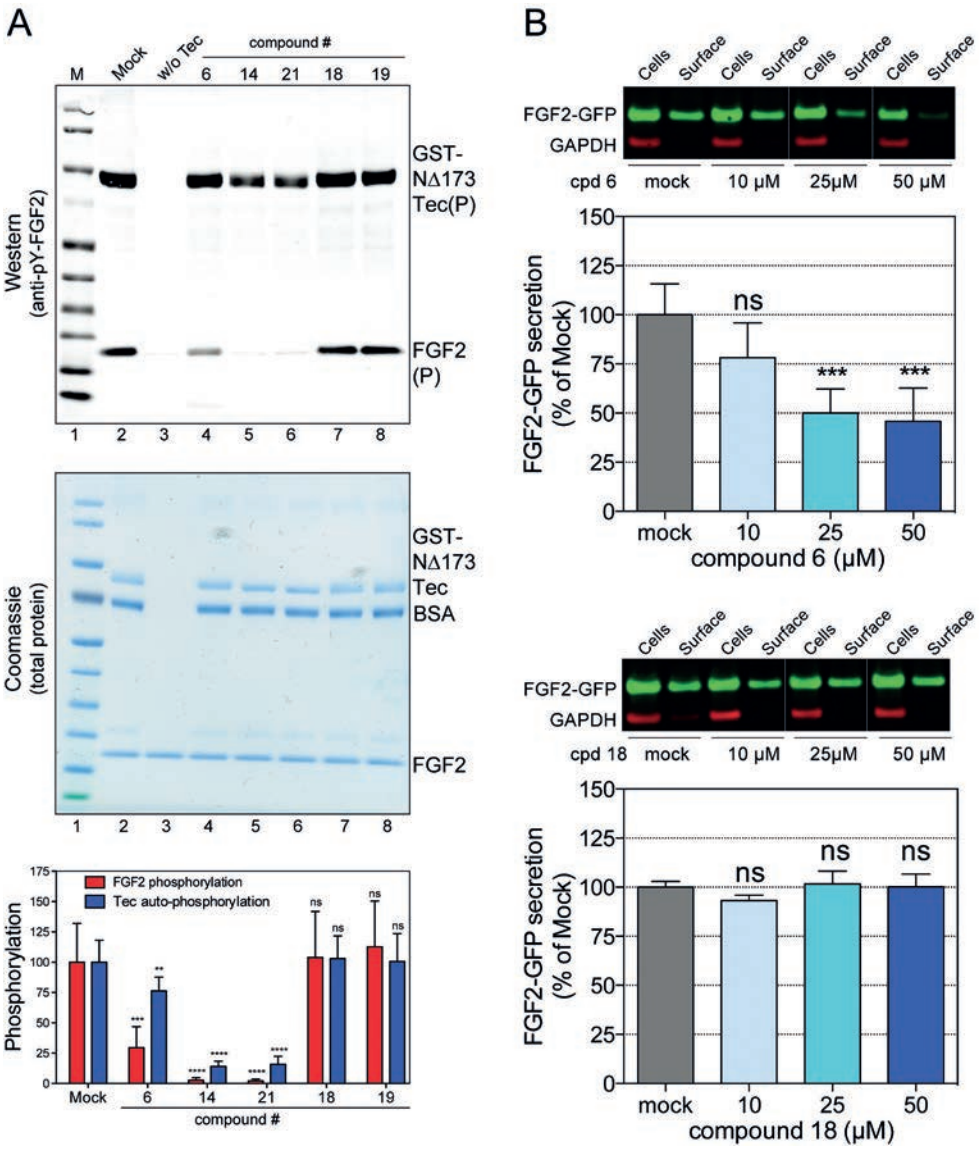

Figure 3: Small molecule protein-protein interaction inhibitors of the Tec/FGF2 complex block unconventional secretion of FGF2 (La Venuta et al, 2016).

(A) *Inhibition of Tec kinase mediated tyrosine phosphorylation of FGF2 in the presence and absence of active (cpds 6, 14 and 21) as well as inactive compounds (cpds 18 and 19).*

(B) *Inhibition of FGF2 secretion from cells in the absence and presence of compound 6 (active) and 18 (inactive).*

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Carmen Ruiz de Almodóvar

Molecular and Cellular Mechanisms of the Neurovascular Link

Goal

Our research group aims to understand the molecular mechanisms of vascular and neurodevelopment and the communication between both networks within the central nervous system.

Background

Despite their distinct functions, the nervous and vascular systems share many more similarities and common principles than previously anticipated. Recent research has demonstrated that both networks develop using similar molecular mechanisms and guidance cues and that communication between them is essential for proper formation and function of each of them. These observations bring up the new concept of an existing **Neurovascular link**. The Neurovascular link highlights the significance of a shared-tight molecular regulation between the vascular and the nervous system and underlines the importance of studying both systems together and not as separate entities. Moreover, identifying the cellular and molecular mechanisms of neuro-vascular communication is essential to understand how both systems develop and function within such complex organs as the brain or spinal cord, as well as to intervene in pathological situations.

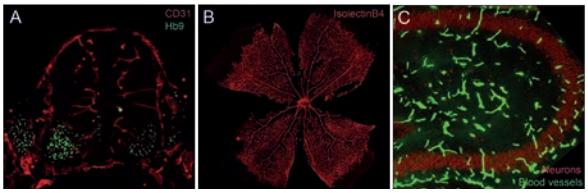

Figure 1. Model systems to study neuro-vascular communication during CNS development,
A) *Image of a spinal cord cross-section from a E11.5 mouse embryo where blood vessels are labeled with the endothelial cell marker CD31 (red) and motor neurons with the HB9 marker (green).*
B) *Whole mount image of a mouse retina from postnatal day 6 where blood vessels are labeled with IsolectinB4 (red).*
C) *Image of a developing mouse hippocampus at postnatal day 10 (blood vessels shown in green and neurons in red).*

Research Highlights

While other embryonic tissues undergo primary vascularization, it is unique that only the central nervous system (CNS) becomes secondarily vascularized by sprouting angiogenesis from a surrounding vascular plexus. Another exclusive feature of the CNS vasculature is the formation of a blood brain barrier (BBB) that restricts the passage of substances between the circulating blood and the cerebrospinal fluid and is essential for neuroprotection. Acquisition of BBB properties occurs concomitantly with developmental CNS vascularization. However, despite the fundamental and critical importance, it is surprising that very little is known about the molecular mechanisms that specifically control CNS vascularization. We are therefore interested in studying the signals that the developing nervous system sends to the growing vasculature in order to control CNS angiogenesis. As model systems we use the devel-

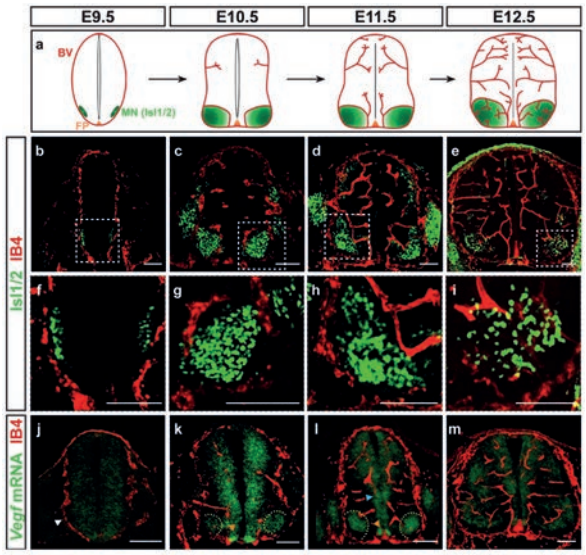

Figure 2. Motor neurons control blood vessel patterning for a specific time window during spinal cord development

A) *Scheme of SC vascularisation during mouse development (E9.5 till E12.5), showing blood vessels (BV, red), the floor plate (FP, orange), and the MN columns (green).* ***B-E)*** *Representative images of SCs at the developmental stages indicated, showing labelled endothelial cells (IB4+) and post-mitotic MNs (Isl1/2+).* ***F-I)*** *Higher magnifications of insets in (B-E). Note blood vessels stay outside the Isl1/2+ domain till E12.5.* ***J-M)*** *Representative images of blood vessel staining (IB4+) in the SC combined with ISH for Vegf from E9.5 till E12.5 in mice. At E9.5 (J), Vegf is uniformly expressed in the entire SC. From E10.5 till E12.5 (K-M) Vegf expression becomes restricted to specific neuronal domains (yellow dotted lines: MN columns; orange arrowheads: FP; blue arrowhead: neuronal progenitors).* ***N-Q)*** *Representative images of ISH for Vegf combined with immunostaining for MNs (Isl1/2+) confirming that Vegf expression is highly localised and increased in MN columns from E10.5 onwards. Insets show higher magnifications of MN columns.*

oping mouse CNS (brain, spinal cord and retina) (Figure 1). In addition, we have established in the lab the use of chicken embryos, as they are easily accessible and allow the performance of large genetic screenings in a short period of time.

Our results of the past three years show that the prototypic pro-angiogenic factor vascular endothelial growth factor (VEGF) is expressed in neuronal progenitors, the floor plate and motor neurons in the developing spinal cord. However, the sole presence and expression of VEGF cannot explain the process of spinal cord vascularization as precisely those regions where VEGF is highly expressed remain avascular during a developmental time window (Figure 2). We found that spinal cord motor neurons utilize an autocrine mechanism in order to titrate their own VEGF and thus control the amount of VEGF that the surrounding blood vessels can sense. Like that they prevent blood vessel invasion into the areas where they are located for a specific time frame. The role of other neuronal populations, as well as the signaling mechanisms involved, for CNS vascularization is currently being investigated. Likewise, we are further characterizing the mechanisms by which neuroepithelium-derived VEGF controls blood vessel growth and guidance within the CNS.

Apart of controlling vascular development, VEGF as well as other members of its family, and their receptors, are also expressed in neuronal cells and participate in processes such as neurogenesis, neuronal migration, axon guidance, dendritogenesis and dendrite maintenance. In the last years, we have focused in further elucidating how VEGF might control axon and dendritic branching of developing neurons of the hippocampus. Finally, Angiopoietins and their Tie receptors, another classical vascular ligand-receptor pair, are also expressed in distinct neuronal populations during CNS development. However, whether their expression is only required for proper CNS vascularization or whether they also exert an additional function on developing neural cells remains unknown. Using mouse genetics we are characterizing the specific function that they might have on the nervous system.

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2001 - 2009	Group Leader – European Molecular Biology Laboratory (EMBL), Heidelberg, Structural Biology Programme
since 2009	Professor for Protein Evolution, Excellence Cluster "CellNetworks", Heidelberg University
since 2013	Group Leader - BZH
since 2014	Co-Director BioQuant, Heidelberg University

Rob Russell

Understanding and predicting mechanism of gene or protein variants

Goal

The advent of high-throughput sequencing and proteomics techniques means that thousands of variants or post-translational modifications can be obtained cheaply in a single experiment. While these data have provided new insights processes & diseases, they often fall short of providing a detailed understanding of disease progression or the underlying molecular mechanism that might enable the better design of novel diagnostics or therapeutics. Our group is currently developing and applying methods to understand and predict the impact of these gene or protein changes on protein function.

Background

Mutational events are central to all human genetic disorders and to all human cancers. The advent of high-throughput sequencing techniques (HTS) now means that thousands of mutations (e.g. from a tumour or a genetic disease sample) can be obtained cheaply in a single experiment. Similarly, the advent of high-throughput, sensitive proteomics methods now mean one can also sample the space of post-translational modifications (PTMs), such as phosphorylation or acetylation, just as rapidly. While these data have provided unprecedented insights into the nature of biological processes & diseases, they fall short of providing a detailed understanding of disease progression or the underlying molecular mechanism that might enable the better design of novel diagnostics or therapeutics. The increasing speed and decreasing cost of data generation by improvements in these technologies, moreover, means that the already wide gap between biological states for which data are available and those for which even the beginnings of a mechanistic understanding is known will continue to grow.

Rewiring is a frequently cited analogy for how mutations or PTMs affect biological systems. Proteins are arranged into pathways, complexes and (generally) networks, and mutations or PTMs often affect individual interactions, effectively rewiring the existing circuits. Disease mutations that specifically affect interactions have been termed edgetic to capture the notion that it is the edges (interactions) in a biological network that are affected above the individual nodes (e.g. proteins or genes). To study rewiring in more detail requires extensive knowledge about the molecular mechanism by which proteins interact with other molecules, which ultimately comes from three-dimensional structures. My group has spearheaded methods to rapidly predict protein-protein, protein-chemical, protein-peptide interactions by homology, and most recently to predict the effect of both mutations and PTMs on biomolecular interfaces, including protein-protein, protein-drug/small-molecule and protein-nucleic acids, and our newest approaches have been applied to a

number of cancer mutations and phosphorylation and acetylation datasets revealing, indeed, that it is possible, by a systematic synthesis of mutation/PTM data with interactome and 3D structures, to obtain information about precise rewiring events in diseases.

Research Highlights

Rewiring events mediated by phosphorylation or cancer mutations

To assess the impact of mutations or PTMs on biomolecular interfaces, we have devised a system to assess the Mechanistic Impact of Structural Modifications (mechismo.russellab.org) that scores changes in particular amino acids according to their likely effects on the local structural environment. An important aspect of this work is to survey large datasets to determine general attributes, such as the fraction of data impacting on protein-protein or protein-drug interfaces, or differences between datasets from different disease groups (e.g. different cancers; Figure 1).

We have also applied this approach systematically to the current set of Cancer mutations (COSMIC database) to identify a set of 213 biomolecular interfaces that are most often perturbed in cancers. These show distinct patterns in different cancer types, and some of them correlate with patient attributes, such as the presence of P53 zinc-binding site mutations, which are associated with poorer survival across all cancers considered (Figure 2).

