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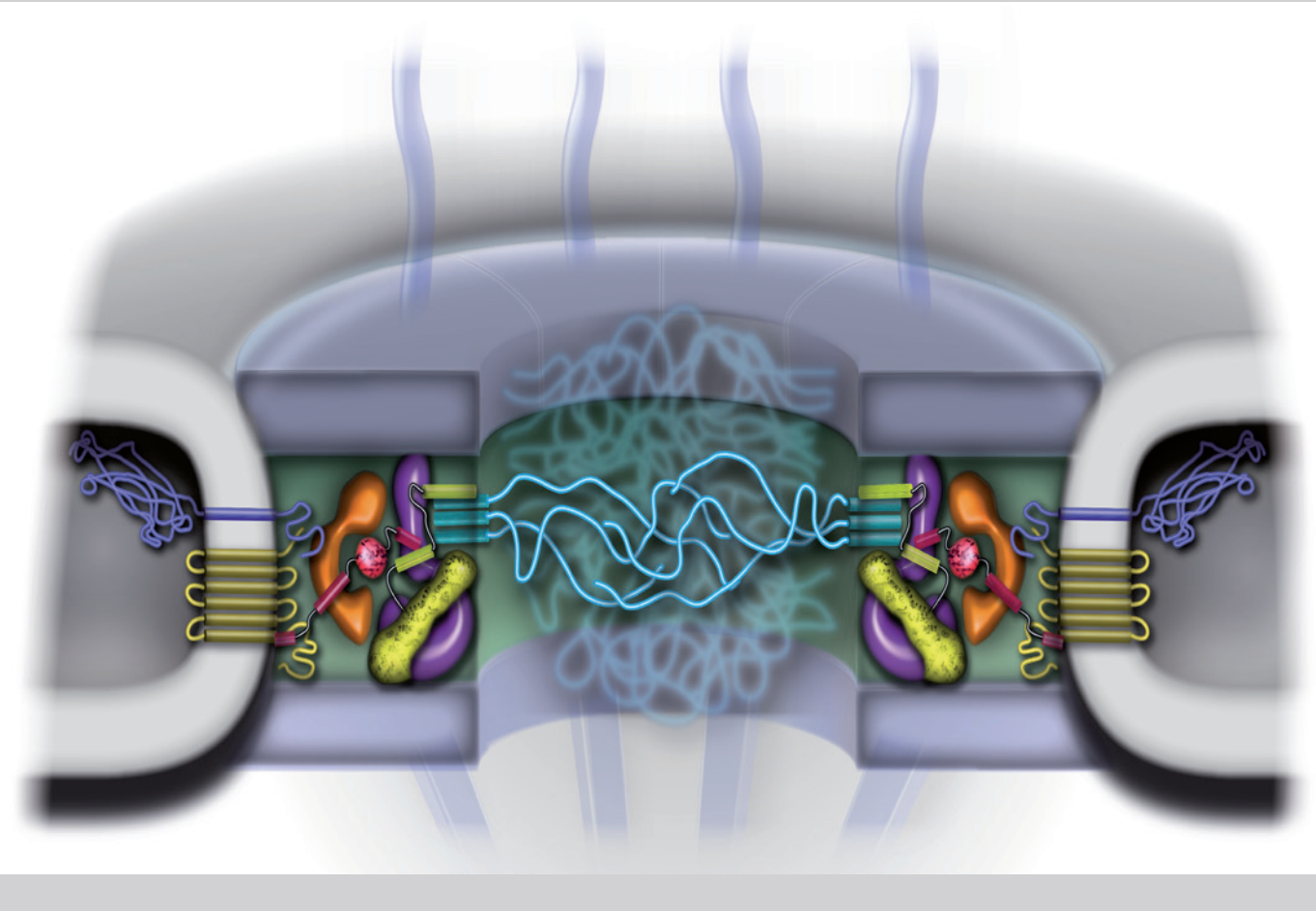

RESEARCH AND EDUCATION IN MOLECULAR LIFE SCIENCES

# HEIDELBERG UNIVERSITY BIOCHEMISTRY CENTER
# REPORT 2011-2013

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## Welcome to the BZH!

Biological machines composed of macromolecular assemblies of proteins nucleic acids control and maintain virtually all functions of a cell. The biogenesis and structure of such molecular machines, as well as their function, regulation and interaction are in the focus of research at the Heidelberg University Biochemistry Center (BZH). The BZH is a central institution for research and teaching. It was founded 1997 and today accommodates 14 research groups, presently including 4 junior groups. Altogether the BZH hosts about 200 scientific and non-scientific coworkers. More than 70% of the scientists are funded by external sources.

Biological processes studied at the BZH include the biogenesis of ribosomes, molecular mechanisms of nucleo-cytoplasmic transport of proteins and RNA, protein translocation into the endoplasmic reticulum, the biogenesis of membrane proteins, the analysis of the machinery for vesicular transport and unconventional secretion of proteins, as well as mechanisms of RNA transport. Furthermore research groups study molecular clocks and the dynamics of gene regulation and chromatin structure. In addition, research groups are concerned with the coordinated development of the vascular and nervous systems and in the characterization of trypanosomes.

Since its establishment the BZH has developed into a leading institute for research in the area of molecular life sciences. A modern department structure with a flat hierarchy and complementary interests of research creates a lively atmosphere.

Research at the BZH is highly recognized on a national and international level. The BZH hosts successful research initiatives: Felix Wieland is the coordinator of a large collaborative research center (SFB 638) and Thomas Söllner is the coordinator of an SFB/Transregio (TRR 83) with participation of 17 research groups in Bonn, Dresden and Heidelberg. Carmen Ruiz de Almodóvar was successful in receiving a highly competitive ERC Starting Grant. Irmi Sinning was awarded the 2014 Leibniz Prize of the German Research Foundation (DFG). Ed Hurt, who had previously received the Leibniz Prize (2001), is now supported by a highly prestigious Reinhart Koselleck Project of the DFG. Furthermore, Michael Brunner and Felix Wieland received the HMLS Investigator Award 2012 and 2013, respectively.

The BZH congratulates our Advisory Board Member Jim Rothman who received 2013 the Nobel Prize for Physiology or Medicine. The BZH has an intimate connection with the Nobel laureate. Felix Wieland was a visiting scientist with Jim Rothman and four BZH group leaders (Brügger, Brunner, Nickel and Söllner) were postdocs in his lab.

Research in molecular life sciences is subject to rapid technological progress and requires elaborate machinery. The BZH has an excellent infrastructure, offering state-of-the-art equipment in a wide range of leading-edge technologies. Under the supervision of Britta Brügger the center accommodates a mass spectrometry facility for the qualitative and quantitative analysis of lipids, and Johannes Lechner leads a

mass spectrometry facility for protein analysis. Managed by Irmi Sinning and Jürgen Kopp the BZH hosts an automated facility for protein crystallization, which is supported by the Heidelberg CellNetworks Cluster of Excellence. Recently, the BZH has established a facility for electron microscopy that is headed by Dirk Flemming. Likewise, the BZH offers confocal light-microscopy and fluorescence activated cell sorting (FACS).

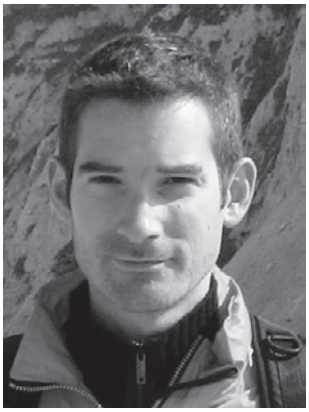
The BZH teaches Biochemistry in Medicine, Biology and Chemistry, and in particular engages in the excellent training of the next generation of molecular life scientists with emphasis on Biochemistry. Annually, about 1000 students are being trained at the BZH including approximately 70 graduate students. All graduate students participate in programs of HBIGS, an international graduate school supported by the German Excellence Initiative, or in an extensive BZH internal graduating program. Within the framework of these programs the graduate students have the opportunity of learning the latest techniques and methods. Since 2012 the BZH hosts a Bachelor program in Biochemistry, which is jointly offered by the Faculties for Chemistry and for Life Sciences. A consecutive Master program in Biochemistry will start in 2015. Rainer Beck serves as coordinator and lecturer of these programs and Michael Brunner as Dean of Studies.

Due to its internal structure and embedded into the research landscape in Heidelberg, the BZH is well set up to ensure, also in the future, an excellent training for the next generations of scientists and to conduct research at an internationally competitive level.

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

Prof. Dr. Michael Brunner

Director, BZH Heidelberg, 31.12.13



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

**Julien Béthune**

# Posttranscriptional regulation of mRNA expression and localization

## Goal

**We are interested in the mechanisms of post-transcriptional gene silencing, notably by miRNAs, as well as in the interplay between membrane trafficking and mRNA regulation and localization.**

## Background

While protein-coding transcripts represent a tiny fraction of the transcriptome of human cells, many non-coding RNAs play a very important role in regulating gene expression. Among them, miRNAs have emerged as key regulators of most cellular functions by post-transcriptionally repressing at least 50% of expressed mRNAs. Not surprisingly, misregulation of miRNAs leads to diseases including cancer. While most mRNA targets of miRNAs end up being degraded, others are kept stable in a silent state until an appropriate stimulus triggers reversal of miRNA-mediated silencing. The latter implies that the action of miRNAs may be used as a temporary process, and suggests that miRNAs may play a role in mRNA transport and localized translation. Membrane-enclosed transport vesicles mediate protein and lipid transport within the secretory pathway, from the ER to the plasma membrane. A surprising and largely unexplored link between mRNA and vesicular transport has been suggested by several studies. Indeed, protein complexes involved in vesicle formation and capture of protein cargo were suggested to bind to mRNAs and regulate their translation. In addition, some mRNAs, and components of the miRNA machinery, were found to associate with purified vesicles. An emerging picture is that vesicular carriers may also transport and help localizing silenced-mRNAs, and thus add another layer of regulation to gene expression.

## Research Highlights

While it is has been known that miRNA-mediated silencing results from the combination of translational repression and mRNA decay, we and others have recently shown that there is a temporal order of silencing. Indeed, targets of miRNAs first undergo a translational repression step that is followed by a stimulated deadenylation step, which then leads to mRNA decay. Such a mechanism involving successive and potentially reversible steps is compatible with a role of miRNA in mRNA transport or storage. However, it is still not clear how certain miRNA targets escape degradation while staying in a silent state. To address this, we are investigating the dynamics of miRNA-mediated repression, both temporally and spatially,

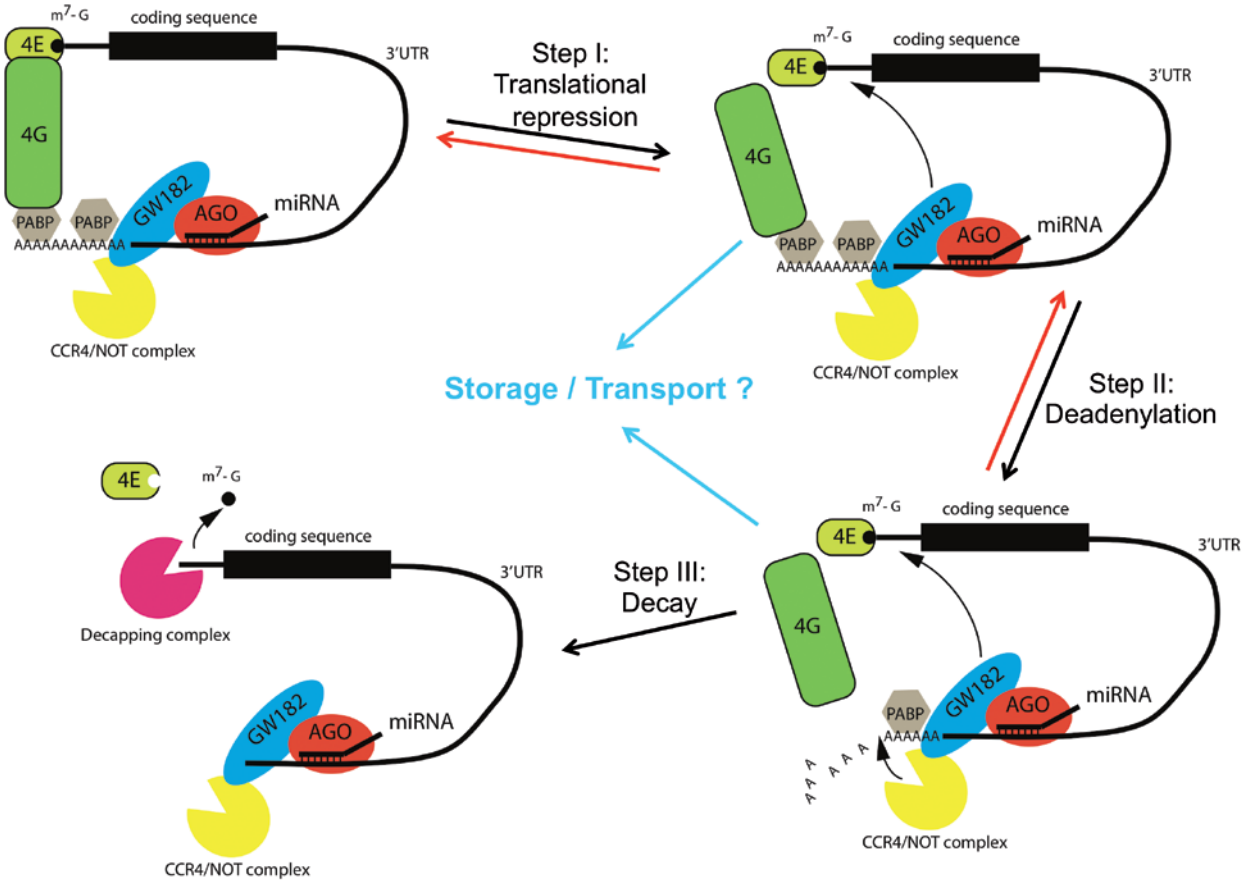


***Fig. 1: The temporal order of miRNA-mediated silencing.*** *The mechanism of action of miRNAs, consisting of three successive major steps, is compatible with a role of miRNAs in mRNA transport or storage.*

and we aim at identifying trans acting factors that modulate silencing on specific mRNAs.

Proper localization of mRNAs is critical for establishing and maintaining cell polarity. As several reports and observations suggest that transport vesicles may help localizing certain mRNAs, we are characterizing the populations of RNAs that are found on different types of intracellular transport vesicles. This is the first step to systematically define an atlas of vesicle-associated RNAs, and will serve as the basis to characterize how specific RNAs are recruited to specific transport vesicles as well as the significance of such an interaction.

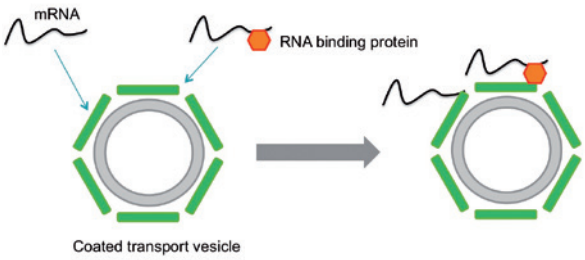


***Fig. 2: A working model for vesicle-mediated mRNA transport.*** *Certain mRNAs may directly, or through a bridging interaction mediated by an RNA binding protein, bind to coat protein complexes found on intracellular transport vesicles. Hence, these mRNAs may hitchhike on the transport vesicles to reach their final destination.*

## Selected Publications


Béthune, J., Artus-Revel, C.G., and Filipowicz, W. (2012). Kinetic analysis reveals successive steps leading to miRNA-mediated silencing in mammalian cells. EMBO Rep 13, 716-723.

Beck, R., Sun, Z., Adolf, F., Rutz, C., Bassler, J., Wild, K., Sinning, I., Hurt, E., Brugger, B., Béthune, J., and Wieland, F. (2008). Membrane curvature induced by Arf1-GTP is essential for vesicle formation. Proc Natl Acad Sci U S A 105, 11731-11736.

Sun, Z., Anderl, F., Frohlich, K., Zhao, L., Hanke, S., Brugger, B., Wieland, F., and Béthune, J. (2007). Multiple and stepwise interactions between coatomer and ADP-ribosylation factor-1 (Arf1)-GTP. Traffic 8, 582-593.

Béthune, J., Kol, M., Hoffmann, J., Reckmann, I., Brugger, B., and Wieland, F. (2006). Coatomer, the coat protein of COPI transport vesicles, discriminates endoplasmic reticulum residents from p24 proteins. Mol Cell Biol 26, 8011-8021.

Béthune, J., Wieland, F., and Moelleken, J. (2006). COPI-mediated transport. J Membr Biol 211, 65-79.



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| 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**

# The Molecular Clock of *Neurospora crassa*

## 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 generates an endogenous robust self-sustained subjective day-night rhythm of approximately 24 h.

In the core of the circadian clock of *Neurospora* is the transcription factor White Collar Complex (WCC), which 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 repressor that inhibits its own synthesis in a negative feedback loop by regulating the activity and abundance of the WCC in rhythmic fashion. FRQ is in complex with the RNA helicase FRH and casein kinase 1a (CK1a) and it inactivates the WCC by facilitating its phosphorylation by CK1a. FRQ also supports accumulation of high levels of the WCC. FRQ is phosphorylated at more than 100 sites, which regulates turnover and function of the clock protein in complex manner and is a crucial process in setting the pace of circadian oscillations. A number of kinases and phosphatases - e.g. CK1a, CK2, PP1 and PP4 - have been implicated in the control of the phosphorylation status of FRQ.

The WCC controls several hundred genes. Amongst these rhythmic genes are about 30 genes encoding transcription regulators, 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.

## Research Highlights

### CSP1 is a global circadian repressor

*CSP1 regulates anti-phasic circadian gene expression*

WCC directly activates morning-specific expression of CSP1, which acts as a transcriptional repressor. We found that newly synthesized CSP1 exists in a transient complex with the co-repressor RCM1/RCO1 and the ubiquitin ligase UBR1. CSP1 is rapidly hyperphosphorylated and degraded via UBR1 and its ubiquitin conjugase RAD6. Genes controlled by CSP1 are rhythmically expressed and peak generally in the evening, i.e. in anti-phase to morning-specific genes directly controlled by WCC (Fig. 1). Rhythmic expression of these second-tier genes depends crucially on phosphorylation and rapid turnover of CSP1, which ensures tight coupling of CSP1 abundance and function to the circadian activity of WCC. Negative feedback of CSP1 on its own transcription buffers the amplitude of CSP1-dependent oscillations against fluctuations of WCC activity. CSP1 regulates predominantly genes involved in metabolism. It controls ergosterol synthesis and fatty acid desaturases and thereby modulates the lipid composition of membranes.

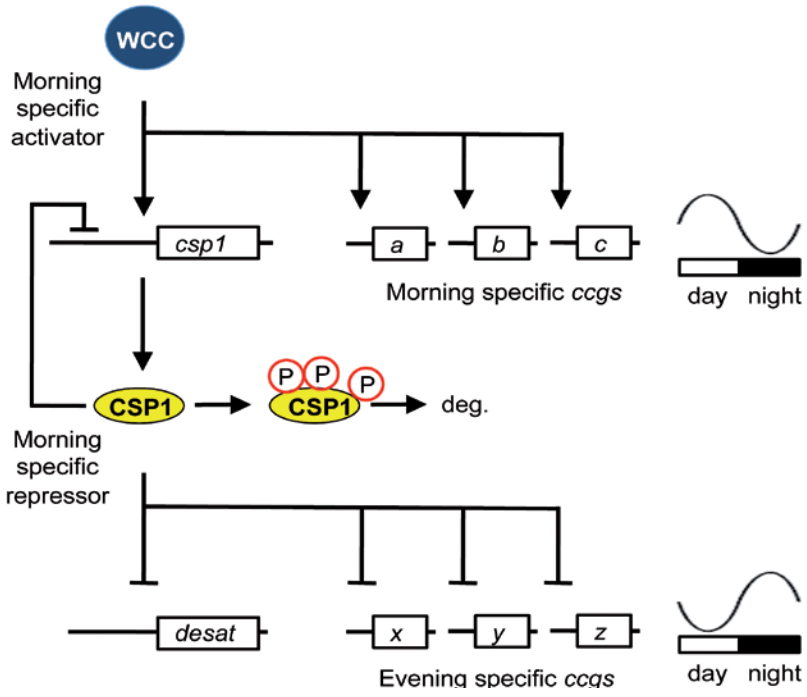


***Fig. 1: Hierarchical organization of clock controlled genes.*** *The WCC, the core transcription factor of the circadian clock, is active in the subjective morning. It activates directly morning specific* ccgs (a, b, c). *One of these genes is* csp1, *which encodes a transcription repressor. Newly synthesized CSP1 is rapidly inactivated by phosphorylation and then degraded (deg). The target genes of CSP1* (desat, x, y, z) *are repressed in the subjective morning. Hence, these second tier* ccgs *genes display an evening specific expression rhythm, i.e. 180° out of phase of first tier* ccgs *directly activated by the WCC. CSP1 inhibits its own gene in a negative feedback loop.*

*CSP-1 regulates metabolic compensation of the clock*

The circadian period length decreases with increasing glucose concentrations in *csp1* mutant strains, while the period is compensated for changes in glucose concentration in wild-type strains. Glucose stimulates CSP1 expression and induced overexpression of CSP1 causes period lengthening and, eventually, complete dampening of the clock rhythm. Our results show that CSP1 inhibits expression of the WHITE COLLAR 1 (WC1) subunit of the WCC by repressing the *wc1* promoter. Glucose-dependent repression of *wc1* transcription by CSP1 compensates for the enhanced translation of WC1 at high glucose levels, resulting in glucose-independent expression of the WCC and, hence, metabolic compensation that maintains a constant circadian period (Fig. 2). Thus, the negative feedback of CSP1 on WC1 expression constitutes a molecular pathway that coordinates energy metabolism and the circadian clock.