Another main aim of the work is to prioritise mutations or PTMs as candidates for future studies. We have already tested numerous phosphorylation sites determined largely by high-throughput studies and identified as novel protein-protein interaction switch candidates using the two-hybrid system and are currently screening several

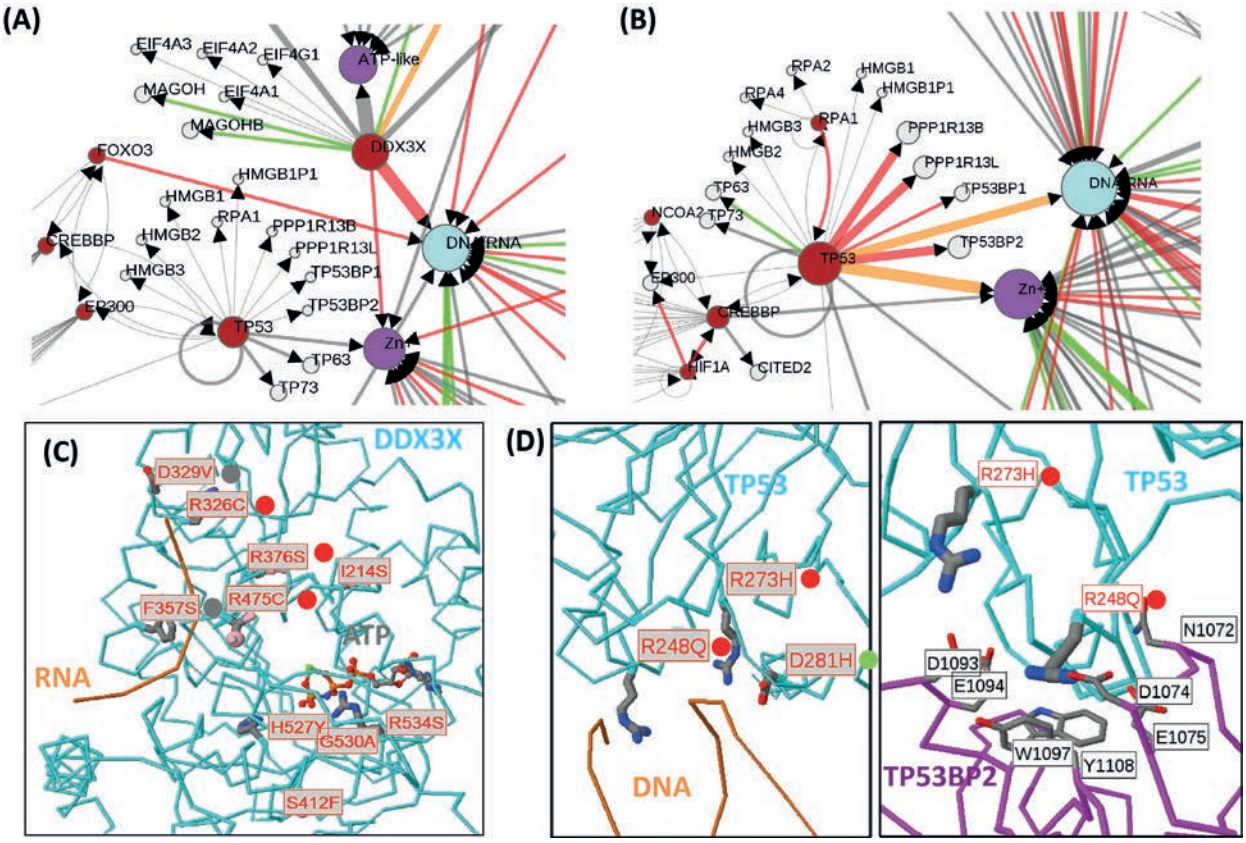

Figure 1: Variants in HTS cancer datasets
(A) *Portions of the wider network of interactions involving proteins (red if mutated, grey if not), chemicals (magenta) and DNA/RNA (blue) affected by mutations identified after sequencing Medulloblastoma tumors and Pancreatic cancer* ***(B).*** *The size of the red protein nodes is proportional to the number of variants contained within them, the size of chemical and DNA/RNA nodes is proportional to the number of sites predicted to interact with them, and the width of edges is proportional to the number of sites affecting them. Red edges are those where the effect of the mutations is predicted to diminish the interaction, green to enhance and orange where different mutations have opposite effects.* ***(C)*** *Structures of DDX3X showing Medulloblastoma mutations affecting DNA or ATP-binding, and* ***(D)*** *mutations in Pancreatic cancer affecting functional interactions of TP53 with DNA and TP53BP2.*

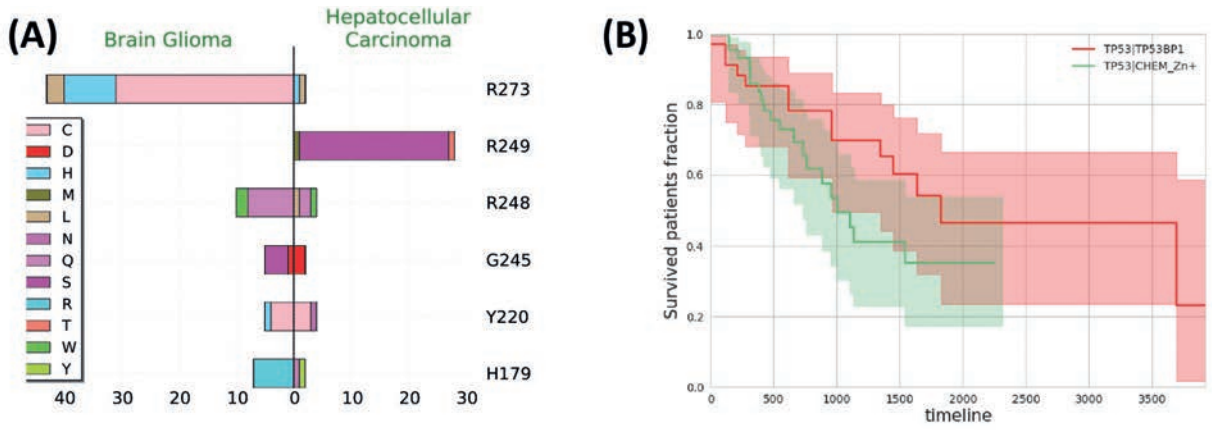

Figure 2: Interrogating mechanism within all cancer types

(A) *Differences in the position and nature of mutations within TP53 in Glioma and Hepatocellular carcinoma. The bars show the number of samples containing a particular motif, with the preferred amino acid substituents coloured as indicated. All of the residues shown (left of figure) are at either the DNA or regulatory protein interfaces.* ***(B)*** *Survival curve showing how tumors having mutation of the TP53 zinc binding site (red) have a poorer survival (across all cancers) compared to those having other TP53 mutations (green).*

dozen cancer mutations predict to have particular impacts on interactions with critical cancer signaling molecules such as GTPases and kinases.

Combining proteomics & genetics to interrogate a disease relevant organelle

Genetic diseases represent another where we use our tools to delineate mechanism and to relate it to disease variants & mutations. As part of an FP7 Systems Biology Project (Syscilia) in collaboration with 15 groups across Europe, combined affinity-proteomics/mass-spectrometry, genetics and cell biology to interrogate cilia: still poorly understood, cell signaling hubs whose defects cause ciliopathies, devastating heterogeneous genetic diseases.

We selected 217 known/candidate human ciliary proteins and using affinity tagging and mass-spectrometry we identified a high resolution landscape of 4905 interactions and 52 complexes involving 1319 proteins. The landscape provides unprecedented details of ciliary signalling and proteostasis, highlighting essential connections to ciliary and vesicle transport, the cytoskeleton, signaling, and ubiquitination (Figure 3).

We extended our Socioaffinity method originally developed for the Yeast proteome and because of the extensive reverse-tagging (i.e. where multiple components of a complex are tagged) and repeated experiments, it was able to delineate sub-complexes, including novel sub-complexes in Intraflagellar transport complex B (IFT-B), which we showed is indeed two complexes by sucrose density centrifugation and by EPASIS, which couples MS-proteomics to increasing concentrations of denaturants. Using the Mechismo approach (above) and binding site hot-spots we also identified several variants in very severe ciliopathies that specifically disrupted different sub-complexes as confirmed by subsequent comparisons between wt and variant proteins.

The interactome included many (over 1000) proteins not previously known to be ciliary overlaps and intriguingly several of these are known, when mutated, to cause genetic diseases not known to be ciliary, and for one (3M Syndrome) we showed that three known causative genes affect ciliogenesis (when knocked-down), and that fibroblasts from patients lack cilia.

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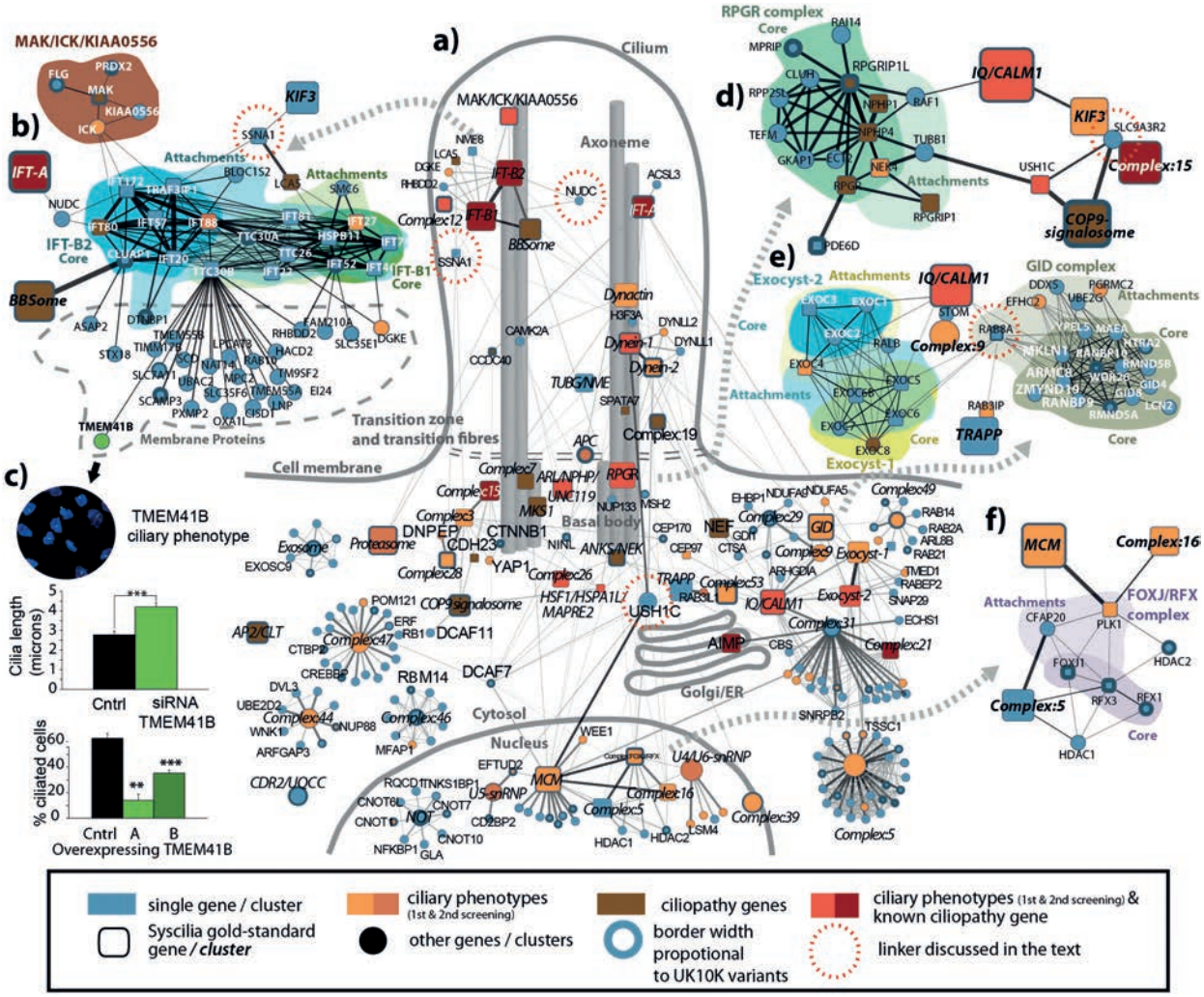

Figure 3: Overview of the ciliary landscape

a) *Complexes/proteins identified in this study. Rounded boxes show complexes (components not shown), circles denote proteins. Edge thickness is proportional to socioaffinity, and proteins/complexes are coloured according to whether they have ciliary phenotypes or variants in UK10K ciliopathy patients.* ***b,d,e,f)*** *show complexes in detail, with core and attachment subunits shaded accordingly.* ***c)*** *effect of TMEM41B siRNA knock-down or over-expression of on ciliary length.*

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2006 - 2009	Director - BZH
2011 - 2013	President of the GBM

Irmgard Sinning

Macromolecular machines in protein targeting and membrane protein biogenesis

Goal

We aim to understand the structure and the functional mechanisms of macromolecular assemblies in protein folding, targeting and membrane protein biogenesis.

Background

Already during protein synthesis at the ribosome, nascent proteins experience numerous interactions with (1) targeting factors, and (2) chaperones and enzymes (**Fig. 1**). We aim at detailed insights into the structure and function of the involved molecular machines by an integrated structural biochemistry approach combining protein crystallography as our key method with biochemical and biophysical techniques.

(1) Our long-term interest is the understanding of the delivery pathways for membrane proteins by the signal recognition particle (SRP) and the system for guided-entry of tail-anchored membrane proteins (Get). The SRP pathway directly couples protein synthesis at the ribosome to membrane targeting and insertion, thereby the exposure of hydrophobic transmembrane domains is avoided. SRP recognizes a signal sequence at the N-terminus of target proteins, while tail-anchored (TA) membrane proteins contain a single transmembrane domain at their C-terminus. This excludes Get substrates from the co-translational SRP pathway. Although the composition of the SRP system differs in the three kingdoms of life, the central SRP core consisting of SRP54 and its cognate binding site on the SRP RNA are conserved. However, the SRP system has also been adapted for specific requirements. Here the post-translationally acting SRP in chloroplasts (cpSRP), which lacks the SRP RNA, is an interesting example. It guides nuclear encoded light-harvesting proteins (LHCPs) to the thylakoid membrane assisted by cpSRP43, a unique component of cpSRP. Over the years, we collected structural snapshots of SRP and SRP receptor (SR) as well as of components of the Get machinery in different functional states with a particular interest in the membrane-associated steps. We aim to finally arrive at a molecular movie of co- and post-translational membrane protein biogenesis in all kingdoms of life. (2) During synthesis at the ribosome, proteins are subject to enzymes for modification and chaperones that assist in folding. These factors seem to share overlapping binding sites at the ribosomal surface close to the tunnel exit. In order to understand the carefully orchestrated interplay of all these different factors, we started to dissect the mechanistic details of eukaryotic ribosome associated chaperones and enzymes.

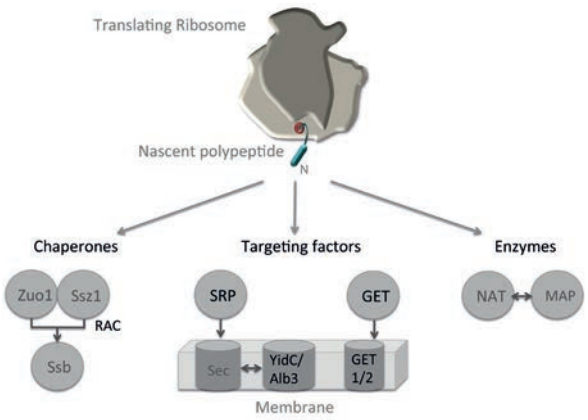

Fig. 1: *Early factors associating with the nascent polypeptide chain at the ribosome – crowding at the tunnel exit.*

Is the ribosome more than a binding platform for factors acting on the nascent chain? Are ribosomal proteins and RNA involved in the selective interaction of all these different factors with specific nascent chains? To answer these questions and to study complex macromolecular assemblies, we combine *in vitro* and *in vivo* analyses.

Research Highlights

Almost three decades after the discovery of SRP, structural and functional studies of the SRP system are rather advanced. However, important data on the bacterial, eukaryotic and chloroplast SRP systems are still missing. Eukaryotic SRP can be divided into an Alu domain involved in the retardation of protein synthesis (so-called elongation arrest) and an S domain responsible for signal sequence recognition, translocon interaction and regulation. The Alu domain is the precursor of the *Alu* elements, which represent more than 10% of the primate genome and play important roles in genome stability. In eukaryotic SRP, the Alu domain consists of half of the SRP RNA and two proteins (SRP9 and SRP14), which are essential for the Alu RNA to adopt a stable fold. In Archaea and most grampositive bacteria the Alu domain is present, but lacks the protein components. The high resolution X-ray structure of the *Bacillus subtilis* Alu RNA answered a number of important questions in the SRP field. The structure shows a conserved, highly complex tRNA-like fold with several inbuilt stabilizing elements (helix 1 and an extended loop-loop pseudoknot) and a novel mode of minor groove interactions (**Fig. 2**). It explains why proteins are dispensable for the archaeal and bacterial Alu domains, and confirmed the “closed” conformation of the Alu domain inferred from previous cryo-EM work and modeling. Placing this structure in the cryo-EM density (with R. Beckmann, Munich) allowed to deduce the mechanism of elongation retardation by competition with the aa-tRNA/EF-Tu complex at the ribosome.