### Phosphorylation of FRQ sets the pace of the Neurospora clock

*FRQ undergoes conformational change triggered by clustered hyperphosphorylation*

In the course of a day the *Neurospora* clock protein FRQ is progressively phosphorylated more than 100 sites and eventually degraded. Phosphorylation and degradation are crucial for circadian time keeping and little

is known how phosphorylation of a large number of sites correlates with circadian degradation of FRQ. We identified two amphipathic motifs in FRQ that interact over a long distance, bringing the positively charged N-terminal portion in spatial proximity to the negatively charged middle and C-terminal portion of FRQ. The interaction is essential for the recruitment of CK1a into a stable complex with FRQ. FRQ-bound CK1a progressively phosphorylates the positively charged N-terminal domain of FRQ at up to 46 non-consensus sites triggering a conformational change, presumably by electrostatic repulsion, which commits the protein for degradation via phosphorylation of the thereby exposed PEST1 signal in the negatively charged central portion of FRQ (Fig. 3).

*FRH is a pacemaker of the CK-1a dependent phosphorylation of FRQ*

FRQ forms a complex with CK1a and FRH, a DEAD box-containing RNA helicase that has a clock-independent essential function in RNA metabolism. Recent findings in our lab strongly suggest that the ATPase of FRH attenuates the kinetics of CK1a-mediated hyperphosphorylation of FRQ. Hyperphosphorylation of FRQ is strictly dependent on site-specific recruitment of a CK1a molecule that is activated upon binding and equilibrates with the unbound pool of less active CK1a. The FRH cycle regulates in cis the access of CK1a to phosphorylation sites in FRQ, suggesting that FRH is an ATP-dependent remodeling factor acting on a protein complex. The affinity of CK1a for FRQ decreases with increasing phosphorylation state, resulting in functional inactivation of the FRQ complex in the negative feedback loop of the circadian clock (Fig. 3).

**Genome-wide analysis of WCC-controlled genes**

Light signaling has profound effects on the development and behavior of Neurospora. We used ChIP-sequencing to uncover direct targets of the WCC. We found that the light-activated WCC binds to hundreds of regions, including promoters of known clock and light-regulated genes. Amongst the genes activated by the WCC are 28 transcription factor genes (Fig. 3). Transcription of most, but not all, WCC target genes is induced by light. Our findings provide links between WC-2 and effectors in downstream regulatory pathways for light-induced behavior. Our data suggest a "flat" hierarchical network in which 20% of all annotated *Neurospora* transcription factors are regulated during the early light response by the WCC, the key transcription factor of the circadian clock.

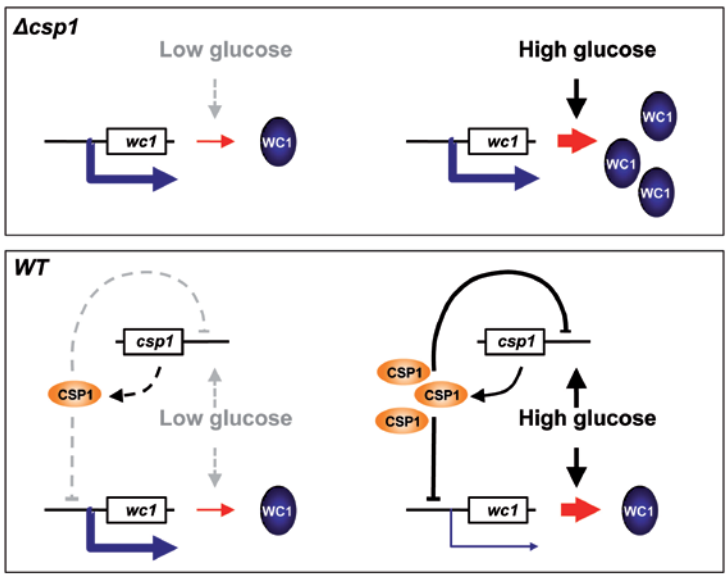


***Fig. 2: Model of glucose-compensated expression of WC1 mediated by CSP1.*** *Expression levels of WC-1 affect the length of the circadian period. Glucose-dependent transcription of* wc1 *RNA and synthesis of WC1 protein in* Δcsp1 *and wild type (WT) are schematically outlined. (*Top *panel) In* Δcsp1*, transcription of* wc1 *is not regulated, and* wc1 *RNA is efficiently produced at low and high glucose levels (thick blue arrows). Glucose affects the general translation rate. At low glucose, translation of* wc1 *RNA is rather inefficient (thin red arrow). The synthesis of WC1 protein per* wc1 *RNA is increased at high glucose (thick red arrow), and hence, WC1 accumulates at an elevated level. (*Bottom *panel) In wild type, CSP1 is a repressor of* wc1 *transcription. Transcription and thus expression of CSP1 are regulated by glucose. Overproduction of CSP1 is limited via a negative feedback of CSP1 on its own transcription. At low glucose, little CSP1 is synthesized, and repression of wc1 transcription is negligible. Hence,* wc1 *RNA is efficiently transcribed (thick blue arrow). Accumulation of CSP1 at high glucose leads to repression of* wc1 *transcription (thin blue arrow). The reduced transcription counterbalances the increased translation efficiency at high glucose (thick red arrow). Accordingly, WC1 levels are similar at low and high glucose.*

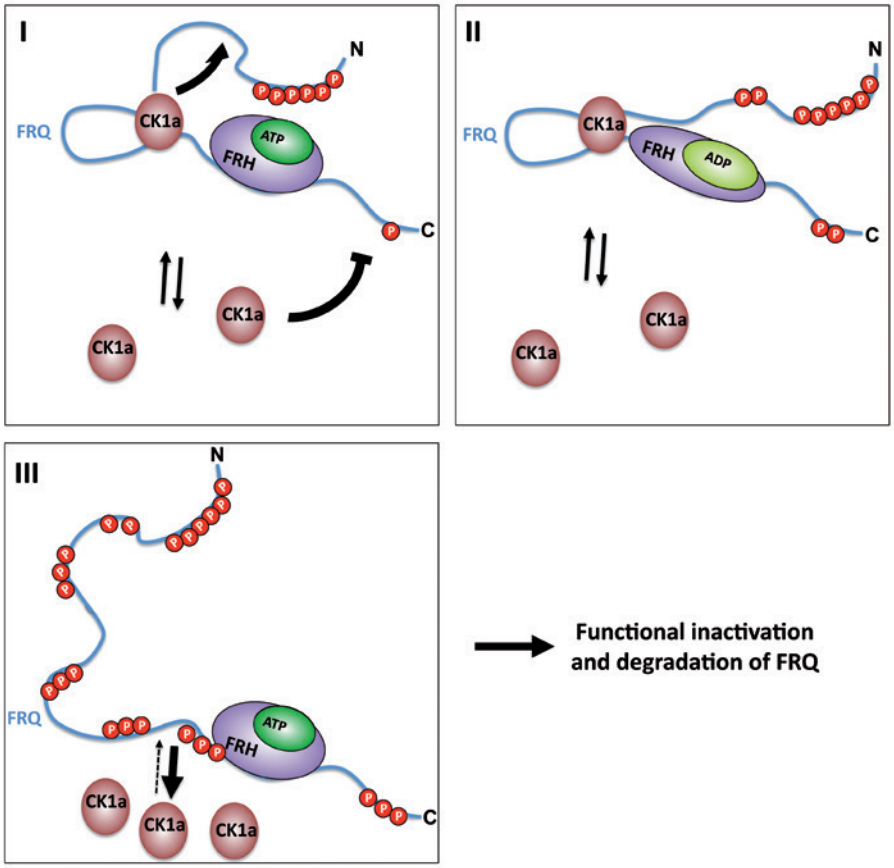


***Fig. 3: FRH acts as a pacemaker in the CK-1a dependent phosphorylation of FRQ***

*FRQ is a largely unstructured protein with a positively charged N-terminal region and a negatively charged middle portion and C-terminus. (I) The potentially flexible random coils of FRQ may eventually reach the catalytic site of a CK1a molecule bound to FRQ. A tightly bound CK1a molecule corresponds to a high local kinase concentration that may support phosphorylation* in cis *of low affinity sites that would not be phosphorylated by free CK1a. (II) ATP hydrolysis by FRH appears to remodel the complex such that the access of bound CK1a to phosphorylation sites in FRQ is compromised. The ATPase cycle of FRH may thus be a pacemaker governing the temporal progression of the phosphorylation of FRQ that is crucial for the molecular timing process. (III) Progressive hyperphosphorylation of FRQ leads to a conformational change and a shift in the binding equilibrium of CK1a with FRQ towards dissociation, which functionally inactivates the complex. Hyperphosphorylated FRQ is eventually degraded via the proteasome.*

## Selected Publications 2011 - 2013


Cesbron F., Brunner M., Diernfellner A.C.R. (2013) Light-dependent and circadian transcription dynamics *in vivo* recorded with a destabilized luciferase reporter in *Neurospora*. PLoS One, in press.

Lee C.-T., Malzahn E., Brunner M. and Mayer M.P. (2013) Light-induced differences in conformational dynamics of the circadian clock regulator VIVID. J. Mol. Biol., in press.

Gin E., Diernfellner AC., Brunner, M., Höfer T. (2013) The Neurospora photoreceptor VIVID exerts negative and positive control on light sensing to achieve adaptation. Mol Syst Biol. 28:9, 667.

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Diernfellner, A.C.R. and Schafmeier, T. (2011) Phosphorylations: making the Neurospora crassa circadian clock tick. FEBS Lett 585, 1461-66.



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| 2006 - 2010 | PostDoc -National Cancer Institute, NIH, MD, USA (Dr. Shiv Grewal) |
| since 2010 | Junior Group Leader - BZH |


## 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

Chromatin compacts and protects the genome, but it also restricts direct access of macromolecules to the DNA. Chromatin modifying activities can open the chromatin structure and provide regulated access to specific genomic loci. Chromatin characteristics, such as position, occupancy and turnover-rate of nucleosomes, incorporation of histone variants, histone modifications and other epigenetic mechanisms, establish an elaborate genomic indexing mechanism, which is responsible for defining functional units in the genome. To identify factors that have a role in repress-

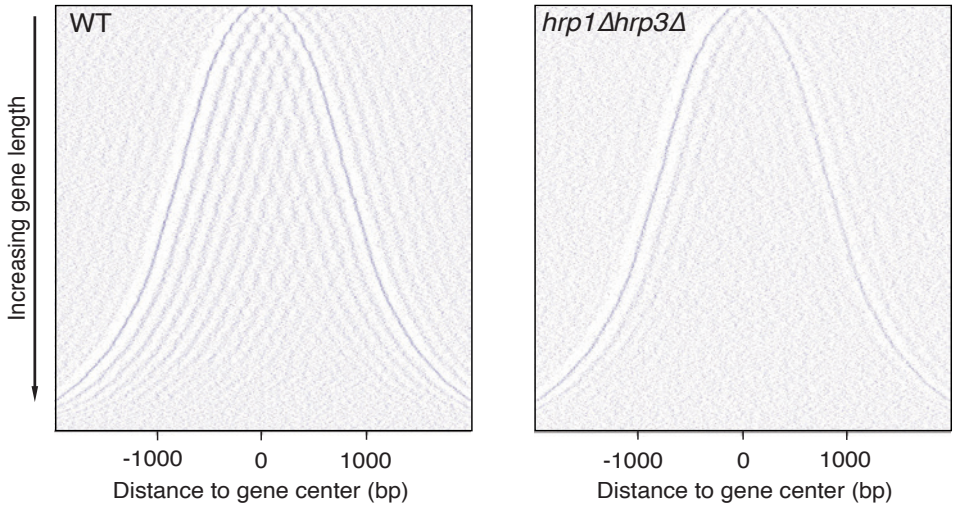


***Fig. 1: The regularly organized nucleosome arrays in gene coding regions are disrupted in the hrp1Δhrp3Δ strain.*** *Two-dimensional plots of nucleosomes along 3,778 genes in WT and hrp1Δhrp3Δ strains. Each row represents a gene; genes were sorted vertically (shortest at the top and longest genes at the bottom) according to the distance between the first and last nucleosome of the gene and were aligned at the mid point. Blue dots correspond to the centre of identified nucleosomes.*

ing cryptic transcription activity in *S. pombe*, we screened a deletion library for chromatin-related factors and tested their effect on cryptic transcription. We found that deletion of the *S. pombe* Chd1 chromatin remodelers, hrp1 and hrp3, results in a dramatic increase in cryptic transcription. To determine the underlying molecular mechanism, we mapped genome-wide nucleosome position and histone acetylation patterns in the Chd1-deficient strain. These experiments uncovered a specific role for Chd1 remodelers in maintaining the highly ordered nucleosome structure within transcription units (Figure 1).

We extended our analysis to other mutations also known to enhance cryptic transcription activity. Although these mutants accumulate cryptic transcripts very similarly to the Chd1-deficient strain, our data showed that the underlying mechanisms are remarkably different.

Another important mechanism to inhibit the accumulation of ncRNAs is their rapid degradation by the nuclear exosome. However, the way that these cryptic RNA transcripts are recognized and targeted to the exosome is not fully understood. We recently identified a multi-subunit complex in *S.pombe* that specifically binds to CUTs and targets them to the nuclear exosome for degradation. Phylogenetic analysis of the subunits shows high similarity to subunits of the human Nuclear EXosome Targeting (NEXT) complex. Deletion or mutation of *S.pombe* NEXT-like (spNEXT) complex subunits leads to dramatic accumulation of CUTs and other aberrant RNAs. spNEXT physically interacts with the nuclear exosome, and with various RNA binding and processing complexes, including the cap-binding, spliceosome, cleavage and poly-adenylation complexes. These results suggest that spNEXT plays a central role in RNA quality control by coupling RNA processing to the RNA degradation machinery.

## Selected Publications 2011 - 2013

Zhu, J.*, Zhou, Y.*, Schermann, G., Ohle, C., Bendrin, K., Sugiyama, T., and Fischer, T. (2013). *Schizosaccharomyces pombe* NEXT-like (spNEXT) complex is a central component in RNA quality control. Submitted (* These authors contributed equally)

Hennig, B.P., and Fischer, T. (2013). The great repression: Chromatin and cryptic transcription. Transcription 4, 97-101.

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Zhang, K., Fischer, T., Porter, R.L., Dhakshnamoorthy, J., Zofall, M., Zhou, M., Veenstra, T., and Grewal, S.I. (2011). Clr4/Suv39 and RNA quality control factors cooperate to trigger RNAi and suppress antisense RNA. Science 331, 1624-27.

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| 11.07.1990 | Habilitation in Biochemistry - University of Regensburg, Germany |
| since 1995 | Full Professor - BZH |
| 2003 - 2005 | Director - BZH |

**Ed Hurt**

# The mechanism of nuclear pore complex and ribosome subunit assembly

## Goal

**We aim to understand how complicated macromolecular assemblies - the nuclear pore complex and the ribosomal subunits - are formed in the eukaryotic cell. To reach these goals we apply** *in vivo* **cell biology and genetic approaches and** *in vitro* **biochemical and structural reconstitution.**

## Background

Nuclear pore complexes (NPCs) are the gateways facilitating nucleocytoplasmic transport across the nuclear envelope. Although the size of the NPC varies between species, the overall structure is evolutionary conserved from yeast to human. Multiple copies (8, 16, 32) of roughly 30 distinct proteins (nucleoporins, or Nups) build up the NPC, which exhibits an eight-fold rotational symmetry and conspicuous substructures such as the spoke-ring complex, cytoplasmic pore filaments and the nuclear basket visualized by different EM techniques. In the past, several model organisms from lower and higher eukaryotes served to study the huge NPC. Although nucleoporins from diverse species differ markedly in their amino acid sequence, their fold-types and domain organization as well as the composition and modularity of the NPC are conserved. The majority of nucleoporins is organized in biochemically stable subcomplexes (e.g. the Y-shaped Nup84 complex), but the mechanism how these modules interact with each other to form the 60-120 MDa NPC assembly is not known. Thus, it is an important goal in our research to reconstitute the entire nuclear pore complex from its individual nucleoporins or derived subcomplexes, and to understand the mechanism of nucleocytoplasmic transport through the active transport channel.

Besides the nuclear pore complexes, ribosomal subunits, which translate mRNAs to synthesize the proteins, are highly sophisticated macromolecular machines with an intricate assembly pathway. During eukaryotic ribosome biogenesis, four ribosomal RNA species and about 80 ribosomal proteins are assembled to form the large 60S and small 40S subunit. This process starts with RNA polymerase I driven transcription of a 35S pre-rRNA species in yeast, which is the precursor to the mature 18S, 5.8S and 25S rRNA. During the subsequent maturation steps, this pre-rRNA is modified, processed by endo- and exonucleases, folded and assembled with the ribosomal proteins and 5S rRNA. Ribosome biogenesis is driven by small nucleolar RNAs (snoRNA) and ca. 150-200 non-ribosomal biogenesis factors. These assembly factors were largely identified by isolation of

pre-ribosomal particles followed by proteomic analysis. Insight into the mechanism of ribosomal assembly came from genetic and biochemical studies in yeast, but recently also structural studies were initiated to characterize nascent ribosomal 60S and 40S subunits by both classical negative stain and cryo-EM. In future studies, the mechanisms of eukaryotic ribosome biogenesis will be unraveled by a combination of different *in vitro* and *in vivo* assays.

## Research Highlights

We aim for the in-depth structure-function analysis of the NPC in *Saccharomyces cerevisiae* and *Chaetomium thermophilum (ct)* by combining, respectively, the excellent genetic and thermophile properties of these two model organisms. In the yeast system, we could develop a non-radioactive pulse-chase method combined with affinity-purification to study the temporal and spatial events during NPC assembly. The principle of this method is to induce a protein-tag of a desired biochemical property on a protein of interest in a very fast responding manner. We took advantage of a specialized orthogonal bacterial tRNA/tRNA-synthetase pair expressed in yeast cells, which incorporates an unnatural amino acid, O-methyl-tyrosine, into proteins in response to a nonsense TAG codon engineered into the ORF. Adding O-methyl-tyrosine to the culture medium will suppress the amber stop codon resulting in an in-frame fusion of the protein-tag to the protein of interest. This method allowed us to pulse/chase label nucleoporins of interest, but also ribosome biogenesis factors (see below) in the 1-15 min range followed by subsequent affinity-purification of the labeled Nup and its associated binding partners. Moreover, in the past three years, we could develop *C. thermophilum* as a source for nucleoporins with improved properties to perform EM, crystallization and *in vitro* reconstitution. Specifically, we have assembled several *ct*Nup modules, the *ct*Nup84, *ct*Nup82 and *ct*Nic96 complexes, and tested for an interaction with the large structural Nups and assembly in higher order structures. The reconstituted complexes were analyzed by negative stain EM. One important breakthrough was the reconstitution of the inner pore ring module with thermophile nucleoporins, consisting of Nup192-Nup170-Nic96-