Another “grey zone” in our understanding of human SRP concerned its two largest protein components, SRP68 and SRP72, which are essential for both elongation arrest and protein translocation. These proteins were notoriously difficult to handle, probably because they are highly flexible. However, we managed to first determine the

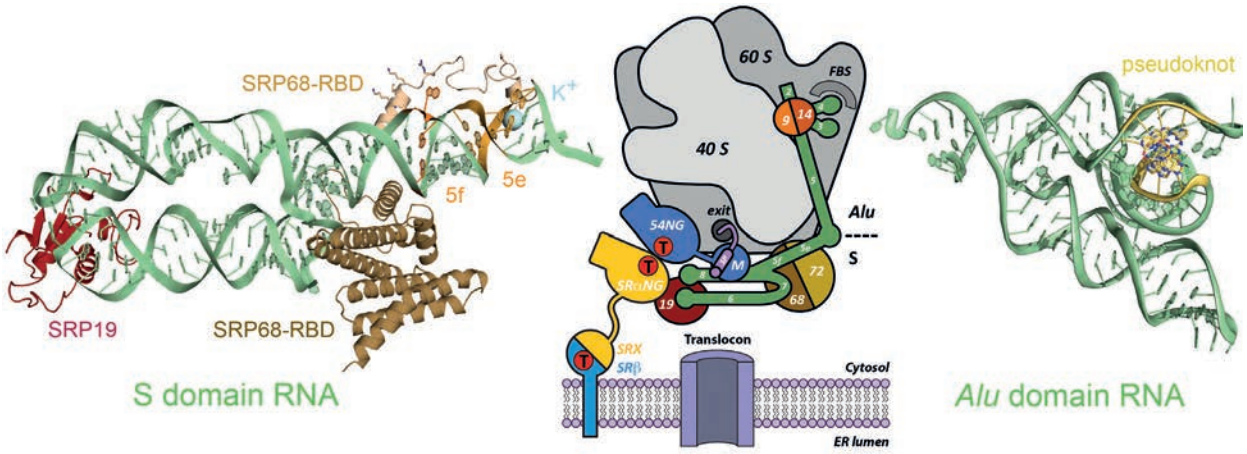

Fig. 2: Insights in to mammalian SRP (middle). Remodeling of the SRP RNA by SRP68/72 (left) *shapes the distal binding site at the 5f-loop.* ***The prokaryotic Alu domain (right)*** *lacks the protein components.*

structure of the RNA-binding domain (RBD) of SRP68 alone as well as of a large part of the human S domain (125 nts RNA, SRP19 and the 68-RBD) (**Fig. 2**). This structure provided a plethora of novel insights into protein-RNA recognition, S domain organization and SRP dynamics. SRP68 modulates the RNA structure in an unexpected way, visualizing why SRP68 is crucial for SRP function. Encouraged by this breakthrough, we continued our efforts on SRP72, and determined the structure of its protein-binding domain (interacting with the SRP68-PBD) and of the SRP72-RBD now in complex with the complete S domain RNA (145 nts RNA, SRP19, 68-RBD, 72-RBD) (**Fig. 2**). The 72-RBD appears as a linear motif that crawls along the "distal site" of the SRP RNA at the 5e- and 5f-loop, with strictly conserved residues (W577, R576, R581) stabilizing the 5e- and 5f-loop geometries. These detailed structural insights in SRP68/72 were crucial to interpret previous cryo-EM data, and allowed for a first detailed model of the human SRP targeting cycle, which involves dynamic rearrangements of SRP and its receptor at the ribosome.

The SRP GTPases form a unique subfamily of the NTP binding proteins, and during protein targeting, the GTPases of SRP54 and the SR (SRα in human SRP, FtsY in bacteria) form a quasi-symmetric heterodimer (the targeting complex, TC). With the structure determination of the archaeal, human and chloroplast TCs, we obtained detailed insights into conservation and specific adaptations of this complex, which allowed us to classify conserved binding sites for external ligands, e.g. the SRP RNA responsible for activation of GTP hydrolysis. However, the human SRP system comprises yet another G protein, SRβ, which is not an SRP GTPase and belongs to the Arf/Sar family of small G proteins. We derived the unique switch cycle of SRβ and integrated the third GTPase into the SRP pathway. Using cross-linking, ribosome binding and translocation studies (with M. Pool, Manchester) we showed that the mammalian SR contains distinct ribosome and translocon interaction sites, and that it switches the Sec61 translocase from Sec62 to SRP-dependent translocation. Thereby, we identified an important function of the SR, which mechanistically links two seemingly independent modes of translocation.

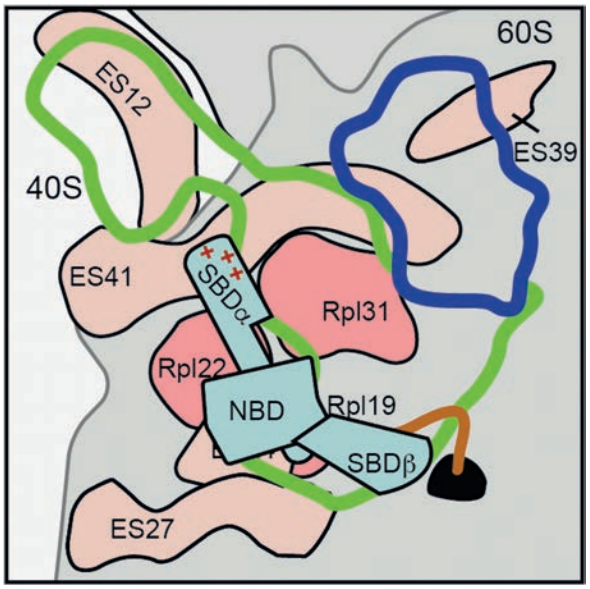

Fig. 3: Ribosome binding of the co-translational chaperone Ssb involves ribosomal proteins and RNA. *The position of RAC is outlined in blue (Ssz1) and green (Zuo1).*

While canonical membrane protein biogenesis requires the co-translational delivery of ribosome-associated proteins to the Sec translocase, the high-throughput delivery of the abundant LHCPs to the Alb3 membrane insertase in chloroplasts occurs post-translationally. Therefore, a transit complex is formed in the stroma consisting of cpSRP54, cpSRP43 and LHCPs. We have previously shown that the delivery of the transit complex to Alb3 involves positively charged motifs in the Alb3 C-terminus. Continuing our studies, we used a hybrid approach involving NMR and X-ray crystallography, and resolved the structural basis of negative cooperativity underlying cpSRP43 chromodomain interactions within the transit complex and with Alb3.

More recent research activities center on interactions of nascent chains with chaperones. The ribosome associated complex (RAC) is a unique chaperone complex, consisting of an inactive Hsp70 (Ssz1) and an Hsp40 protein (Zuo1). After resolving the interaction of RAC with the ribo-

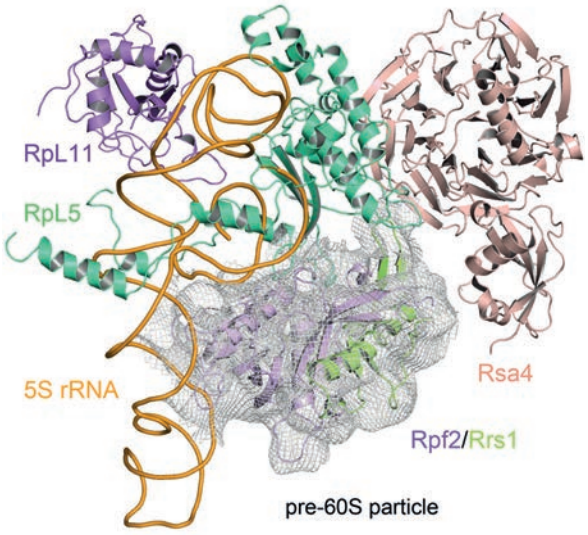

Fig. 4: Eukaryotic ribosome biogenesis. *Snapshot of 5S RNP trapped by its protein neighbours during 60S maturation.*

some by cryo-EM (with R. Beckmann), we now focused on the Hsp70 chaperone (Ssb), which is activated by RAC. Using an engineered disulfide bridge to stabilize the ATP-bound form of Ssb, we determined its crystal structure, and combined the structural information with ribosome-binding and crosslinking (with S. Rospert, Freiburg). By this integrated approach we could show that the Ssb C-terminus is required for ribosome interaction in the vicinity of the tunnel exit, and that Ssb contacts ribosomal proteins and RNA (**Fig. 3**). Our data allowed us to link the conformational rearrangements upon ATP hydrolysis to specific contacts of Ssb with the ribosome.

In the last years, we got interested in the assembly and maturation of eukaryotic ribosomes, which is a complex and highly regulated process, and involves a myriad of biogenesis factors (with Ed Hurt). We embarked on a medium throughput structural genomics project of ribosome biogenesis factors from the thermophilic fungus *Chaetomium thermophilum*, and continued on the nuclear import and assembly of the 5S RNP (consisting of 5S RNA and the two ribosomal proteins L5 and L11). Crystal structures of proteins were determined acting near the 5S RNP, including Rrs1, Rpf2 and Rsa4, which occupy strategic positions in the pre-60S ribosomal subunit to block the rotation of the 5S RNP (**Fig. 4**). Taken together, structural snapshots of ribosomal biogenesis factors are crucial to finally understand eukaryotic ribosome biogenesis.

Selected Publications 2014 - 2016

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Gumiero, A., Conz, C., Valentín Gesé, G., Zhang, Y., Weyer, F. A., Lapouge, K., Kappes, J., von Plehwe, U., Schermann, G., Fitzke, E., Wölfle, T., Fischer, T., Rospert, S. & Sinning, I. (2016) Interaction of the cotranslational Hsp70 Ssb with ribosomal proteins and rRNA depends on its lid domain, Nat. Comms. 7: 13563.

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Kempf, G., Wild, K. & Sinning, I. (2014) Structure of the complete bacterial SRP Alu domain, Nucl. Acids Res. 42: 12284-94.

Grotwinkel, J.T., Wild, K., Segnitz, B. & Sinning, I. (2014) SRP RNA remodeling by SRP68 explains its role in protein translocation, Science 344: 101-104.

Leidig, C., Thoms, M., Holdermann, I., Bradatsch, B., Berninghausen, O., Bange, G., Sinning, I., Hurt, E. & Beckmann, R. (2014) 60S ribosome biogenesis requires rotation of the 5S ribonucleoprotein particle, Nat. Comms. 5: 3491.

Awards and Honors

2016	Member of the Heidelberg Academy of Sciences and Humanities
2014	Heidelberg University Research Award
2014	Gottfried Wilhelm Leibniz Prize
2014	Constitution Medal of Bavaria
2010	HMLS Investigator Award
2010	Member of EMBO
2010	Member of LEOPOLDINA

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1991	Ph.D. - Ludwig-Maximilians-Universität München, Germany
1991 - 1993	PostDoc - Sloan-Kettering Institute, New York, USA
1994 - 1997	Assistant Laboratory Member - Sloan-Kettering Institute
1998 - 2004	Assistant Professor - Sloan-Kettering Institute
2004 - 2005	Associate Professor - Sloan-Kettering Institute
since 2005	Full Professor - BZH
since 2016	Director - BZH

Thomas Söllner

Regulated Membrane Fusion: Molecular Mechanisms and Machinery

Goal

To solve the entire reaction cascade mediating regulated exocytosis on a functional and structural basis. Using neurotransmitter release as a model system, the physiological events shall be reconstituted in an *in vitro* liposome fusion assay employing purified proteins and defined lipid species to reveal the concerted interplay of the components at distinct reaction steps.

Background

Regulated exocytosis at the neuronal synapse is a multistep process starting with synaptic vesicle tethering at the active zone followed by vesicle priming and culminating in fast calcium-triggered membrane merger. Distinct protein-protein interactions forming macromolecular assemblies control individual steps and sequentially bring the two membranes into close proximity creating a hemifusion intermediate, which finally leads to fusion pore opening and dilation. The key protein machinery has been identified: vesicle tethering: Rab3, Rim1; priming: Munc13-1 and Munc18-1; calcium regulation: synaptotagmin1 and complexin; fusion machinery: SNARE proteins (syntaxin1, SNAP-25, VAMP2). In addition, specific lipids such as phosphoinositides and diacylglycerol play distinct functions in the assembly reaction (see Figure 1).

In more detail, following the initial tethering step, Munc13-1 and Munc18-1 control the initial pairing of syntaxin1 and SNAP-25 on the plasma membrane with VAMP2 on synaptic vesicles, thereby contributing specificity to membrane trafficking. Subsequently, the trans SNARE complex (SNAREpin) starts to zipper into a four-helix bundle. This protein folding reaction provides the driving force for membrane fusion. Complexin stabilizes a half-zippered SNAREpin but blocks the zippering of the membrane proximal SNARE motifs. Synaptotagmin1, the calcium sensor contributes to this block in an ill-defined manner and in the presence of calcium confers fast synchronized membrane fusion. How these proteins are exactly arranged in the distinct prefusion intermediates and how they affect the lipid environment and *vice versa* are key questions in cell biology. Furthermore, the opening and structure of fusion pores remain obscure events.
Reconstituted membrane fusion assays, including purified proteins and liposomes are instrumental to reveal structural and functional contributions of individual components in a well-defined lipid bilayer system.

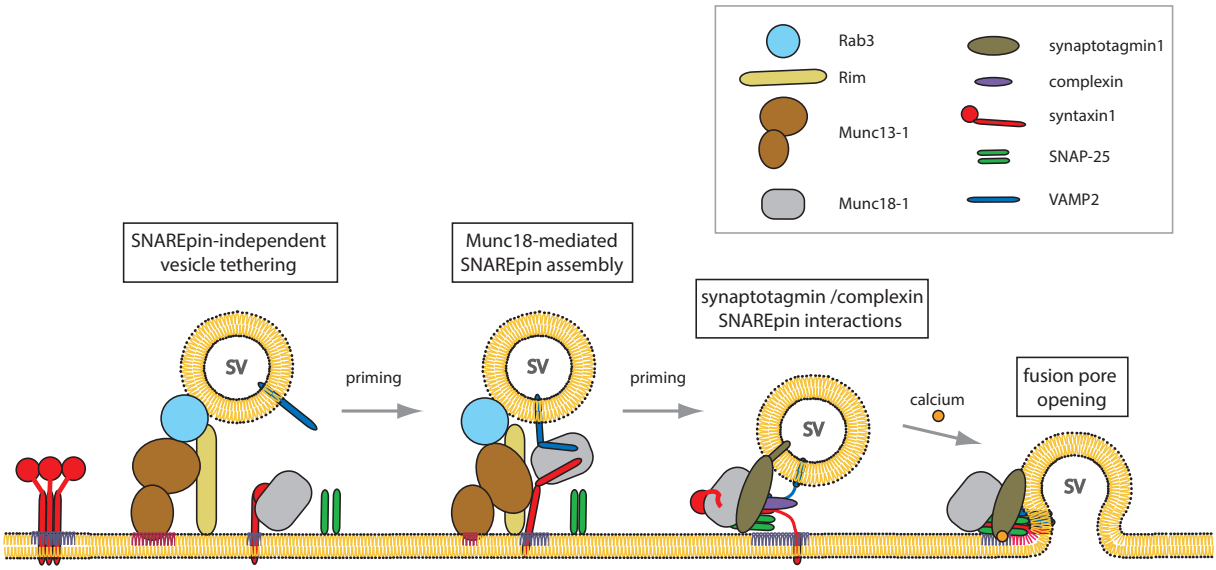

Figure 1: Components and reaction steps involved in regulated exocytosis at the neuronal synapse. *Please note that only functionally relevant components are shown at the distinct reaction steps. Protein-lipid interactions are depicted by colored lipids.*

Research Highlights

By reconstituting preassembled syntaxin1/SNAP-25 into giant unilamellar vesicles (GUVs, mimicking the plasma membrane) and VAMP2, together with synaptotagmin1 into small unilamellar vesicles (SUVs, mimicking synaptic vesicles) and adding complexin and Munc18-1, we were able to functionally reconstitute the equivalent of a readily releasable pool of vesicles, which fuses in a calcium-synchronized manner. Using cryo electron tomography in collaboration with John Briggs at the EMBL, we could demonstrate that this late prefusion intermediate results in the formation of membrane protrusions on GUVs, which likely lower the activation energy for membrane fusion, permitting calcium-triggered fusion pore opening on a sub-millisecond time scale.

To allow such a fast response, the prefusion intermediate relies on weak protein-protein and protein-lipid interactions. Considering the membrane constraints and the weak interactions, it is technically challenging to identify the relevant binding sites. To determine interaction sites on a single amino acid level, we started to scan the accessory helix of complexin, which contributes to the fusion-clamp. Using a site-specific photo-crosslinking approach, we introduced the unnatural photo-activatable amino-acid p-benzoyl-l-phenylalaine (BPA) at every position covering amino acids 23-49 of complexin II (see Figure 2). Most of these substitutions, with the exception of E23 had only minor effects on the clamping function of complexin in the reconstituted fusion assay. However, upon photo-activation, crosslinks to the nearest neighbor impaired significantly calcium triggered lipid mixing. The systematic analysis of the crosslink products revealed that the accessory helix of complexin provides two interacting surfaces: one providing a binding site for the membrane proximal region of VAMP2, the other one an interaction site for the C-terminal region of the second SNARE motif of SNAP-25. Thus, the interactions with the C-terminal regions of VAMP2 and SNAP-25 apparently block final SNAREpin zippering. Considering the weak interaction, the simultaneous temporal C-terminal “inactivation” of two SNAREs could provide an efficient clamp not compromising the fast calcium trigger. Indeed, independent quadruple mutants of the relevant complexin residues covering the VAMP2 and SNAP-25 binding sites separately resulted in a significant loss of the fusion clamp. The role of E23 in fusion clamping could be confirmed *in vivo* in living neurons (collaboration with Christian Rosenmund, Charite-Universitätsmedizin Berlin).