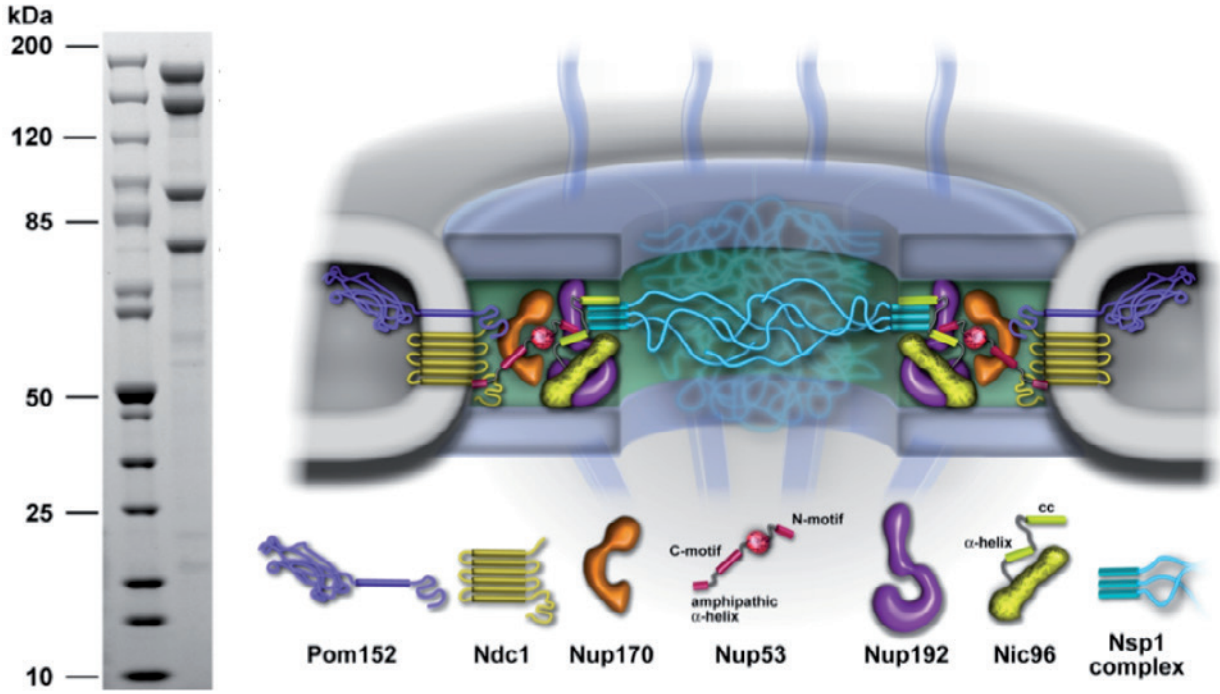


***Fig. 1: Reconstitution of the inner pore ring module from a thermophilic eukaryote.*** *a, SDS-PAGE of the reconstituted Nup192-Nup170-Nic96-Nup53 complex. b, Model of the innner pore ring complex spanning form the nuclear transport channel to the nuclear pore membrane.*

Nup53 (Figure 1). This module will serve as seed to further dock it to other NPC subcomplexes and eventually reconstitute the entire NPC. Moreover, we are currently testing in collaboration with the Hoelz lab (California Institute of Technology, Pasadena, USA) the large structural *ct*Nups and their individual domains for crystallization. From

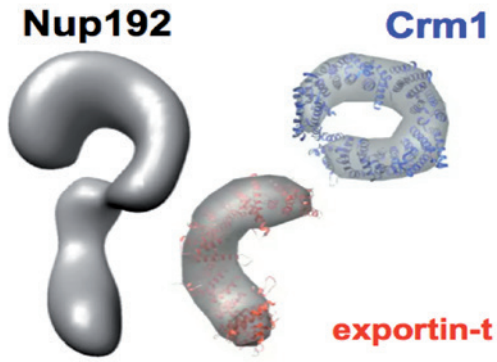


***Fig. 2: Comparison of the structure of ctNup192 with shuttling karyopherin transport receptors*** *(Crm1, exportin-t).*

all these studies, we could get new insight into the structure and assembly of the conserved nuclear pore complex.

When comparing the unusual "S"-like morphology of the α-helically predicted Nup192 molecule to known structures, we noticed that each of the two half circles of Nup192 resembles, in both shape and curvature, the karyopherin transport receptors (e.g. exportin-t or Crm1) that share a superhelical architecture of tandem HEAT repeats (Figure 2). Accordingly, Nups and karyopherins may have a common evolutionary origin. Hence, we speculate that an ancestral Nup (e.g. Nup192) may have lost its ability to get stably incorporated into the NPC, but instead gained binding pockets on the solenoid surface for recruiting export or import cargoes that carry short and exposed targeting signals (e.g. NLS, NES). These evolutionary considerations are supported by findings from a collaborative lab (Ficner group at the University of Göttingen) revealing the x-ray structure of the nuclear export receptor Crm1 from *Chaetomium thermophilum*.

In our research investigating the mechanism of ribosome assembly, we have observed an unforeseen link between nuclear protein import and ribosome assembly. We have discovered a novel import adaptor (Syo1) that synchronously binds two related ribosomal proteins, Rpl5 and Rpl11, which eventually become neighbors on the 60S subunit through their adjacent binding to 5S rRNA. A stoichiometric Syo1-Rpl5-Rpl11 complex was reconstituted *in vitro* that recruited the import receptor Kapβ2 via a PY-NLS at the N-terminus of Syo1, and was imported into the nucleus (Figure 3). The crystal structure of the Syo1-Rpl5 complex (obtained in collaboration with the Sinning lab, BZH) revealed the binding

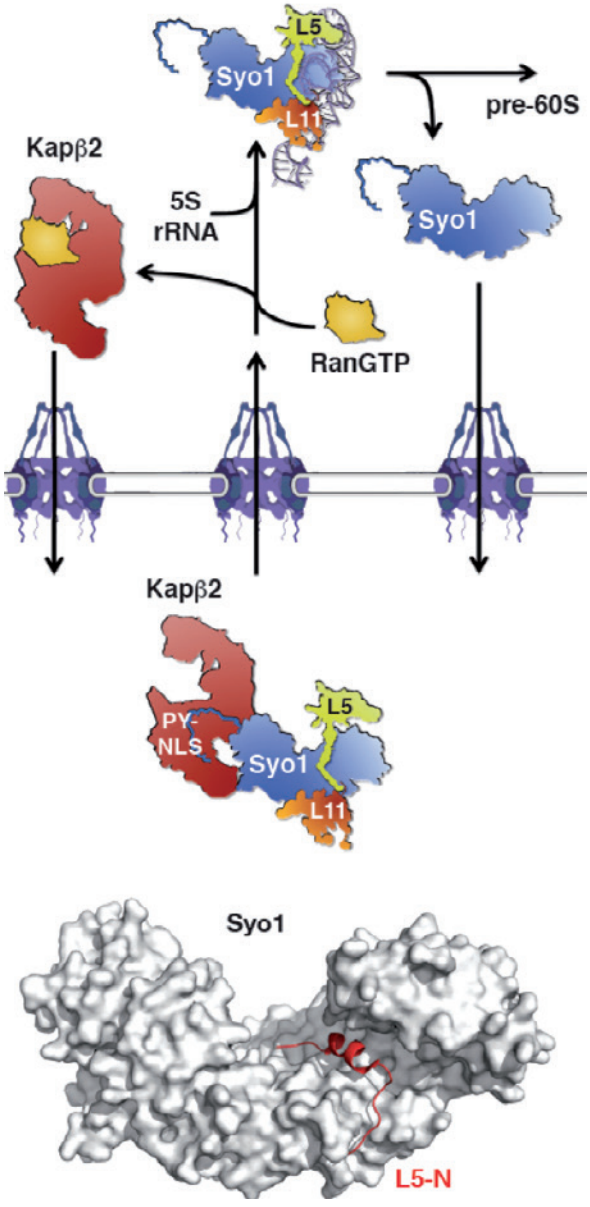


***Fig. 3: Model of the Syo1-Rpl5-Rpl11 import into the nucleus*** *(adapted from Commun Integr Biol. 6:e24792. doi: 10.4161/cib.24792; 2013).*

of the Rpl5 N-terminal peptide motif to a groove on the α-solenoid surface of Syo1. Altogether, the genetic, structural and *in vitro* reconstitution studies have indicated that co-import of Rpl5-Rpl11 coupled with *en bloc* transfer from its import adaptor Syo1 to 5S rRNA could ensure a coordinated and stoichiometric assembly of the 5S RNP onto the evolving pre-60S subunit.

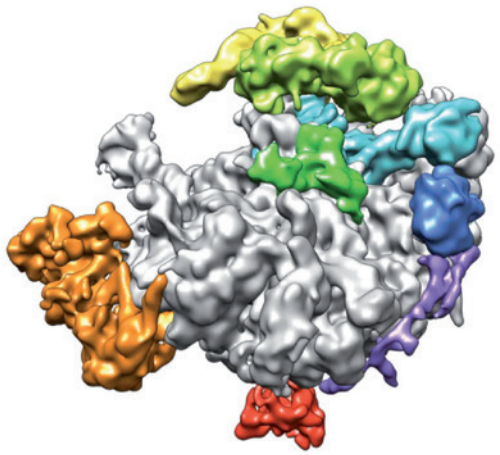

***Fig. 4: Cryo-EM structure of the pre-60S ribosomal subunit*** *(picture provided by C. Leidig and R. Beckmann, Gene Center, University of Munich).*

Despite the advances in our compositional understanding of pre-ribosomal particles, only little structural data is available for nascent ribosomes. Hence, we purified the pre-60S particle and could obtain the first cryo-EM structure of a native pre-60S subunit in association with the assembly factor Arx1 at 9 Å resolution (in collaboration with the Beckmann lab, Gene Center of the University of Munich). This study revealed major structural differences between pre-60S particles and the mature 60S subunit (Figure 4) demonstrating the importance of comprehensive structural investigations of such native pre-ribosomal particles. Our ultimate goal is to come to pseudo-atomic models of pre-ribosomal particles, which will not only be snapshots of the evolving nascent ribosome, but give also insight into the mechanism of ribosome assembly in space and time.

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Bettina Bradatsch, Christoph Leidig, Sander Granneman, Marén Gnädig, Bettina Böttcher, David Tollervey, Roland Beckman and Ed Hurt: Structural insight into the late pre-60S ribosomal subunits with bound nuclear export factor Arx1
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Yoshihira Matsuo, Sander Granneman, Matthias Thoms, Rizos-Georgios Manikas, David Tollervey and Ed Hurt: Quality control of 60S pre-ribosome export involves coupling of a conformation-sensing GTPase to a remodeling AAA-ATPase
Nature, doi: 10.1038/nature12731. Epub 2013 Nov 17.