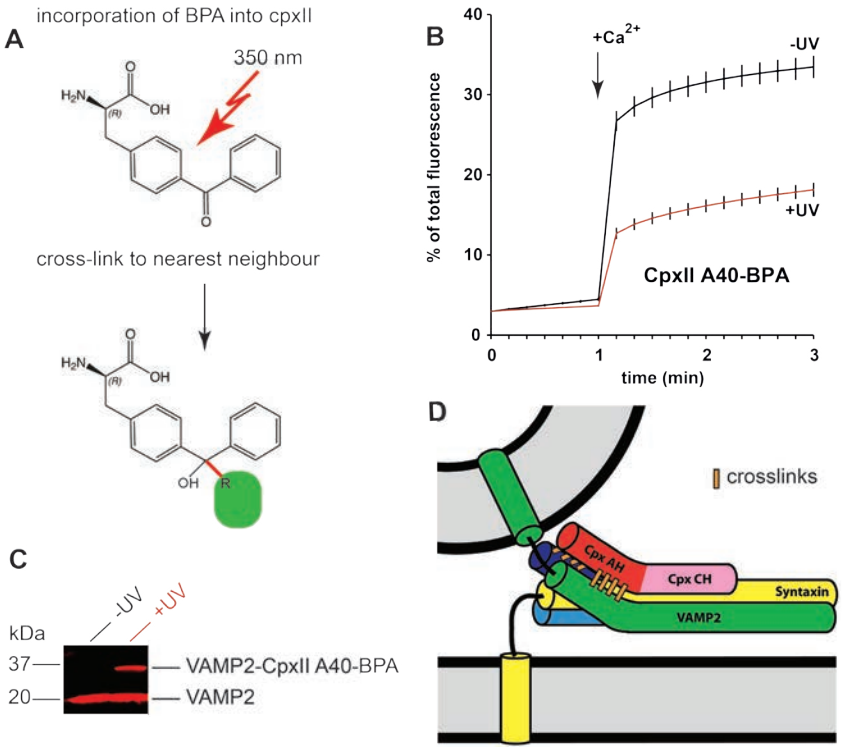

Figure 2: Site-specific arrest of the fusion machinery by crosslinking of the complexin II accessory helix to VAMP2
(A) *Scheme showing the general p-benzoyl-l-phenylalanine (BPA) photoreaction.*
(B) *BPA in position 40 impairs fast calcium-triggered lipid mixing upon photo-activation. GUVs containing preassembled syntaxin1/SNAP-25 and SUVs containing synaptotagmin1 and VAMP2 were preincubated on ice in the presence of mutant complexin II (CpxII A40BPA). One sample was UV irradiated at 350 nm for 15 seconds and subsequently fusion kinetics were recorded at 37°C. After 1 minute, 100 µM free Ca2+ was added to trigger fusion.*
(C) *CpxII A40BPA cross-links to VAMP2 as determined by Western blot analysis.*
(D) *Model, summarizing the results of a series of crosslinking experiments (systematic BPA incorporation), reveals a bipartite interaction of the Cpx accessory helix (Cpx AH) with the membrane-proximal regions of VAMP2 (green) and SNAP-25 (dark blue).*

This photo-crosslinking approach will now be extended to other proteins of the fusion machinery.

To identify further functional interaction sites in the fusion machinery we also introduced mutations at distinct positions of synaptotagmin1 and SNAP-25. In synaptotagmin1, we could show that the positively charged amino acids R398/R399, which are opposite to the calcium-binding site, play a postdocking role in membrane fusion. R398/R399 interact with the SNAREs and presumably directly couple the calcium-dependent synaptotagmin1-lipid interaction to rearrangements in the SNARE machinery. Collaborations with the groups of Matthijs Verhage at the University of Amsterdam and Jakob Sorensen at the University of Kopenhagen demonstrated the physiological relevance of this interaction in neuronal cultures. Along the same line we also probed the other side of the interaction by introduced mutations in both helices of SNAP-25. Our *in vitro* experiments in combination with electrophysiological measurements in neurons (in collaboration with Jakob Sorenson), revealed that interactions between SNAP-25 and synaptotagmin1 are involved in vesicle priming, clamping spontaneous releaseand stimulating evoked neurotransmission.

Using GUVs containing syntaxin1 instead of preassembled t-SNAREs, we were also able to analyze earlier priming steps in SNARE complex assembly, which are controlled by Munc18-1. We could demonstrate that Munc18-1 phosphorylation at Y473 shuts off neurotransmitter release without affecting vesicle docking. This phosphorylation selectively impairs VAMP2 binding. A collaboration with Matthijs Verhage demonstrated that this tyrosine phosphorylation impairs the formation of the readily releasable, “sucrose–sensitive” vesicle pool.

We also established a single vesicle fusion assay using SUVs immobilized on a cover slip and FRET based lipid- and content-mixing using TIRF microscopy (see Figure 3). This assay will now allow us to resolve the role of regulatory components in vesicle docking und subsequent membrane fusion at the single vesicle level.

Overall, we have set up *in vitro* reconstituted lipid- and content-mixing assays at the biochemical and microscopic level. Crosslinking approaches have been developed to detect direct interactions

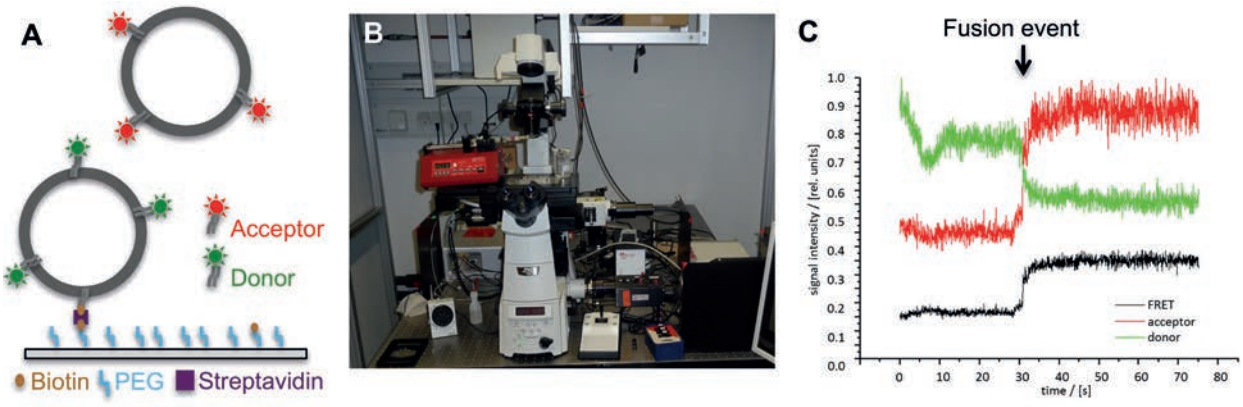

Figure 3: Single vesicle fusion assay using FRET-based lipid mixing and TIRF microscopy. (A) *SUVs containing e.g. t-SNAREs and a lipid-coupled donor fluorophore (e.g. an Atto dye) are immobilized on a cover slip using a lipid-biotin/streptavidin/ biotin-PEG sandwich. SUVs containing v-SNAREs and the acceptor fluorophore are added in the presence/absence of regulatory components.* ***(B)*** *Reactions are analyzed by TIRF microscopy.* ***(C)*** *Docking can be monitored by the colocalization of the donor and acceptor dyes. Fusion results in increased FRET and quenching of the donor fluorophore.*

at the single amino acid level allowing us to probe the local environment of distinct components in a stage-specific manner. However, we still need to establish the mass spectrometry analysis to identify the interacting peptides/residues. Collaborations with groups using electrophysiology with its exquisite temporal resolution in living cells (neurons, chromaffin cells) will demonstrate the physiological relevance. Cryo-electron microscopy employing single particle analysis and tomography using direct electron detectors should provide high-resolution structural insights into the structural organization of fusion machinery assembly intermediates and fusion pore dynamics. Thus, systematic functional and structural analyses of the membrane fusion machinery should be in reach.

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J. Biol. Chem. 289: 9639-9650.

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1978	Ph.D. - Ludwig-Maximilians-Universität München, Germany (Max Planck Institute of Biochemistry, Martinsried)
1978 - 1986	PostDoc and Group Leader - University of Regensburg, Germany
1986 - 1988	Visiting Scientist - Dept. of Biochemistry, Stanford University, USA
1988 - 1997	Full Professor and Chairman of Biochemistry I - Heidelberg University, Germany
1991 - 2003	Chairman SFB 352
1997 - 2002	Director - BZH
since 2001	Managing Editor FEBS Letters
2005 - 2007	President of the GBM
2004 - 2015	Chairman SFB 638
2014 - 2015	Director - BZH

Felix Wieland

Molecular mechanisms of COPI transport

Goal

We are interested in the molecular mechanisms that underlie the formation and uncoating of coated vesicles, and the structure of their coats. (Since establishing her own group Britta Brügger continues research on lipid/protein interactions and on lipidomics).

We are characterizing the components of vesicular coats and their coordinate action that allow formation, fission and uncoating of Golgi- or semi-intact cell derived coated vesicles. This includes proteomics and lipidomics, functional in vitro assays and reconstitution of individual functional steps in a chemically defined liposomal system.

Background

In the eukaryotic cell, vesicular transport represents the basic mechanism for i) maintaining the homeostasis of the endomembrane system, ii) biosynthetic transport of newly synthesized proteins and lipids, and iii) the uptake and intracellular transport of exogenous macromolecules. Three classes of coated vesicles are well established to mediate transport in the exo- and endocytic pathway: COPII vesicles for ER export, COPI vesicles for retrograde Golgi-ER and bidirectional intra-Golgi transport, and various Adaptor Protein-containing clathrin-coated vesicles operating in the late secretory and endocytic pathways. Coat components are involved in multiple tasks such as cargo selection, curvature formation at the donor membrane, vesicle fission and initiation of uncoating. We are interested in the molecular mechanisms underlying such vesicular transport..

In our view, the formation of a COPI transport vesicle involves the following minimal set of components: donor membranes with transmembrane proteins acting as coat and/or cargo receptors (e.g. members of the p24 family), cytosolic Arf1, cytosolic coatomer and auxiliary enzymes that serve activation on the membrane of Arf1 (GBF1) and the activation of GTP hydrolysis by Arf1 (Arf GAPs).

A schematic view of individual steps in COPI vesicle biogenesis is given in Fig. 1. In contrast to COPI, where the heptameric coat component coatomer is recruited to the membrane en bloc, the COPII-and clathrin coats are recruited in two subsequent steps.

Research Highlights

Proteomics of COPI and COPII vesicles

In order to further understand the functions of COP vesicle isoforms, we have cloned and expressed in insect cells the various isoforms of both the mammalian COPI and COPII systems.

Vesicle preparations were obtained from semi-intact cells with the isoformic coats and subjected to quantitative comparing proteomic analysis by SILAC. Whereas clear differences are observed with regard to cargo proteins in isoforms of COPII vesicles, very surprisingly the protein compositions of all four isoforms of COPI vesicles are strikingly similar, even in three different cell types (Adolf et al.,2016, Adolf et al, in prep., Rhiel et al., in prep.). In light of a heterogeneous partition to the various Golgi stacks of COPI isoforms this homogeneity poses a puzzling problem. In this regard, Julien Bethune describes for the first time evidence of a basic cellular function exclusively of COPIγ1 isoforms (see report Bethune group).

Reconstitution of Clathrin coated vesicles (CCVs)

In the last period we have expressed in insect cells clathrin heavy and light chains as well as complete and functional AP1 and AP2 complexes, together with key molecules described for the formation and scission of AP2 CCVs. Presently we are establishing reconstitution of the corresponding vesicles from liposomes, in order to study their mechanisms of scission from their donor membranes. Together with Frank Adolf and John Briggs we plan to solve the structure of AP1 CCVs by cryo EM.

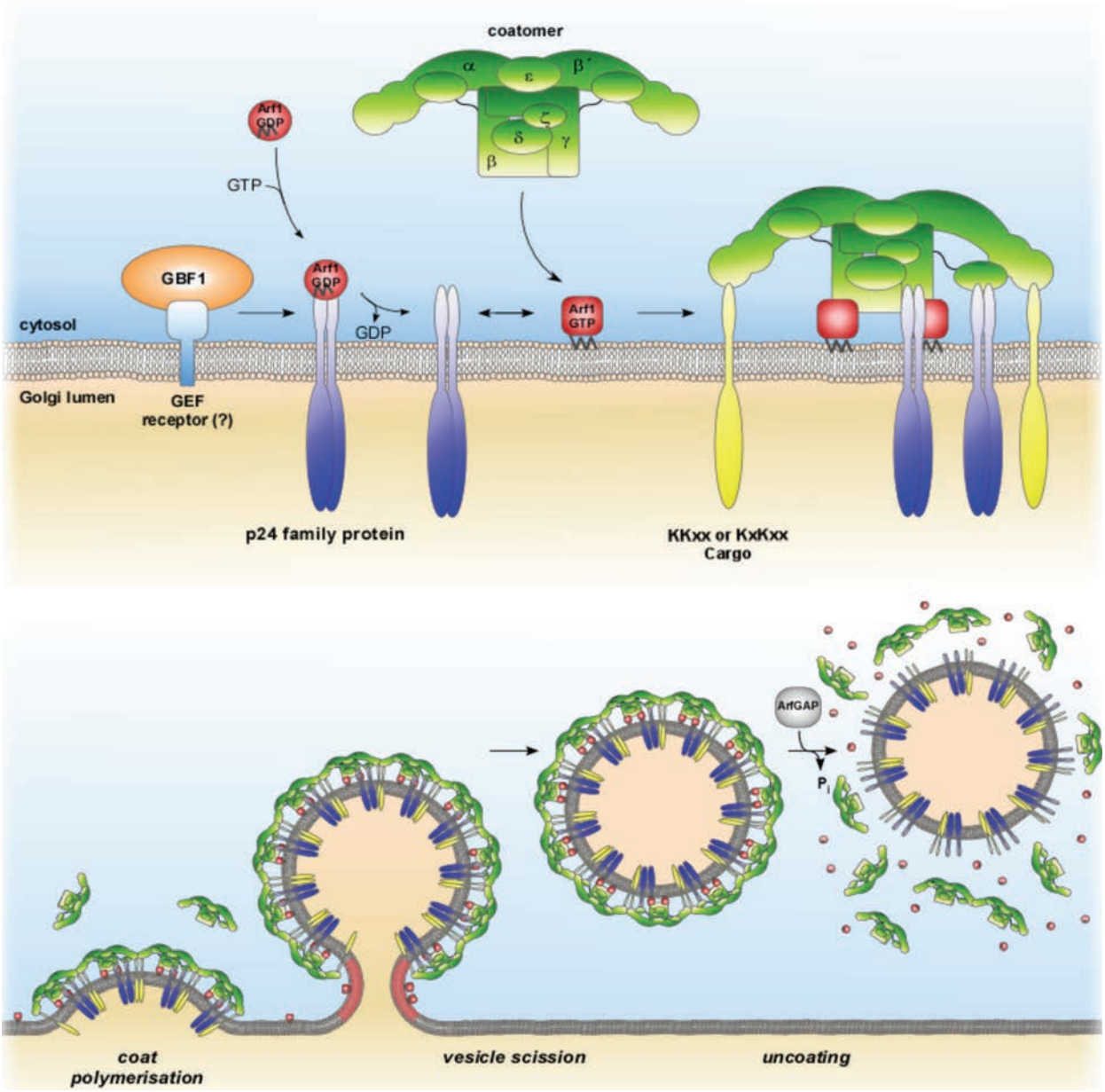

Fig. 1: Upper panel: *Arf-cycle for coat recruitment and dissociation (for details see text).* ***Lower panel:*** *Vesicle scission and ArfGAP-dependent dissociation of the coat.*

Structure of the COPI coat and molecular mechanisms of its dissociation

High resolution structure of the COPI coat

Together with John Briggs' group at the EMBL we are investigating the structure of the coatomer shell on coated vesicles. With the first data of a coat on a membrane, a structure has emerged that is strikingly different from those of the COPII and the clathrin systems as delineated from protein assemblies in solution. The basic unit of the coat lattice turned out to be a coatomer triade. Triades can be arranged on the vesicular membrane in various patterns that are defined by variable vertices at the contact sites of triades (Faini et al., 2012 Science). Higher resolution cryo EM (Dodoneva et al., 2015, Science) combined with x-ray crystallography in collaboration with Irmi Sinning's group has allowed us to unequivocally attribute all masses found to individual subunits and structural elements thereof, including a βδ-COP subcomplex resolved by x-ray crystallography at 2.7A°. From these data a stoichometry emerges of 2Arf to 1coatomer, and surprisingly different chemical environments for the two GTPase molecules emerge. We propose that the different sites of Arf within the coat reflect different functions in the process of coating and uncoating (Dodoneva, Aderhold et al. in preparation, and see Fig.2.