## Awards and Honors

| | |
|---|---|
| 2007 | Feldberg Prize |
| 2001 | Gottfried Wilhelm Leibniz Prize |
| Since 2010 | Editorial Board of EMBO Journal |
| Since 2007 | Member of ACADEMIA EUROPAEA |
| Since 2005 | Member of LEOPOLDINA |
| Since 1994 | Member of EMBO |


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| 1998 - 2002 | Ph.D. - European Molecular Biology Laboratory (EMBL) Heidelberg, Germany and Charles University in Prague, Czech Republic |
| 2002 - 2008 | PostDoc - Wellcome Trust Centre for Cell Biology, University of Edinburgh, Great Britain |
| since 2008 | Junior Group Leader - Excellence Cluster “CellNetworks”, BZH |

**Martin Koš**

# Ribosome biogenesis

## Goal

**Our aim is to understand how ribosomal RNAs are processed, correctly folded and assembled with proteins to form functional 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 (Figure 1). Pre-rRNA processing takes place within very large particles (>2MDa) called pre-ribosomes, where the rRNA is properly processed, folded and assembled with ribosomal proteins. The process of ribosome maturation is very complex and highly dynamic; it takes only 6 minutes to make a functional mature ribosome. At least 180 non-ribosomal proteins and 70 small nucleolar RNAs (snoRNAs) have been implicated in ribosome biogenesis in yeast. However, the precise function of most of the factors remains unclear. The goal of the lab is to extend our understanding of the molecular mechanism underlying ribosome biogenesis and its regulation.

## Research Highlights

*RNA helicases in ribosome biogenesis*

Mature rRNAs have a very complex structure that appears to be incompatible with their processing and assembly. Unsurprisingly, 19 RNA helicases were found to be essential for ribosome biogenesis in yeast. We investigate their role in rRNA processing. We could show that the RNA helicase Has1 has a dual and independent role in the synthesis of both small and large ribosomal subunits. Using RNA-protein crosslinking and affinity purifications, we have found that two molecules of Has1 bind co-transcriptionally to the pre-rRNA at two distinct sites and are required for pre-rRNA cleavage at the A2 site, which separates the small and large subunit pathways. After cleavage, the two Has1 molecules remain associated with their respective substrates (20S and 27S rRNAs) and likely facilitate recycling of associated ribosome biogenesis factors.

*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

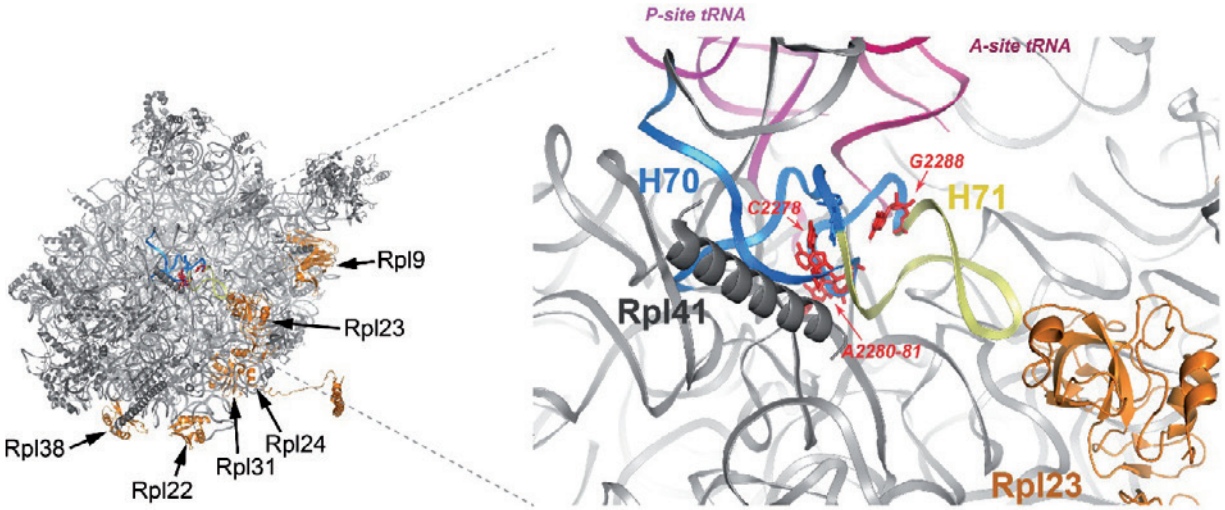


***Fig. 1. Proteins affected in ribosomes lacking methylations of nucleotides C2278 and G2288.*** *Left: crystal structure of the 60S ribosomal subunit. Proteins that are lost from ribosomes lacking rRNA methylation are highlighted in orange. Right: Detailed view of the domain IV of 25S rRNA. The methylated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

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. We could then show 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 (Fig. 1).

*Timing of ribosome biogenesis events*

At least 180 biogenesis factors and 82 ribosomal proteins are known to be required to build a mature ribosome. Affinity purification of various proteins led to the identification of a large number of intermediate pre-ribosomal complexes. Their overlapping composition allowed the various factors to be roughly assigned different stages of ribosome biogenesis. However, more precise information about when they join and leave the pathway is lacking for most of these factors. We attempted to tackle this problem from another direction, to purify pre-ribosomes directly through the pre-rRNA. We created strains with truncated rRNA genes (native promoter and terminator context), each with an RNA affinity tag at its 3'end. This allows us to express pre-RNAs truncated at a chosen position and thus purify "arrested" pre-ribosomes with a theoretical nucleotide resolution. Our system faithfully reproduces pre-rRNA processing and provides information about timing of all cleavages. We can also analyze timing of rRNA modifications and joining of individual processing factors. We are currently optimizing purification of the associated complexes.

## Selected Publications

Gigova, A., Duggimpudi S., Polex T., Schaefer M. and Koš M. (2013). A cluster of methylations in 25S rRNA is required for ribosome stability. (submitted)

Gnanasundram S. and Koš M. (2013). Has1 RNA helicase is required for two distinct processing events during ribosome biogenesis. (submitted)

Kos-Braun,I.C., Jung, I. and Koš M. (2013). Timing of ribosome biogenesis with nucleotide resolution using truncated rDNA genes. (submitted)

Ross,D.A., Barrass, J.D., Dichtl,B., Koš,M., Obtulowicz,T., Robert,M.-C., Koper,M., Karkusiewicz,I., Mariconti,L., Tollervey,D., Dichtl,B., Kufel,J., Bertrand,E. and Beggs,J.D. (2010). RiboSys, a high-resolution, quantitative approach to measure the *in vivo* kinetics of pre-mRNA splicing and 3'-end processing in *Saccharomyces cerevisiae*. *RNA* 16, 2570-2580.

Boon,K.-L. and Koš,M. (2010). Deletion of Swm2p selectively impairs trimethylation of snRNAs by Trimethylguanosine synthase (Tgs1p). *FEBS Lett.* 584, 3299-3304.

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Bohnsack, M.T., Koš,M. and Tollervey,D. (2008). Quantitative analysis of snoRNA association with pre-ribosomes and release of snR30 by Rok1 helicase. *EMBO Rep.* 9, 1230-1236.

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| 1989 | Habilitation in Biochemistry |
| 1995 - 2003 | Apl. Professor for Biochemistry - BZH |
| 2002 | Call for a professorship for Pharmaceutical Chemistry (Marburg), declined |
| since 2003 | Professor for Biochemistry - BZH |

**Luise Krauth-Siegel**

# The parasite-specific trypanothione redox metabolism

## Goal

**Aim of our work is to unravel the unique trypanothione-based thiol redox metabolism of trypanosomatids in atomic detail and to contribute to the development of new antiparasitic drugs on the basis of specific enzyme inhibitors and by identifying novel target molecules.**

## Background

Trypanosomatides, the causative agents of various tropical diseases, possess an unusual redox metabolism. The main non-protein thiol is the bis(glutathionyl)spermidine-conjugate trypanothione which is an essential metabolite for a wide variety of parasite pathways (Fig. 1; Krauth-Siegel and Leroux 2012).

## Research Highlights

**Dissecting the mechanism of trypanothione synthetase under *in vivo*-like conditions**

All proteins of the trypanothione metabolism are indispensable for the viability of African trypanosomes. A final conclusion as to which protein plays a main role in the pathway control yet would need a reliable model based on the kinetic parameters of all enzymes obtained under conditions that reflect the milieu in which the pathway is operating. The kinetic analysis under such *in vivo*-like conditions together with computational modelling revealed that trypanothione synthetase follows a ter-reactant mechanism, releases the intermediate glutathionylspermidine between the two catalytic steps and undergoes both substrate and product inhibition suggesting a tight *in vivo* regulation (Leroux et al. 2013).

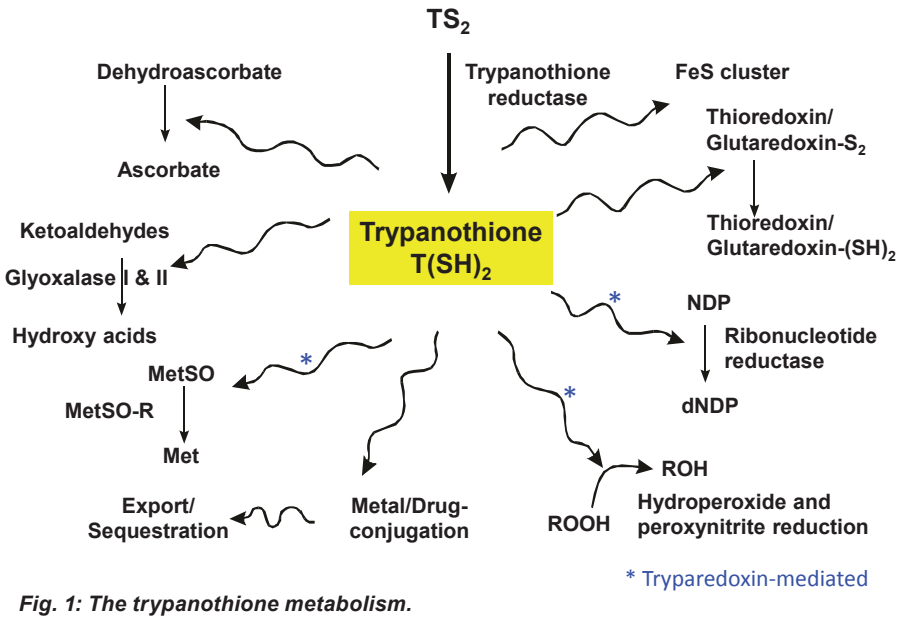


*Fig. 1: The trypanothione metabolism.*

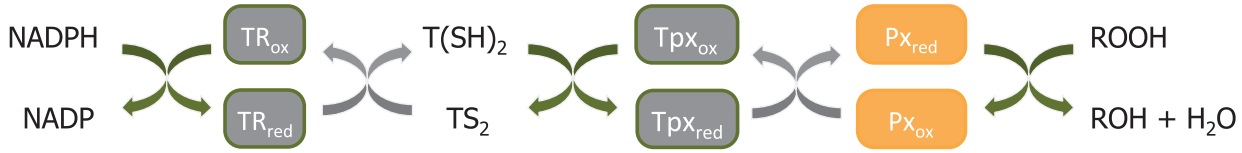


***Fig. 2: Detoxification of hydroperoxides (ROOH) by the trypanothione redox cascade.***

**Detoxification of lipid hydroperoxides in trypanosomes**

In *Trypanosoma brucei*, glutathione peroxidase-type (Px) enzymes, obtaining their reducing equivalents from the T(SH)$_2$/tryparedoxin (Tpx) system (Figs 1 and 2), are responsible for the detoxification of lipid hydroperoxides. Deletion of the individual px genes revealed that the cytosolic isoenzymes, but not the mitochondrial one, are essential. Parasites lacking the cytosolic peroxidases show an extremely rapid cell lysis (Fig. 3). Their proliferation, however, can fully be rescued by supplementing the medium with the vitamin E analog Trolox (Diechtierow and Krauth-Siegel 2011). Recently we could show that the cellular damage originates from disintegration of their lysosome (Hiller et al., submitted).

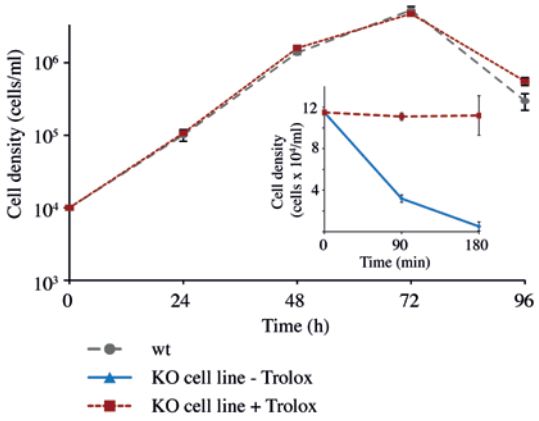


***Fig. 3: Bloodstream T. brucei lacking the cytosolic peroxidases are highly sensitive to cell lysis.***

**The infective form of African trypanosomes requires lipoamide dehydrogenase for DNA synthesis**

Lipoamide dehydrogenase (LipDH), a member of the FAD disulfide oxidoreductase family which also comprises TR, GR, and ThioR, is a component of four mitochondrial multienzyme complexes. Bloodstream *T. brucei* rely exclusively on glycolysis for energy supply. These parasites have an only rudimentary mitochondrion devoid of cytochromes and most enzymes of the citric acid cycle. Thus the role of LipDH in these cells remained elusive. We could show that deletion of *lipdh* resulted in cells with an absolute need for exogenous thymidine. This strongly suggests that bloodstream parasites require LipDH as component of the glycine cleavage complex which generates methylene-tetrahydrofolate for dTMP and thus DNA synthesis (Roldán et al. 2011).

## Selected Publications 2011 - 2013

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Füller, F., Jehle, B., Putzker, K., Lewis, J. D. and Krauth-Siegel, R. L. (2012) High-throughput screening against the peroxidase cascade of African trypanosomes identifies antiparasitic compounds that inactivate tryparedoxin. J. Biol. Chem. 287, 8792-8802.

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| 1985 | Ph.D. - University of Regensburg, Germany |
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| 1987 - 1990 | PostDoc - University of California, Santa Barbara, USA |
| 1994 | Habilitation - University of Regensburg |
| 1994 - 1999 | Group Leader - University of Regensburg |
| since 1999 | Group Leader - BZH |

**Johannes Lechner**

# Kinetochore and Mitosis

## Goal

**To understand kinetochore structure and function.**

## Background

Reliable chromosome segregation depends on the correct attachment of the two sister chromatids to microtubules (MTs) emanating from the opposing spindle poles. The chromosomal structures that mediate this interaction are the kinetochores (KTs). They are composed of several conserved multiprotein complexes that either bind to the centromere DNA, constitute the KT–MT interface or serve as linkers between these two. In addition, proteins that regulate kinetochore function interact with the core components in a mitosis stage specific manner. These include the proteins that regulate the spindle assembly checkpoint (SAC), a signaling mechanism that prevents the initiation of anaphase as long as unattached kinetochores (uaKTs) are present. A second group of proteins that may serve a regulatory function at the KT are proteins that influence the dynamics of MTs. These include the CLASP protein family. CLASP proteins contain two or more TOG-like domains (TOGL). TOG or TOGL domains consist of 6 HEAT repeats that can bind to alpha/beta tubulin dimers via their intra HEAT repeat loops. CLASPs exploit this feature probably to promote MT rescue. A prominent example in this respect is the stabilization of the mitotic spindle. In anaphase, CLASPs promote the stability of interpolar MTs (iMTs) by localizing to the spindle midzone. For metaphase however the principals of CLASP localization and function are not well understood. At the KT, CLASPs are thought to regulate kinetochore microtubule (kMT) dynamics and KT-MT interaction. However, the regulation and dependencies of KT localization as well as the exact function at the KT are unclear.

## Research Highlights

We have analyzed how the individual domains (Fig. 1) of the *S.cerevisiae* CLASP, Stu1, contribute to the localization and function of Stu1 in space and time. In prometaphase Stu1 is sequestered (possibly via oligomerization) at uaKTs via the TOGL1 domain and with the assistance of the CL domain. Thus Stu1 is not available for the stabilization of spindle MTs (Fig. 1A, B, E). Sequestering Stu1 at uaKTs therefore serves as a checkpoint that prevents the formation of a stable spindle and keeps the spindle poles in close proximity in prometaphase. This guarantees fast bipolar attachment of newly captured KTs. Stu1 is important for the capturing process. It is however unclear whether this requires the localization of Stu1 at uaKTs. Upon capturing of an uaKT, Stu1 moves with the KT to the pole and subsequently a majority of Stu1 relocates to spindle microtu-

bules. In metaphase Stu1 localizes to the overlap of iMTs (Fig. 1A, C). This depends on a basic, serine rich sequence (ML domain), that confers lateral MT interaction but not on Ase1, the protein that establishes the spindle midzone. We thus assume that Stu1 interacts with the MTs directly in metaphase. Furthermore, dimerization of Stu1 (via the endogenous D4 domain or an ectopic dimerization domain) is important for efficient localization to the overlaps of the iMTs in metaphase. We thus assume that Stu1 crosslinks iMTs in metaphase and thus assists the formation of a stable metaphase spindle. In addition, localizing Stu1 to iMT overlaps allows microtubule rescue. Surprisingly only one of the two TOGL2 domains present in Stu1 (TOGL2) is required for this activity and consistently only TOGL2 binds a tubulin dimer. As observed for uaKTs, TOGL1, enables Stu1 to interact with the KT in metaphase (Fig1A, F). In addition, this localization requires lateral MT interaction. Localization of Stu1 to metaphase KTs is essential to stabilize kMTs (that is kMTs shorten dramatically if KT localization is compromised). Moreover Stu1 at metaphase KTs apparently regulates kMT length in correlation to the tension at the KT-MT interface. One model how this may be achieved is depicted in Fig.1F: TOGL2, located between the KT-interacting TOGL1 and the MT-interacting ML, may be displaced from the MT plus ends in the absence of tension at the KT-MT interface and thus allow MT depolymerization. Upon tension TOGL2 may be placed at the MT plus end to support MT rescue. With the beginning of anaphase Stu1 detaches from the MT lattice and binds to the Ase1 dependent midzone via the D4 domain (Fig. 1A, D). This relieves the iMT crosslinking by Stu1 while guaranteeing the continued localization of the (MT-rescuing) TOGL2 to the iMTs overlap. Thus the strategy of Stu1 localization in meta- and anaphase fits the needs: To stabilize metaphase spindles when tension is applied at the unresolved sister chromatids and to allow MT gliding in anaphase. Stu1 also dissociates from KTs in anaphase (Fig. 1A, G) and consistently the kMTs shorten (anaphase A). The dissociation of Stu1 from KTs at the metaphase to anaphase transition therefore is sufficient to regulate the initiation of anaphase A.

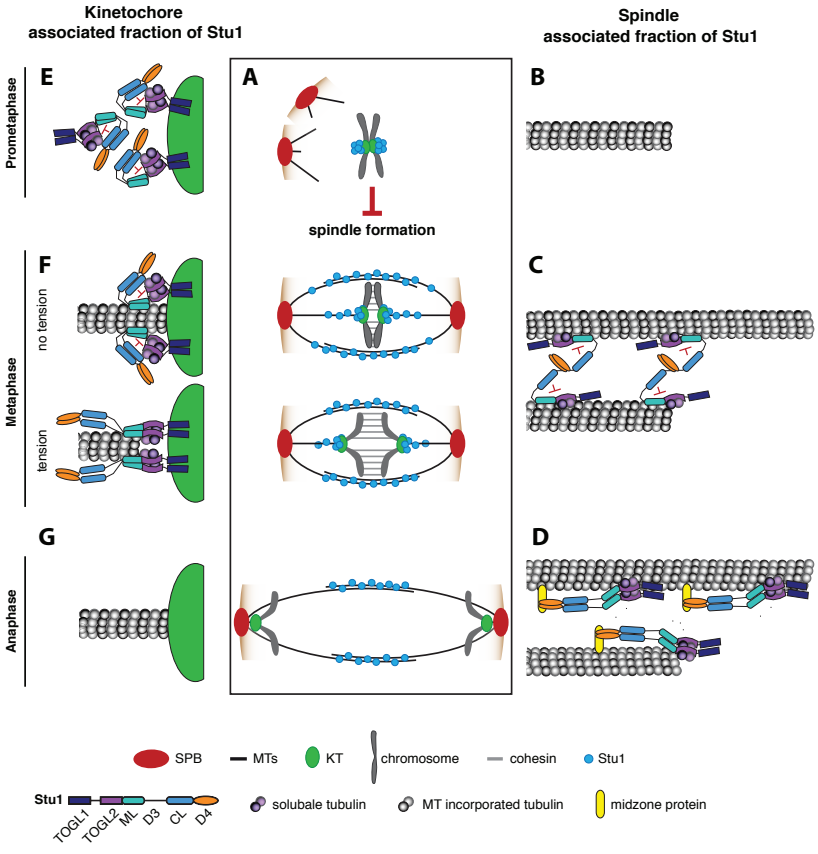


***Fig. 1: Model of Stu1 localization and function during mitosis.***

## Selected Publications


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Ortiz, J., Funk, C., Schäfer, A., and Lechner, J. 2009. Stu1 inversely regulates kinetochore capture and spindle stability. *Genes Dev* 23(23): 2778-2791.