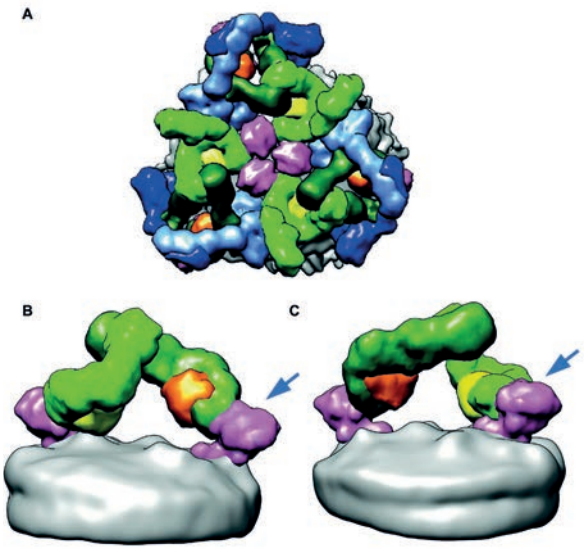

Fig. 2: 2)Top: *A triade of coatomers with Arf molecules in pink (three center Arfs linked to γ-COPs, and three peripheral Arfs linked to β-COPs), viewed from top.* ***Bottom:*** *Side views of β-Arf (arrow in B) and of γ-Arf (arrow in C).*

Role of Arf1 in coat dissociation

Fusion of a transport vesicle with its target membrane requires prior dissociation of its protein coat. As outlined above, the small GTPase Arf in its GTP-loaded state anchors coatomer to the membrane by different interactions: one Arf via the trunk domain of β-COP together with δ-COP (Dodoneva et al. in preparation), and another Arf by the trunk domain of γ-COP. According to the current view of the field, auxilliary Arf-GTP-hydrolysis activating proteins (ArfGAPS) stimulate GTP hydrolysis in Arf and thus render the G-protein soluble to dissociate from the membrane, followed suit by coatomer. This concept does not explain how during uncoating the various interactions of coatomer within and between triads are dissociated and how the membrane-associated conformation(s) of the complex is reversed to its soluble form. We have investigated ArfGAP mediated coat dissociation in a fluorescence burst analysis in real time, with Arf and coatomer labeled with different fluorescent dyes. Full length ArfGAPs efficiently cause dissociation of Arf, followed by coatomer after a lag time. The catalytic domain of the ArfGAPs alone also efficiently dissociates Arf from the vesicles. Strikingly, however, the coatomer network remains on the membrane in a meta-stabile state. Subsequent addition of the non-catalytic domain or of full length ArfGAPs did not dissociate coatomer. Thus, ArfGAP together with Arf must connect to the coat in order to drive coatomer dissociation at the expense of GTP hydrolysis (Ganeva et al., in preparation, see Fig.3). Specific interactions of ArfGAP non-catalytic domains with coatomer described in the literature are candidates for such "point levers". (see Fig.3)

Various mechanistic aspects

Vincent Popoff during his postdoctoral work in our group has revealed a mechanism by which cargo

can be taken up efficiently into a COPI vesicle by bridging to coatomer via Golgi phospho protein 3 (Eckert et al. 2014).

Based on our recent finding of an unexpected specificity of trans-membrane segments of proteins with lipid molecular species, Andreas Ernst during his postdoctoral work has discovered that the transmembrane span of the Ebola virus glycoprotein specifically binds cholesterol. Cells expressing this transmembrane domain drastically shrink when cholesterol is depleted. We conclude that cytotoxicity of the virus can be alleviated by cholesterol inhibiting drugs (Hacke et al., 2015).

Further results on protein-lipid interactions are described in the report of Britta Brügger.

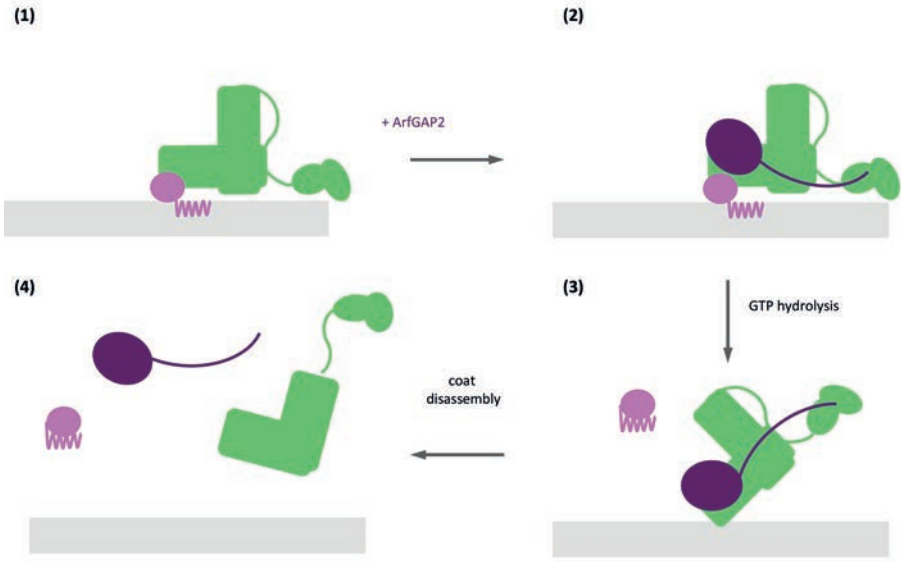

Fig. 3: Model of point-lever action of ArfGAPs. 1: *detail of COPI coat with Arf (pink) on the membrane (gray) in contact with γ-COP trunk domain (green triangle) and appendage domain (green rest).* ***2:*** *ArfGAP2 (dark red) binding to Arf with its catalytic domain (ball), and to γ-COP appendage domain (bow).* ***3:*** *Hydrolysis of GTP in Arf converts Arf into its soluble form and concomitantly induces a change in conformation in γ-COP. As a consequence the coat is disassembeled and coatomer is rendered soluble.*

Our research is supported by the German Research Council (SFB 638: Dynamics of macromolecular complexes in biosynthetic transport (-2015), GRK 1188: Quantitative analysis of dynamic processes in membrane transport and translocation (-2015), Grants WI 654/11-1, WI 654/12-1) and CellNetworks Heidelberg.

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E.S. P. Eckert , I. Reckmann, A. Hellwig , S. Röhling ,A.E.Battari, F.T. Wieland, V. Popoff (2014) Golgi phosphoprotein 3 triggers signal-mediated incorporation of glycosyltransferases into coatomer-coated (COPI) vesicles. J. Biol. Chem.289 (45): 31319-31329

M.J. Gerl, T. Sachsenheimer, M. Grzybek, U. Coskun, F.T. Wieland, B. Brügger. (2014) Analysis of transmembrane domains and lipid modified peptides with matrix-assisted laser desorption ionization-time-of- flight mass spectrometry ACS Publ. 86 (8):3722-3726

P. Björkholm, A.M. Ernst, M. Hacke, F. Wieland, B. Brügger, G. von Heijne, (2014) Identification of novel lipid-binding motifs in mammalian membrane, Biochimica et Biophysica Acta-Biomembranes 1838, 2066-2070

M. Hacke, P. Björkholm, A. Hellwig, P. Himmels, C. Ruiz de Almodóvar, B. Brügger, F. Wieland, A.M. Ernst (2015) Inhibition of Ebola virus glycoprotein-mediated cytotoxicity by targeting its transmembrane domain and cholesterol. Nat Commun. July 9; &.7688. doi:10.1038/ncomms8688 PMID2615

S.O. Dodonova, P. Diestelkoetter-Bachert, A. von Appen, W.J.H. Hagen, R. Beck. M. Beck, F. Wieland, J.A.G. Briggs (2015) A structure of the COPI coat and the role of coat proteins in membrane vesicle assembly Science 349 (6244), 195-198.

F. Adolf, M. Rhiel, I. Reckmann, F.T. Wieland. (2016) Sec24C/D-isoform-specific sorting of the preassembled ER-Golgi Q-SNARE complex. Mol Biol Cell. 27(17):2697-707. doi: 10.1091/mbc.E16-04-0229. Epub 2016 Jul 13.

Awards and Honors

1991-92	Dean of the Faculty of Science in Medicine, Heidelberg University
1993	Honorary Member of Charité, Medical Faculty of the Humboldt University, Berlin
2000	Elected EMBO Member
2001	Heinrich-Wieland Award
2003	Member of Deutsche Akademie der Naturforscher Leopoldina
2006	Feldberg Foundation Award
2011	Elected Member of the Academia Europea
2013	HMLS Award

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1988	Ph.D. - ETH Zürich, Switzerland
1989 - 1991	PostDoc - Yale University School of Medicine, New Haven, USA
1992 - 1999	PostDoc - Institute of Biochemistry I, Heidelberg University / BZH
1999	Habilitation in Biochemistry, Heidelberg University, Medical Faculty
2000 - 2002	Scientific Director - German Cystic Fibrosis Association
since 2002	Head of the teaching unit and lecturer - BZH

Cordula Harter

Teaching and Education

BZH's activities in biochemical education are unique: We train more than 1000 undergraduate students of three faculties and offer an elaborate graduate program for around 60 PhD students. In addition, we engage in the development of modern curricula and novel teaching formats.

The teaching unit

BZH's teaching activities are centrally coordinated. Our office provides services for students and teaching staff, manages courses and examinations, maintains the electronic learning platform, and cooperates with the deans' offices in curricular and interdisciplinary affairs. Our teaching laboratories are managed by technicians who set up the students' experiments and take care of the infrastructure. Our lab space can accommodate up to 120 students and is equipped with instruments for biochemical analyses as well as for large scale preparations. For advanced courses a cell culture lab, a cold room and a dark room are available. In a computer room with 14 workstations students can use special software and online tools, like databases for gene and protein analysis.

Undergraduate Program

Approximately 800 medical students, 190 biology students and 120 chemistry students participate in courses each year. In addition, 25 students are trained each year in a selective biochemistry study program which is described in a separate section of this report. All students attend obligatory courses which consist of lectures, seminars and practicals and are individually organized for the students of the respective subject. In addition, students can choose among various electives.

Medicine

The medical students' courses extend from the second throughout the fourth semester. They are systematically structured from fundamental biomolecules, metabolic pathways, cell and organ functions, to the molecular basis of diseases.

As a novel teaching format, we recently introduced the "inverted classroom", a concept which moves the lecture outside the classroom – as an online lecture - and allows the students to prepare a topic at home and deepen the knowledge later in the classroom. Another innovative teaching format are virtual patients: We integrated electronic cases in our curriculum in order to emphasise the clinical relevance of biochemistry and to foster self-directed learning.

The preclinical curriculum at Heidelberg University is likewise unique in that topics are taught interdisciplinary with other preclinical subjects like anatomy and physiology but also with clinical subjects, e.g. pharmacology. The high quality of our curriculum is confirmed by very good results in internal as well as national examinations:

Heidelberg continues to rank among the top three German medical faculties (out of 31) in the national state examinations.

Medical students prepare selected topics in a interactive seminar.

Biosciences

For students of biosciences, we offer courses at the Bsc and Master level. Our Bsc program starts in the first semester with a seminar "Current topics of the life sciences". In the second semester, the foundations of biochemistry, e.g. enzymatic catalysis and metabolism, are taught in a core course which consists of lectures and a practical. For students of the third to fifth semester courses at an advanced level are offered, e.g. a practical where students apply technologies to regulate gene expression. At the master level, the BZH participates in the major "Molecular and Cellular Biology (MCB)" of the international master program "Molecular Biosciences". BZH group leaders offer lectures on topics of their current research and lab rotations. Upon completion of the course program, students have the opportunity to perform a thesis in one of the research groups and later on to apply for a graduate program.

Chemistry

For chemistry students, we offer two modules at the bachelor level: one obligatory for students of the third semester and one elective for students of the fourth and fifth semester. In the obligatory module fundamental biochemistry is taught in lectures and seminars, and students learn to handle basic lab equipment and to analyse various types of biomolecules. In the elective module students get insight into more sophisticated techniques, like protein crystallography and structural analyses. Master students perform a lab rotation and participate in research seminars. Our electives are in great demand which gives us the privilege to choose the best students for a bachelor thesis and afterwards for the master or doctoral program.

Practical work in small groups is an essential element of our educational program.

Graduate program

To offer excellent research opportunities in a stimulating and supportive atmosphere and to prepare our graduates for a career in academia or industry are our demands on graduate education. To this end, we provide not only state-of-the-art laboratories for our approximately 60 graduate students, but also comprehensive training in our BZH graduate program or in one of the other graduate programs on campus, like the Hartmut Hofmann-Berling international graduate school of molecular and cellular biology (HBIGS). Graduate students' program includes supervision by an advisory committee, progress reports in our department seminar and participation in a program which offers a variety of courses ranging from laboratory techniques to soft skills. Interaction among the BZH graduate students is further intensified by regular social and scientific meetings, like the yearly doctoral retreat, which is organized by our board of PhD students. In addition, opportunity is given to discuss science issues in guest speakers' seminars and at international conferences.

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2008	Ph.D. - BZH
2008 - 2012	PostDoc - Yale University, New Haven, USA
since 2012	Study Coordinator, Lecturer Biochemistry - BZH

Rainer Beck

Bachelor and Master Study Programs in Biochemistry

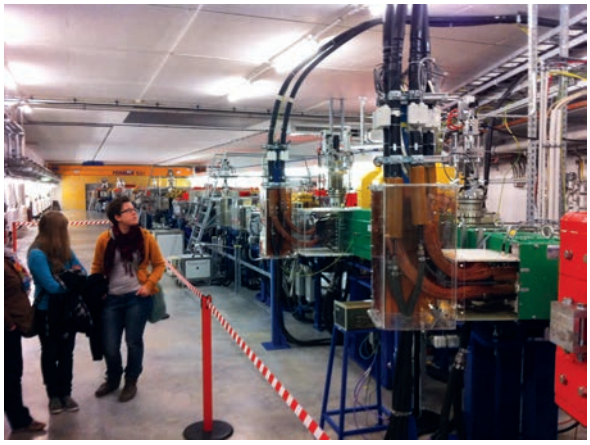
Since winter 2012, the Faculty of Biosciences and the Faculty of Chemistry and Geosciences, offer a joint study program Biochemistry Bachelor. The goal is to prepare excellent students for a career in applied research in Life Sciences. Since winter 2015, the consecutive study program Biochemistry Master started.

Lecture Biochemistry II.

Capacities and Selection of Applicants

Biochemistry Bachelor in Heidelberg is a very selective study program. Between 700 and 1100 candidates per year applied for the 25 slots available. This gives us the privilege to draw the most ambitious and talented applicants.

Initial selection is based not only on grades (focus on math and science), but also on additional qualifications, such as extended laboratory practicals and participation scientific competitions.



Students of Biochemistry visiting the Heidelberg Ion-Beam Center.

Based on this initial ranking, the top 75 candidates are invited to the BZH for the second round of the selection process: Interviews are conducted by a member of the Faculty of Biosciences together with a member of the Faculty of Chemistry.

Biochemistry Bachelor

In the first years, students of Biochemistry in Heidelberg undergo a very thorough training in Chemistry; they complete all courses mandatory for students of Chemistry.

Starting in the 2nd year, the students commence their education in Biochemistry:
We made a new lecture series Biochemistry I-III for this study program. Our goal is to offer our students unique lectures, where we can take the time to thoroughly explain and, together with the

Biochemistry Practical A.

students, explore molecular mechanisms underlying biochemical processes. We put special emphasis on the relationships of structure and function of biomolecules, and describe biochemical processes quantitatively.

We often sidestep and reference to techniques and laboratory experiments, in an effort to demonstrate how textbook knowledge was initially discovered, and how such data is experimentally generated.

In the 3rd year, students of Biochemistry will start conducting research practicals in research groups of the BZH and affiliated mural and extramural institutions.

By this time, the students will have acquired a thorough understanding of Chemistry and Biochemistry. The Bachelor program concludes with a Bachelor thesis and an oral exam on topics of Chemistry and Biochemistry.

Biochemistry Master

The consecutive Master program extends over two years and aims at preparing the students for a subsequent training as PhD students.
In contrast to the tightly structured Bachelor program with little freedom of choice, Master students are to choose freely combinations of in total four modules from a list of nine chemical and biological subjects. Out of the nine modules, two were created specifically for Master Biochemistry, one being Pathobiochemistry (coordinated by Britta Brügger and Walter Nickel), the other being Bioinformatics/Molecular Dynamics (coordinated by Rob Russel).

Each module consists of a lecture/seminar, as well as a research practical of 8 weeks. The main focus is on the practical trainings, to this end our students can choose freely the institutions and laboratories where the research practicals will be carried out, including research at the pharmaceutical industry.

After completion of the four modules and their associated research practicals, the students will be prepared to choose the group in which they want to work during the next 6 months for their master thesis. Before commencing the laboratory work, the students will, with the help of respective group leader, formulate a detailed research proposal about their project.

The Master program concludes with a disputation of the Master thesis.

Biochemistry Bachelor students visiting CERN.

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PhD Board and BZH Meeting

PhDs for PhDs

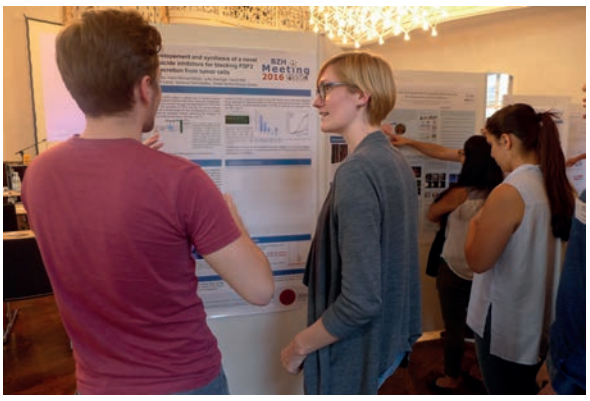
The PhD board of the BZH is a committee of PhD students - at least one person from each group at the BZH - that takes over some tasks for the benefit of all PhD students. The PhD board organizes social and scientific events for the BZH community, and arbitrates between PhDs and group leaders within the Biochemistry Centre of Heidelberg. Here, the core events in which the PhD board is involved are presented:

The group leader meeting

As part of the BZH family, we have a reserved slot in the group leader meeting to introduce topics or new ideas and to raise awareness of challenges that PhD students might face.

The BZH lecture speaker, invited by PhD students and PostDocs

On behalf of all BZH PhD students, we invite and host one speaker per year for the BZH lecture series. The PhD students choose speakers in a ranked poll. One of those speakers is then hosted



Poster session.

by the PhD board and is asked to share his or her scientific experience with junior scientists such as PhD students and young PostDocs.

Students' lunch with the BZH lecture guest speakers

As part of every guest speakers' visit at the BZH lecture series, the PhD board organizes a common lunch with each speaker for up to ten interested PhD students and young PostDocs to meet the speaker in an informal setting. While

Lecture hall during poster session.

usually some scientific questions are addressed, the discussion focuses on the career of the speaker and his/her opinion on the research field, its future perspectives or the involved scientific community. Of course, in this context advice about scientific career planning and professional orientation in academia are extremely valuable.

Science forum

Every one or two months on a Friday evening the PhD board organizes a relaxed get-together for PhDs and PostDocs. This gives PhD students from the different groups of the BZH the opportunity to get to know each other, talk to each other, exchange ideas, get new contacts and get a feeling of how to tackle the challenges of a PhD student's life. Furthermore, it provides the PhD students with the possibility to meet people outside their group and to get to know alternative scientific approaches to overcome their experimental obstacles.

The BZH Meeting

The BZH meeting is an annual retreat for PhD students and young PostDocs of the BZH that takes place for an extended weekend. The PhD board organizes its scientific programme. During

the meeting, one PhD student from each group introduces the main topics of their group and presents their work in front of an audience of about hundred scientists in a formal presentation. Additionally, three national and international guest speakers are invited to give a keynote lecture and to participate in the rest of the scientific activities during the meeting.

Round table discussions.

Not only the speakers have the opportunity to present their work, but also all other PhD students do in the form of a poster presentation.

The BZH Meeting also includes the highly appreciated round table discussions. Here, five to six people from different groups sit together at a table and present their projects in a simple and yet comprehensive way, without technical support (slides, etc) in order to maximize feedback from other participants.

The BZH meeting serves the purpose of interconnecting the different groups of the BZH not only scientifically but also personally, which is also encouraged through the social events of the meeting such as canoeing, hiking or board gaming.

The first BZH meeting was held in 2016 from 12th to 14th June in Kloster Schöntal (Baden-Württemberg). There were more than 50 participants with over 40 posters and ten presentations from BZH students and postdocs, in addition to three keynote lectures from guest speakers from Germany, France, and the USA.

PhD Board
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Group photo of the participants of the BZH Meeting 2016 at Kloster Schöntal..

Facilities and Platforms

Microscopy

In the BZH researchers have access to the following microscopic systems:

Zeiss LSM 510 META spectral imaging confocal laser scanning system. The system can be used for 3D reconstruction and time-lapse (4D), FLIP, FRAP, dynamic FRET and linear unmixing. It permits the precise separation of fluorophores with highly overlapping emission spectra. Up to 32 channels can be acquired simultaneously in 1.2 seconds. The LSM510 is also equipped with a motor-driven XYZ scanning stage with Mark & Find and Tile Scan (Mosaic Scan) functions and with software for time-lapse imaging of living cells.

Olympus CellR Imaging Station (resources of SFB 638). It enables fast 3D multicolor time-lapse fluorescence microscopy. The microscope is fully automated and is equipped with a piezo drive for all objectives, an automated XYZ-stage, an emission filter wheel and a sensitive ORCA/ER cooled CCD camera.

Zeiss Axiovert 200 inverted widefield fluorescence microscope. Suitable for fluorescence / phase or DIC imaging. Equipped with an Axiocam MRm camera and filters for Cy5, Rhodamine, EGFP and DAPI. This microscope is also equipped with an Axiocam MRc color camera with a high dynamic and color range of more than 1: 2200 at 3 x 12 bit RGB.

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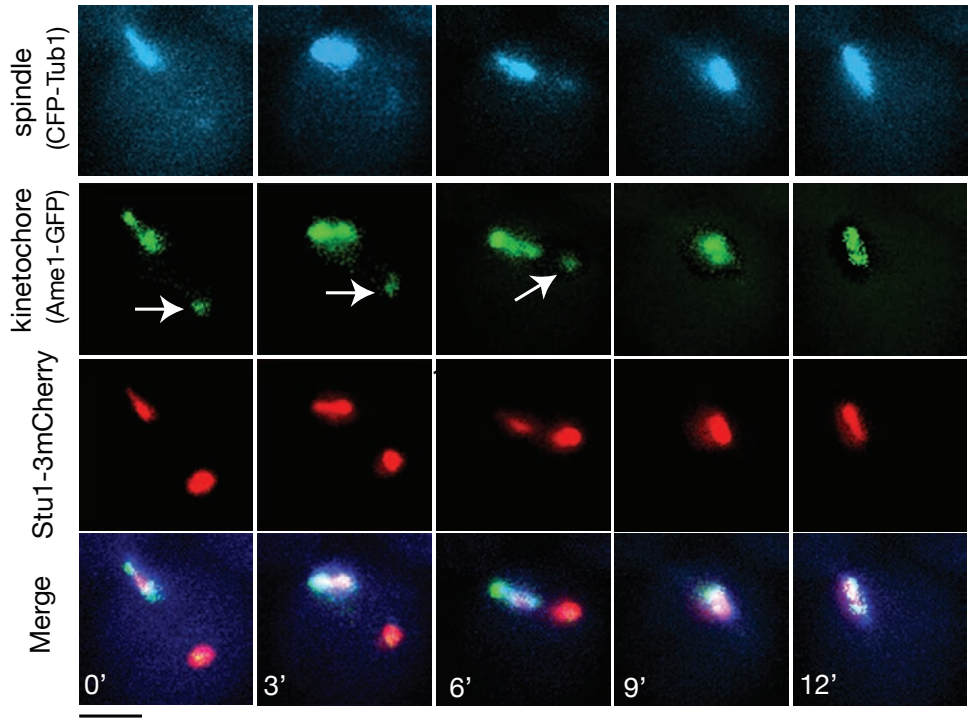

Figure: Stu1/CLASP is recruited to unattached kinetochore and facilitates their capture to the mitotic spindle. *The unattached chromosome state to the attached state is shown in the budding yeast with time lapse fluorescence imaging performed on an Olympus CellR Imaging Station. Kinetochores are shown in green (GFP), the white arrows point to unattached kinetochores, whereas the larger GFP signal indicates the attached kinetochores. The mitotic spindle is shown in blue (CFP). The conserved midzone protein Stu1/CLASP (shown in red; 3m-cherry) is recruited to the unattached kinetochore, then proceeds to travel with the captured kinetochore to the mitotic spindle. (Image courtesy of C. Funk & J. Lechner).*

Lipidomics platform

Lipidomics aims to analyze cellular lipid metabolic pathways and signaling networks by quantitatively and comprehensively defining the lipidomes of biological systems. Given the depth and wealth of the structural information desired, mass spectrometry is the state-of-the-art method of analysis. Over the last two decades we have continuously expanded our methods and tools towards a comprehensive and quantitative analysis of lipids. Our platform provides expertise in identifying and quantifying lipid classes and species from any organism and any type of sample. This includes samples ranging from protein-lipid assemblies to whole pro- and eukaryotic organisms. Capitalizing on our lipidomics approach we address in collaborations a multitude of fundamental cell biological questions. The lipidomics platform is available for groups on Heidelberg Campus and beyond. Lipidomics analyses include sample extraction, measurement and data evaluation via our web-based software application. For publications see page 11.

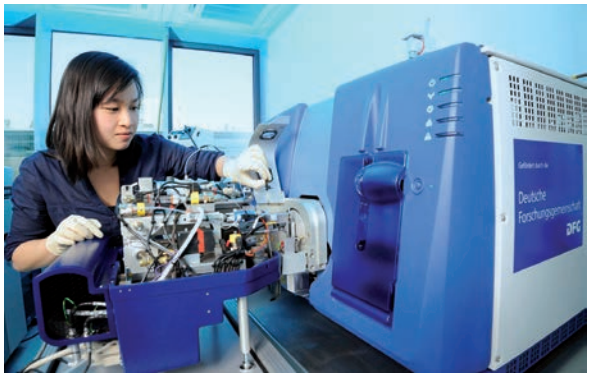

Fig. 1: A hybrid triple quadrupole-linear ion trap mass spectrometer.

Instrumentation:

The Lipidomics platform is supported by the Cluster of Excellence CellNetworks Heidelberg and builds on unique expertise in qualitative and quantitative lipid analysis by state-of-the-art nano-mass spectrometry. Depending on the scientific question, five complementary nano-platforms are available, including quadrupole-Orbitrap, quadrupole linear ion trap, quadrupole time-of-flight, and triple quadrupole mass spectrometers. Measurements are performed in direct injection mode via an automated nano-device or following chromatographic separation by liquid, supercritical fluid or differential ion mobility chromatography.

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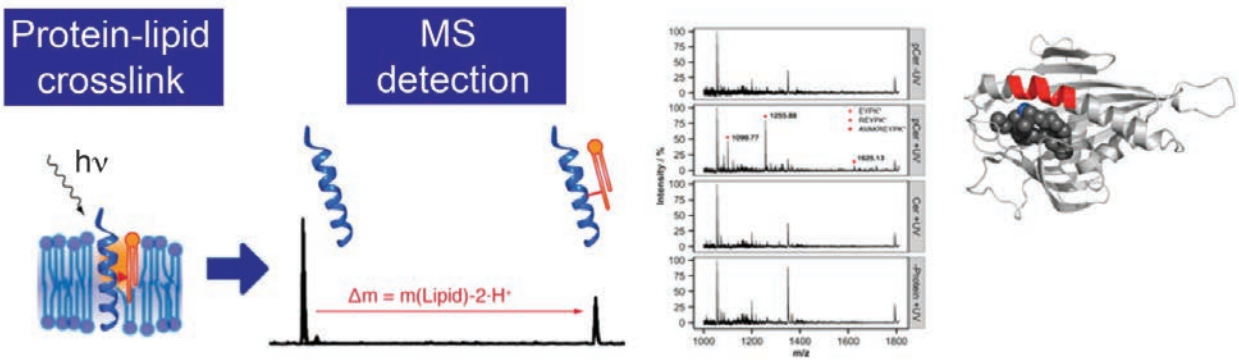

Fig. 2: Photoactivatable lipids as tools to identify protein-lipid interactions. Cells labeled with functionalized lipids are UV-irradiated to form covalent protein-lipid crosslink products, which are then identified via mass spectrometry.

Electron Microscopy (EM)

Transmission electron microscopy (TEM) of biological macromolecular assemblies combined with single-particle image processing is a powerful method for structure determination.

Information on the size, shape, homogeneity, and plasticity of macromolecular complexes or single proteins can be achieved from negatively stained samples. High-contrast structural information, mainly about the outer envelope of a molecule, can be achieved to a resolution of up to 20 Å by this method. Embedding the sample in vitrified ice (Cryo-EM) is currently gaining attention for the ability to calculate structures that reach sub-5 Å resolution due to improved microscopes, better detectors, and more advanced software. This yields density maps with sufficient detail to deduce the atomic structure for a range of specimens. For an overview with a focus on the importance of the biochemical properties see Takizawa, 2016, "While the revolution will not be crystallized, biochemistry reigns supreme".

At the BZH we provide technical and scientific support; from sample preparation using e.g. the GraFix method, labeling of proteins using e.g. the DID-Dyn2 label to the preparation of the negatively stained specimen and first quality check with an EM10 (Zeiss) microscope.

In close cooperation with the Electron Microscopy Core Facility (EMCF) we have access to microtomes, freeze-substitution machines and scanning EMs. Also several high-end transmission micoscopes (including a state-of-the-art Krios with direct electron detecor) are in close proximity, helping the groups of the BZH to solve questions from cell to high resolution structural biology.

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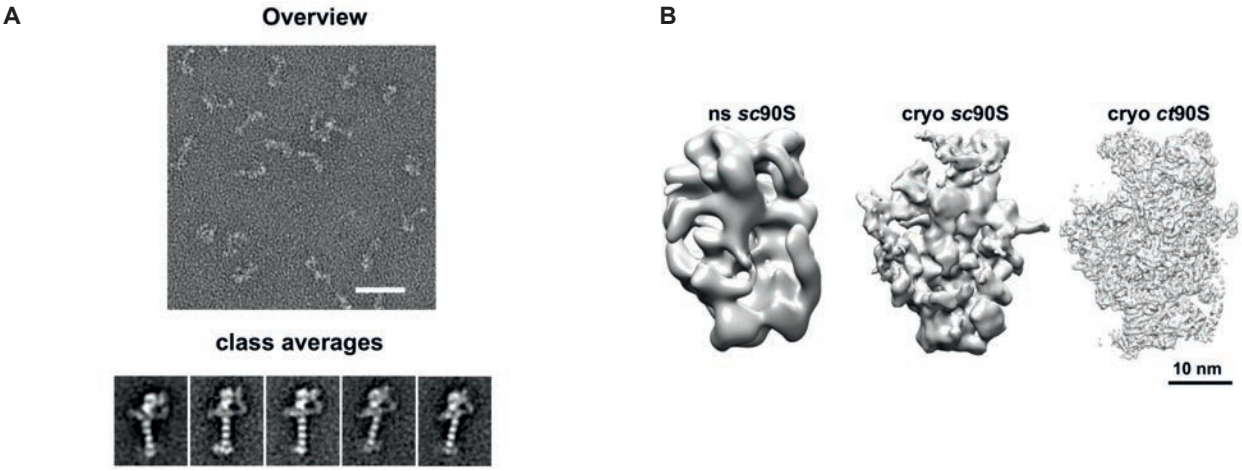

Figure: (A) *Overview of a negative stain electron micrograph and class averages of an NPC sub-complex* ***(B)*** *Three-dimensional reconstruction of the 90S Pre-ribosome illustrating the methodical gain of resolution.*

Protein Crystallization Platform

In 2008, the Cluster of Excellence:CellNetworks and Prof. I. Sinning/BZH have established a state-of-the-art high-throughput crystallization platform for biological macromolecules. The platform is equipped with an ArtRobbins Phoenix nano-liter dispensing robot which allows screening

of thousand crystallization conditions with as little as 100 microliters of concentrated protein sample. The crystallization plates are stored at strict temperature control in Rigaku Minstrel HT incubators with a total capacity of 800 plates. Crystallization experiments are automatically imaged at storage temperature and images can be immediately viewed, analyzed, and annotated via a web interface. A specialized Formulatrix UV microscope allows distinguishing between protein and salt crystals by detecting the fluorescence signal of tryptophan residues at a very early stage of the project. Predefined crystallization screens are available for soluble proteins, RNA-protein and other complexes as well as for membrane proteins. For refinement of initial crystallization hits, finescreens are designed using the Rigaku CrystalTrak software and then produced with a Perkin-Elmer Janus 4-channel liquid handling robot using the in-house stock solutions. The ArtRobbins Gryphon dispensing robot is specialized in handling highly viscous solutions at nano-liter scale and crystallizing membrane proteins in lipidic cubic phase. More information can be found at http://xtals.bzh.uni-heidelberg.de.

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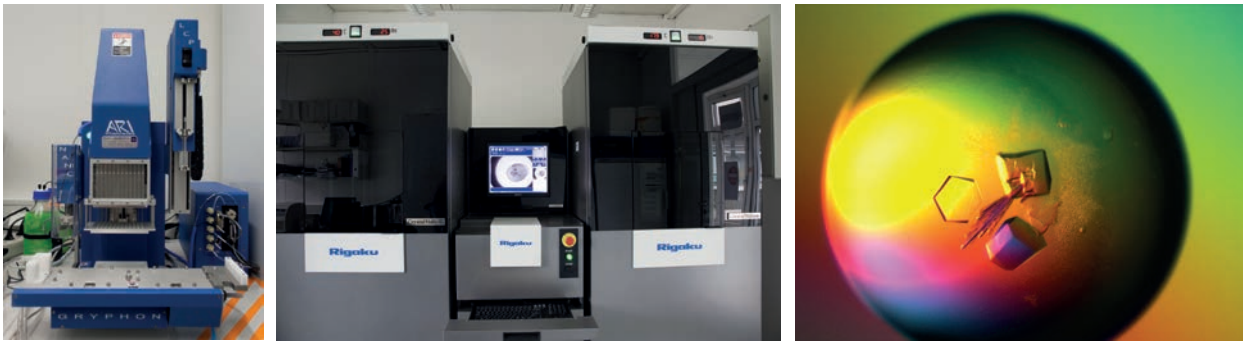

Figure: (left) *ArtRobbins Gryphon pipetting robot,* ***(middle)*** *Rigaku Minstrel storage and imaging system,* ***(right)*** *protein crystals of transferase domain.*

Protein Mass Spectrometry

We provide the following analytical service:

- Protein identification by MALDI-TOF mass spectrometry using Peptide Mass Fingerprint and Post Source Decay data (LIFT).
- Protein identification by LC-MS/MS (Orbitrap) mass spectrometry with equipment located at the ZMBH.
- Analysis of posttranslational protein modification by LC-MS/MS (Orbitrap) mass spectrometry.
- Quantitative mass spectrometry by LC-MS/MS (Orbitrap) focusing on SILAC (stable isotope labelling with amino acids in cell culture) and “label-free” methods.
- Determination of the molecular mass of various biological molecules (peptides, oligonucleotides, RNA) by MALDI-TOF mass spectrometry.

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Funding

Sonderforschungsbereich (SFB) 638: Dynamics of macromolecular complexes in biosynthetic transport

Coordinator: Felix Wieland, Heidelberg University Biochemistry Center (BZH)

Cells are highly dynamic structures that can be compared with factories full of sophisticated machines. In the last decades many individual parts of these machines have been identified and characterised. At present and in the years to come the most exciting challenge will be to decipher how individual building blocks are put together in variable ways to perform the cell´s dynamic functions. This is done in an iterative way: one first tries to com- bine the single parts to functional assemblies; once an assembly is defined functionally, such assemblies are combined to even higher aggre- gates at a next layer of complexity, again func- tionally characterised, and so on. With a hundred thousand or so different proteins that build up a human cell, and up to 200 parts comprising a macromolecular complex (a functional unit), it is evident that there is still a long way to go in order to completely understand not only the compositions of all possible functional units, but also their interplay, i.e. their dynamics. With this knowledge complete, we would understand the molecular basis of life, and to prove our understanding, we would have to reconstitute a living cell from its defined building blocks. This would have to occur not only by adding each component in exactly the correct concentration, but also in a defined sequence, because of their dynamics many of the assemblies can only function correctly in a time- dependent manner. Needless to say that such a task could be solved only by the activity of many scientists worldwide, and final success, if possible at all, lies in the far future.

Along this way, the SFB 638 “Dynamics of Macromolecular Complexes in Biosynthetic Transport” has initiated an interdisciplinary approach to investigate the structural and dynamic behaviour of complexes of up to 100 or so components within a cell. Even if none of the scientists involved is likely to reach this final goal, we believe that many important lessons can be learned during this journey. We have made quite some progress on our way from 2004 to 2015 to learn about functions of macromolecular assemblies in a cell by studying the dynamics of such complexes employed in steps of intracellular transport. Our focus was on the analysis of mechanisms in *transport between nucleus and cytoplasm* and on *biosynthetic transport from the ribosome to the endomembrane system.* To this end we capitalised on the ample know how available within Heidelberg Molecular Life Sciences and specifically combined basic molecular research on viral functions with biophysical, biochemical, molecular biological and structural approaches mainly in eukaryotic cells.

Whereas at the outset our knowledge of individual building blocks as part of transport machinery was quite complete, only limited information was available as to more sophisticated assemblies of such building blocks and their dynamics, i.e. their functional interplay. In this summary examples are given of how analyzing structures of higher assemblies combined with biochemical approaches led to novel and original insight.

Most of the ca. 30 individual components of the nuclear pore were already categorized at the onset of this CRC, but their interactions and assembly into higher order complexes were poorly understood. Within the SFB, not only was the better part of the nuclear pore reconstituted from many of its individual protein components as a basis to understand its structure, but in addition unexpected functional interactions were defined that led to novel concepts of RNA transport and ribosome biogenesis.

Likewise, novel concepts were reached based on studies of the mechanism of action of ribosome-associated chaperons in co-translational folding and transport of proteins. Trigger factor (TF) is the first chaperon to interact with the nascent protein chain in bacteria. TF binds and stabilizes partial protein folds while distant interactions are prevented. Ribosome proximity and TF binding limit conformational sampling of nascent chains, suggesting TF can avoid early misfolding or rescue misfolded protein species generated co-translationally. The TF interactome was assessed by ribosome profiling and revealed that most of the nascent polypeptides are clients of TF, but only after they reached about 100 aa residues. This allows enzymatic processing of nascent chains by the essential ribosome interacting enzymes peptide deformylase and methionine aminopeptidase. From studies on maturation of a hetero-dimeric model protein it was observed that assembly of its subunits occurs close to the site of synthesis, involving co-translational interactions of nascent subunits. As a novel concept a role was attributed to TF in delaying the onset of co-translational interactions until the subunit dimer interface is fully exposed on the ribosomal surface.

In the field of vesicular transport we have for the first time characterised a coat lattice on a membrane, the envelope of COPI transport vesicles, by cryo electron microscopy. As a result we discovered how two molecules of the small GTPase Arf serve to anchor the coat protein complex coatomer on the membrane, and thus organize it into triades that serve as a building block to form the coat network. At the same time the resulting geometry governs the curvature of the membrane within the vesicle. These results suggest new concept-driven biochemical projects, e.g. to elucidate the molecular mechanisms that underlie the process of coat release from a COPI vesicle. In addition, a variety of data in the literature about partial structures of the coat protein could either be fitted into the coat network or were proven not to exist in the coat on a membrane.

Studying dynamics of macromolecular assemblies being in the focus of each single project has solicited a continuous mutual interest, and consequently many lively and fruitful discussions and collaborations, including intense and uninhibited exchange of materials and methods. Many of these collaborations are persisting beyond the termination of the CRC end of 2015.

Sonderforschungsbereich (SFB) / Transregio 83: Molecular Architecture and Cellular Functions of Lipid/Protein Assemblies

Coordinator: Thomas Söllner, Heidelberg University Biochemistry Center (BZH)

Biological membranes provide a variety of functions, ranging from simple barriers to elaborate signalling transduction platforms. About 1/3 of the genome-encoded proteins are membrane proteins and hundreds of distinct lipid species have been described as constituents and building blocks of membranes creating unique boundaries and reaction sites at the level of cells, organelles and membrane microdomains. However, we are just beginning to obtain an initial glimpse of how specific interactions are formed within the hydrophobic core and at the aqueous interface of a lipid bilayer. Thus, the central aims of the TRR 83 are to characterize the molecular composition and structural organization of distinct membrane domains, to elucidate their physical and chemical properties and to understand their physiological functions. The TRR 83 brings together scientist from three locations – Bonn, Dresden, and Heidelberg – and has significantly advanced the current status in lipid analysis, lipid imaging and lipid cross-linking by developing

various new chemical tools. For example, TRR 83 members have synthesized novel functionalized lipids, which led to the identification and characterization of a highly specific interaction with a single sphingolipid species. Based on this discovery an interacting protein signature motif has been deduced and candidate proteins have identified, whose function/structure relationships are now studied. Furthermore, photo-caged lipids have been synthesized providing photo-switches to bypass steps in signal transduction chains, revealing the propagation of downstream intracellular signal events in physiological relevant processes such as insulin release. Such functionalized lipids are now used by many researchers world-wide. Concerning other highly specific protein-lipid interactions, an inhibitory role of a specific ganglioside and the stimulatory function of polyphosphorylated phosphoinositides in growth hormone receptor signalling at the plasma membrane were elucidated in detail. The role of membrane microdomains in HIV budding and controlling membrane trafficking in HIV-infected cells has been determined. Concerning vesicle tethering/fusion, it could be shown that the interaction of an endosomal Rab protein with its specific vesicle tethering protein leads to the collapse of the tether bringing the two membranes in close proximity, a prerequisite for the subsequent assembly of the membrane fusion machinery. Here, the use of a reconstituted assay and optical tweezers allowed a detailed biophysical analysis measuring molecular forces. The lipidomics platforms have been further developed to analyse critical, but hard to detect lipid species, and have established comprehensive and quantitative lipid analysis ranging from distinct protein/lipid assemblies to entire organisms. Thus, by joining experts in fields as different as e.g. synthetic organic chemistry, biochemistry, cell biology, virology, and immunology (as well as many others), and making advanced technologies like lipidomics, optical tweezers and super resolution microscopy available, the TRR 83 provides unique opportunities to address challenging questions, which could not be solved by individual approaches. In general, the tool sets are used to elucidate a contribution of specific lipid environments to signal transduction, immune system activation, translocation of proteins across membranes, to the intracellular formation of virus particles, lipid droplets, and endosomes, and to the complex events that allow the controlled fusion of membranes. Analyses in tissue culture and in whole organisms are complemented by *in vitro* reconstitution of functional membrane protein complexes from completely defined lipids and proteins. Combining these approaches will eventually allow to unequivocally attribute specific functions to individual components of such lipid/protein assemblies and to reveal the underlying interaction principles inherent to biological membranes.

The TRR 83 is now in its second funding period (January 2014 – December 2017) and consists of 18 research groups. The research team includes scientists of the BZH, the Heidelberg University Hospital, the European Molecular Biology Laboratory (EMBL) in Heidelberg, the Biotechnology Center (BIOTEC) and the Paul Langerhans Institute of the TU Dresden, the Max Planck Institute for Molecular Cell Biology and Genetics in Dresden, the Life & Medical Sciences Institute (LIMES) and the Institute of Innate Immunity of the University of Bonn, the German Centre for Neurodegenerative Diseases (DZNE) and the CAESAR research centre in Bonn. Four research groups (Brügger/Wieland, Nickel, Sinning, Söllner), the coordination, and administration of the TRR 83 are located at the BZH.

Sonderforschungsbereich (SFB) / Transregio186:
Molecular Switches in the Spatio-temporal Control of Cellular Signal Transmission

Coordinator: Walter Nickel, Heidelberg University Biochemistry Center (BZH)

A hallmark of cell physiology is the coordination of signal transmission across space and time. Cells employ molecular switches to control all principal steps of their signaling pathways. The corresponding cellular responses are characterized by a wide spectrum of time scales ranging from milliseconds to hours. For example, ultrafast processes such as neurotransmission occur in milliseconds. Slower responses are protein secretion and receptor-proximal signaling occurring at a time scale of seconds to minutes. Global transcriptional modulation by the circadian clock is even slower with response times of hours. The identities and mechanisms of many cellular components acting as molecular switches such as kinases and phosphatases are known in detail. However, it is poorly understood how they operate in space and time to regulate cellular responses at different time scales in living cells. On the one hand, this deficit can be overcome by the relatively recent development of adequate tools to acutely activate or deactivate molecular switches in living cells. On the other hand, the emerging availability of quantitative live cell imaging combined with super-resolution techniques now allows for experimental read-outs providing high spatio-temporal resolution. Therefore, a principal aim of this research network is to take advantage of a broad spectrum of novel chemical biology and optogenetic tools to study cellular signaling processes governed by molecular switches with sophisticated microscopic methods. Biological questions were selected to systematically compare a wide range from ultrafast (ms) to slow (hours) cellular responses. The researchers of SFB/TRR 186 aim at a quantitative description and dissection of individual steps of regulated protein secretion and endocytosis, ligand- and stress-dependent receptor down-stream signaling and the regulation of gene transcription and splicing in living cells. This goal will be achieved by quantitative measurement of the time-dependent

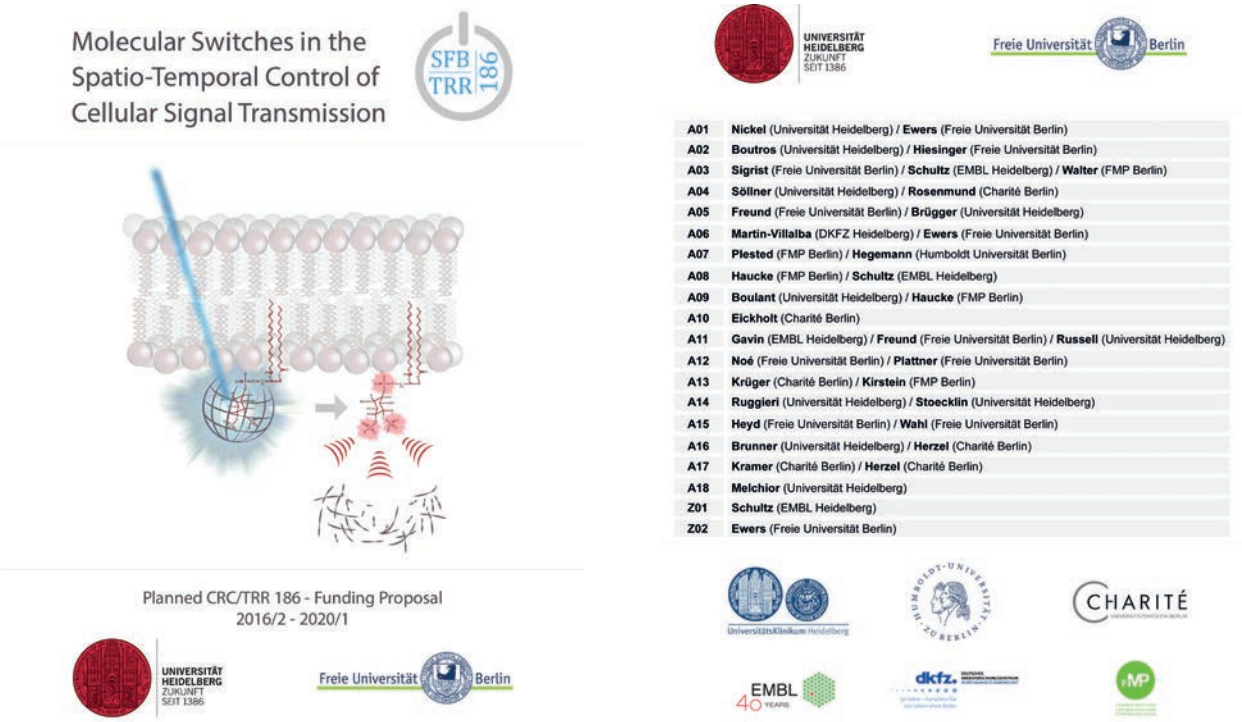


Fig. 1: Researchers and institutions involved in SFB/TRR 186: "Molecular switches in the spatio-temporal control of cellular signal transmission".

localization, assembly and activation state of signaling molecules with appropriate temporal and spatial resolution in living cells, following controlled interference by light or by small molecules. As a long-term goal, these experimental data will be used to establish theoretical models aiming at a quantitative description of how living cells translate the activation states of a limited set of molecular switches into a broad spectrum of cellular responses. This will permit the discovery of principles that govern the spatio-temporal regulation of signaling networks.

Using advanced chemical biology tools such as photoactivatable membrane lipids along with experimental systems that allow for reading out signalling processes with spatio-temporal resolution in intact cells, a team of researchers from various University and external institutions in Heidelberg and Berlin started this endeavour on July 1, 2016.

Research Training Group (Graduiertenkolleg; GRK) 1188: Quantitative Analysis of Dynamic Processes in Membrane Transport and Translocation

Coordinator: Walter Nickel, Biochemie-Zentrum der Universität Heidelberg (BZH)

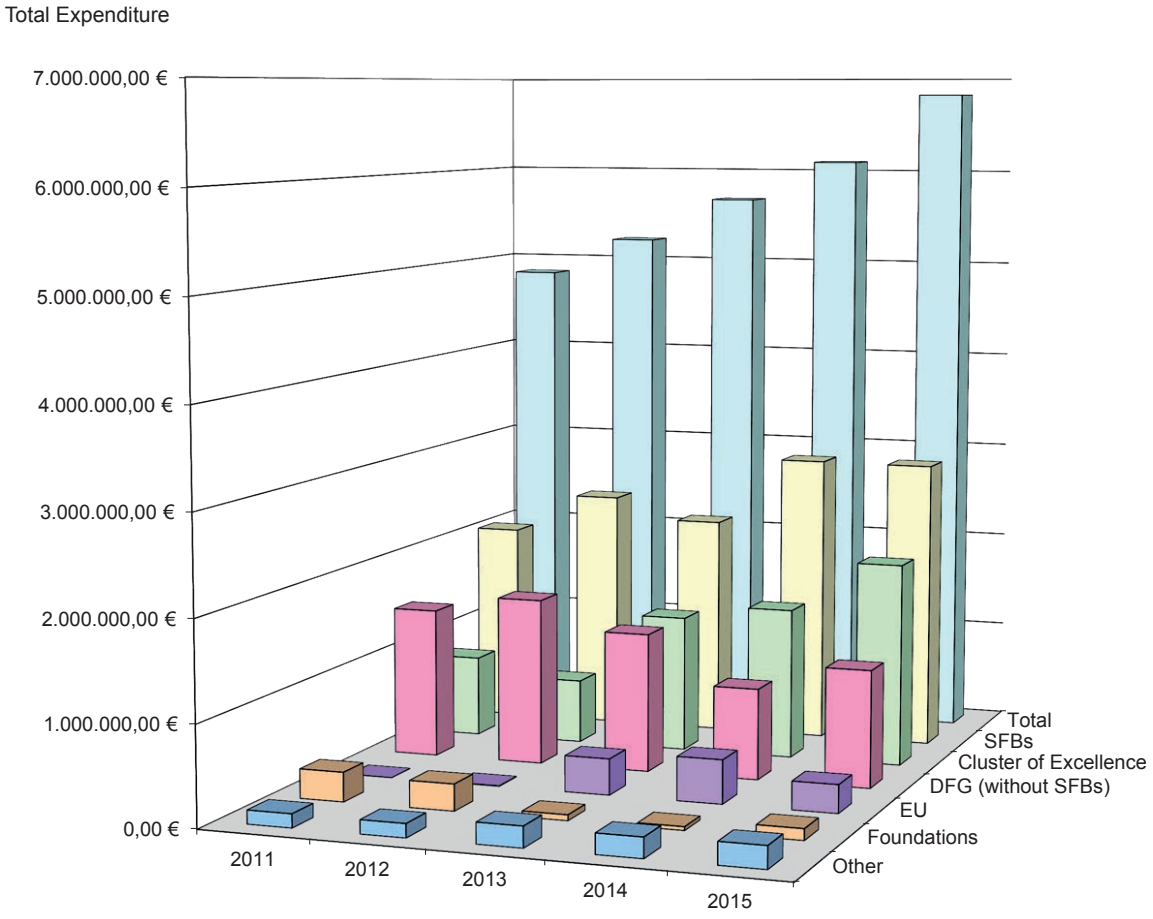
The Research Training Group "GRK 1188" was supported by the German Research Foundation (DFG) for the maximum of two funding rounds from October 1, 2005 until September 30, 2015. GRK 1188 integrated the molecular analysis of structure-function relationships of individual components with a more global understanding of the dynamic interactions between a large number of different macromolecules. These efforts were facilitated by novel key technologies that enabled us to understand the dynamics of molecules in living cells. Employing state-of-the-art screening technology, it became possible to identify those protein or lipid components that specifically govern a given biological process out of the enormous complexity of the eukaryotic cell. The GRK 1188 graduate program selected intracellular transport and membrane translocation processes in eukaryotic cells as its central theme, a classical field in molecular cell biology that particularly benefited from these technical advances. The growing complexity of biologically relevant information derived from such studies demanded an increasingly sophisticated and interdisciplinary knowledge. The mission of GRK 1188 was therefore to provide PhD students of the Heidelberg Biosciences that enter the research area of membrane transport with a specifically tailored qualification program in the context of an excellent research environment. The main focus of this training program was on specific methodology that the fellows directly applied to their own research. These activities were complemented by measures to promote soft skills and scientific independence of the PhD students. GRK 1188 integrated laboratories at the Heidelberg University Biochemistry Center (BZH), the Zentrum für Molekulare Biologie (ZMBH), the Department für Infektiologie des Universitätsklinikums Heidelberg and the European Molecular Biology Laboratory (EMBL). Furthermore, to promote the international visibility of GRK 1188, the program collaborated with the University of Manchester and organized three international conferences between 2005 and 2015.

Excellence Initiative: CellNetworks and Institutional Strategy

CellNetworks is an interdisciplinary research cluster with a focus on the analysis of complex biological networks at various levels of complexity. It comprises research groups of the DKFZ, EMBL,

Max-Planck-Institute for Medical Research and Heidelberg University. To provide state-of-the-art technology CellNetworks supports several core facilities. The BZH hosts the CellNetworks protein crystallization platform, which is headed by Irmi Sinning and Jürgen Kopp and the CellNetworks lipidomics platform headed by Britta Brügger. In 2014, Britta Brügger accepted a call for a professorship in Chemical Biology/Biochemistry to strengthen the interaction between Chemistry and Life Sciences on the Heidelberg Campus. The professorship is supported by CellNetworks and Institutional Strategy funds of Heidelberg University. Michael Brunner (BZH) together with Jochen Wittbrodt (COS) are steering the CellNetworks deep sequencing facility at Bioquant. CellNetworks supported the BZH junior group of Martin Koš and presently supports the BZH junior group of Julien Béthune. Irmi Sinning and Ed Hurt coordinate a program that focuses on the structural characterization of molecular machines. This initiative is one of the five emerging collaborative topics supported by CellNetworks.

External Funding BZH 2011 - 2015



	2011	2012	2013	2014	2015
SFBs	2.061.196,78 €	2.471.715,63 €	2.254.587,68 €	2.960.716,92 €	2.955.925,82 €
DFG (without SFBs)	1.519.314,46 €	1.692.638,98 €	1.408.174,71 €	923.247,96 €	1.186.811,98 €
Cluster of Excellence	825.684,95 €	651.296,71 €	1.392.149,72 €	1.539.603,26 €	2.069.645,16 €
EU	806,72 €	5.237,62 €	363.320,42 €	439.938,33 €	283.388,91 €
Foundations	294.815,47 €	267.347,07 €	57.958,97 €	37.201,29 €	113.082,61 €
Other	137.014,79 €	137.360,79 €	207.760,33 €	200.822,37 €	219.467,19 €
Total	**4.838.833,17 €**	**5.225.596,79 €**	**5.683.951,83 €**	**6.101.530,13 €**	**6.828.321,67 €**

Expenses according to SAP (01.01. - 31.12.)

Theses 2014 - 2016

2014

Thorsten Michael Beitlich, Fluorescence Spectroscopic Investigation of Folding and Unfolding of Cytidine Monophosphate Kinase.
Group Leader: Schirmer

Francois Cesbron, Time-resolved biochemical analysis of transcriptional discontinuity.
Group Leader: Brunner

Caroline Funk, Distinct functions of the two TOGL domains in CLASP orchestrate mitosis in *S. cerevisiae*.
Group Leader: Lechner

Monika Gaik, Structural and Functional Characterization of the Conserved Nup82 Subcomplex Located on the Cytoplasmic Side of the Yeast Nuclear Pore Complex.
Group Leader: Hurt

Andriana Gigova, Novel RNA methyltransferase Rcm1 is required for ribosome stability.
Group Leader: Koš

Bianca Hennig, Identification and characterization of mechanisms involved in controlling cryptic transcription in gene-coding regions.
Group Leader: Fischer

Linda Lauinger, The RNA helicase FRH attenuates CKIa-dependent phosphorylation of the clock protein FREQUENCY.
Group Leader: Brunner

Rizos-Georgios Manikas, Nug1 is a potassium-stimulated GTPase affecting the association of early 60S assembly factors in ribosome biogenesis.
Group Leader: Hurt

Daniel Parisotto, An extended helical conformation of Munc18-1 domain 3a provides a template for SNARE complex assembly.
Group Leader: Söllner

Maximilian Pfau, Establishment of a novel lipid and content mixing assay to resolve the regulatory effect of Munc18-1 and CpxII on SNARE-mediated membrane fusion of reconstituted VAMP2/Syt1 SUVs and isolated rat brain synaptic vesicles.
Group Leader: Söllner

Simone Röhling, Interaktionen der kleinen GTPase Arf1 mit Coatomer.
Group Leader: Wieland

Cigdem Sancar, GATA type transcription factors SUB1 and WCC cooperate in nucleosome dynamics and transcription activation.
Group Leader: Brunner

Verena Schmeiser, Regulated by the interplay of multiple CLASP domains, the localization of the TOGL2 domain to microtubules drives spindle formation in *Saccharomyces cerevisiae.*
Group Leader: Lechner

Sivakumar Vadivel Gnanasundram, Dual role of Has1 RNA helicase in ribosome biogenesis in *Saccharomyces cerevisiae.*
Group Leader: Koš

Sonja Zacherl, A Role for ATP1A1 in Unconventional Secretion of Fibroblast Growth Factor 2.
Group Leader: Nickel

2015

Lyudmila Dimitrova, The role of TREX-2 and the nuclear basket in mRNA export.
Group Leader: Hurt

Annemarie Horn, The chloroplast signal recognition particle in post-translational membrane protein targeting.
Group Leader: Sinning

Bhalchandra Jadhav, Structural and functional characterisation of the eukaryotic SRP receptor.
Group Leader: Sinning

Cagakan Özbalci, Bottom-up and top-down lipidomics approaches to study cellular lipids.
Group Leader: Brügger

Corinna Schaffroth, Tryparedoxin peroxidases protected African trypanosomes from iron-induced damages of dinstinct cellular membranes.
Group Leader: Krauth-Siegel

Christoph Schneider, Clock controlled gene-9 is a trehalose phosphorylase which does not contribute to growth or development in *Neurospora crassa.*
Group Leader: Brunner

Felix Weyer, Structural and functional characterisation of ribosome associated factor.
Group Leader: Sinnnig

Yang Zhou, The MTREC complex is the major nuclear exosome-targeting factor for CUTs and unspliced pre-mRNAs in fission yeast.
Group Leader: Fischer

2016

Patrick Aderhold, On the structure and function of the COPI coat protein complex coatomer.
Group Leader: Wieland

Katharina Beckenbauer, Identification and characterization of novel cholesteroylated proteins using a click chemistry based approach.
Group Leader: Brügger

Katja Bendrin, Characterization of the mRNA export complexes TREX-2 and the novel Nsc3 complex in *Schizosaccharomyces pombe*.
Group Leader: Fischer

Iva Ganeva, Molecular Mechanism Underlying Dissociation of the Coat of COPI Vesicles.
Group Leader: Wieland

Satyavati Kharde, Structural and biochemical characterisation of ribosome assembly factors involved in 5S RNP biogenesis.
Group Leader: Sinning

Giuseppe La Venuta, Targeting Unconventional Secretion of Fibroblast Growth Factor 2 by Small Molecule Inhibition.
Group Leader: Nickel

Blessing Musunda, Dissecting the physiological roles of dithiolic glutaredoxins in *Trypnosoma brucei*.
Group Leader: Krauth-Siegel

Severino Urban, The role of VEGF in hippocampus development.
Group Leader: Ruiz de Almodovar

Marcel Zeitler, Identification of common key properties in unconventional secretion of Fibroblast Growth Factor 2 (FGF2), HIV-1 Transactivator of transcription (HIV-Tat), and Interleukin-1β (IL-1β).
Group Leader: Nickel

Publications 2014 - 2016

2014

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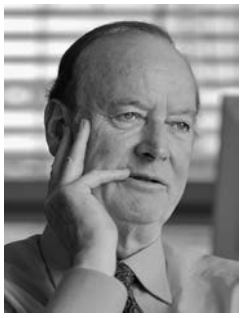

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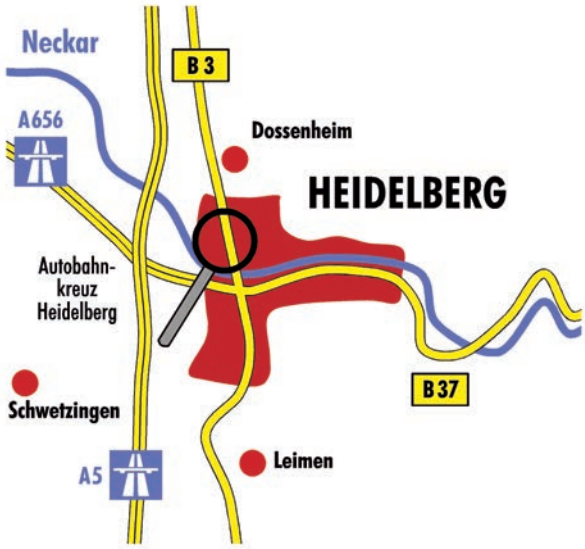

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