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|---|---|
| 1988 - 1993 | Chemistry Studies, University of Athens, Greece |
| 1994 - 1998 | Ph.D. - Zentrum für Molekulare Biologie Heidelberg (ZMBH), Germany (Prof. Stefan Jentsch) |
| 2000 - 2005 | PostDoc - Institute of Biochemistry, ETH Zürich, Switzerland (Prof. Yves Barral) |
| 2006 - 2013 | Junior Group Leader - BZH |
| since 10/2013 | Group Leader, CRBM Montpellier, CNRS France |

**Dimitris Liakopoulos**

# Spindle positioning in yeast

## Goal

**To study the mechanisms that bring the spindle to its correct position during asymmetric cell divisions.**

## Background

Polarized cells have two options when they divide: they can either divide symmetrically, or asymmetrically. Asymmetric divisions are encountered whenever the goal is generation of cellular diversity, for example during embryonic divisions or the divisions of stem cells. Factors that determine cell fate are asymmetrically segregated in one of the two daughters, that consequently differentiates.

In an asymmetric cell division, the cytokinetic machinery must cleave the cell perpendicular to the polarized material, resulting in unequal segregation of the polarized factors. At the same time, the cytokinetic actomyosin ring cleaves the cell midway through the mitotic spindle to ensure equal segregation of chromosomes between daughters. Coordination of cell cleavage with chromosome segregation depends on interactions of astral spindle microtubules (aMTs) with the cortical actin cytoskeleton. A complex network of proteins involving non-motor microtubule plus-end tracking proteins (+TIPs), kinesins, dynein and actin-interacting proteins mediate these interactions (Fig. 1).

Our lab mainly studies the mechanisms and regulation of astral spindle microtubules with the cortical cytoskeleton using one of the simplest asymmetrically dividing organisms, the yeast *S. cerevisiae*.

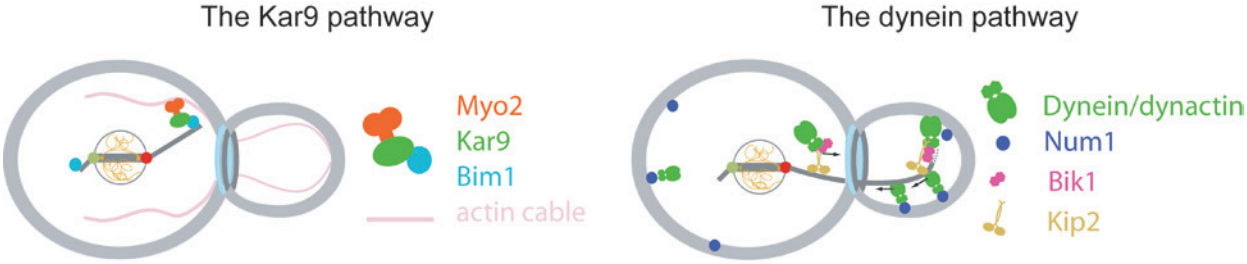



***Fig.1: Spindle positioning in yeast.*** *Thick grey bar: metaphase spindle, red, green spots: old and new microtubule organizing centers (SPBs), thin grey lines: aMTs. Left: The protein Kar9 forms a bridge between aMTs and actin cables through its interaction with Bim1 and Myo2. Myo2 pulls aMTs from the old SPB and the metaphase spindle towards the bud. Right: Dynein is transported by the kinesin Kip2 to the (+)-ends of aMTs and binds to Num1 at the cortex. Num1-immobilized dynein pulls aMTs and orientates the spindle, because its motor activity is directed towards the minus-ends of aMTs that are anchored at the SPB. The blue-gray ring is the actomyosin-based cytokinetic apparatus and the future site of cytokinesis. Only metaphase spindles aligned with the mother-bud axis are able to elongate and partition half of the chromosomes into the bud in anaphase, so that cytokinesis can occur later midway through segregated chromosomes.*

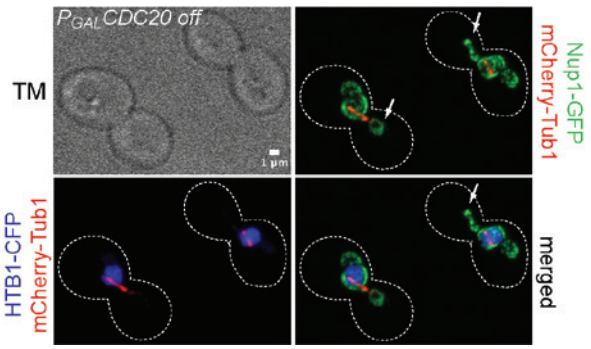
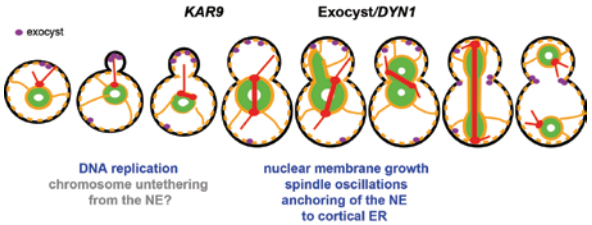



***Fig. 2:*** *Images of living cells showing the relative positions of the nuclear envelope (marked with the nucleoporin fusion Nup1-GFP), the mitotic spindle and the DNA (visualized with the histone H2B fusion HTB1-CFP). Two cells with nucleopodia (arrows). In all cases, the mass of the DNA is associated with the spindle. In dumbbell nuclei, spindle and DNA are inserted in the bud neck, whereas NP enter the bud while the spindle and the bulk of the DNA are still in the mother cell.*

## Research Highlights

Our project concerning the mechanics of nuclear migration during closed mitosis led to the discovery of a novel, auxiliary mechanism of nuclear migration in budding yeast (Kirchenbauer et al., 2013). We investigated changes in nuclear morphology during nuclear migration and showed that in pre-anaphase cells, nuclear protrusions (nucleopodia, see Fig. 2) extend into the bud, preceding insertion of chromosomes into the bud neck. Interestingly, formation of nucleopodia did not depend on the Kar9 or the dynein pathway, but instead required nuclear membrane expansion, an intact actin cytoskeleton and the exocyst complex. In addition, DNA replication was also required for generation of nucleopodia. We thus proposed that nuclear membrane expansion, DNA replication and exocyst-dependent anchoring of the nuclear envelope to the bud affect nuclear morphology and facilitate correct positioning of nucleus and chromosomes relative to the cleavage apparatus (Fig. 3). We are now following this project investigating the mechanism that connects DNA replication with nuclear migration.




We had recently found that the protein Kar9, the yeast functional equivalent of the Adenomatous Polyposis Coli (APC) tumor suppressor that links astral microtubules with actin, is regulated by phosphorylation, sumoylation and ubiquitylation (Kammerer at al. 2010). We were now able to identify the enzymatic machinery that mediates Kar9 ubiquitylation and we have elucidated the pathway that connects the three posttranslational modifications which control Kar9 function.

In addition, we initiated the reconstitution and study of Kar9 complexes *in vitro*, in order to generate quantitative information on the function and properties of these complexes.

Finally, we have made additional progress on the regulation of the kinesin Kip2 by the GSK-3 kinase. Yeast GSK-3 phosphorylates Kip2 and reduces its affinity for microtubules and consequently, transport of dynein and Kar9 on aMT ends. Interestingly, Kip2 is able to stabilize microtubules. We collaborated with the lab of J. Howard (Dresden) which has made significant progress on understanding the mechanism through which Kip2 increases microtubule stability.

Since October 2013, the lab has moved and will continue its projects to the CRBM (Centre de Recherche de Biochimie Macromoléculaire)-CNRS in Montpellier, France. We are thankful for all the exciting time in the BZH!

## Selected Publications 2011 - 2013

Kirchenbauer, M. & Liakopoulos, D. An auxiliary, membrane-based mechanism for nuclear migration in budding yeast. *Mol Biol Cell* 24, 1434-1443 (2013).

Stevermann,L. and Liakopoulos,D. (2012). Spindle positioning: structures and new concepts. *Curr. Opin. Cell Biol.* 24, 816-24 (2012).

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| 1994 | Ph.D. - University of Göttingen, Germany |
| 1994 - 1997 | PostDoc - Institute for Biochemistry I, University of Heidelberg, Germany (Prof. Felix Wieland) |
| 1997 - 2000 | PostDoc - Memorial Sloan-Kettering Cancer Center, New York, USA (Prof. James E. Rothman) |
| 2001 | Group Leader - BZH |
| since 2004 | Professor of Biochemistry - BZH |

**Walter Nickel**

# Unconventional Protein Secretion

## Goal

**To reveal the molecular components and mechanisms involved in unconventional secretion of fibroblast growth factor 2 from tumor cells as well as to identify small molecule inhibitors of this process to develop a novel class of anti-angiogenic drugs.**

## Background

The vast majority of extracellular proteins is secreted through the classical ER/Golgi-dependent secretory pathway, however, numerous exceptions have been identified. As opposed to proteins that are transported along the classical route, unconventional secretory proteins lack a signal peptide and their export from cells is not affected by brefeldin A, an inhibitor of ER-to-Golgi trafficking. Several kinds of unconventional secretory pathways have been described some of which involve intracellular vesicles such as secretory lysosomes or multi-vesicular bodies. By contrast, unconventional secretion of fibroblast growth factor 2 (FGF2) has been shown to occur by direct translocation across plasma membranes resulting in its association with heparan sulfate proteoglycans on cell surfaces. Using biochemical reconstitution experiments and genome-wide RNAi screening approaches, our laboratory aims at a functional dissection of the unconventional secretory pathway of FGF2 at the molecular level. We further make use of these insights to develop small molecule inhibitors as lead compounds for the development of novel drugs that inhibit tumor-induced angiogenesis as well as prevent anti-apoptotic effects of FGF2 that cause resistance of tumor cells against chemotherapy.

## Research Highlights

In recent years, we have revealed a number of key steps of the molecular mechanism by which FGF2 translocates across the plasma membrane to reach the extracellular space (Fig. 1). Following the initial observation that FGF2 can physically traverse the plasma membrane, we identified a binding pocket for the phosphoinositide $PI(4,5)P_2$ in the crystal structure of FGF2. Further studies revealed that FGF2 secretion is initiated by $PI(4,5)P_2$-dependent recruitment of FGF2 at the inner leaflet of the plasma membrane. This process causes FGF2 to homo-multimerize driving membrane insertion of a FGF2 oligomer that has been hypothesized to have a ring-like, membrane-pore-forming structure. This view is supported by reconstitution experiments with model membranes where $PI(4,5)P_2$-dependent oligomerization and membrane insertion of FGF2 causes both membrane passage of small fluorescent tracers and transbilayer diffusion of

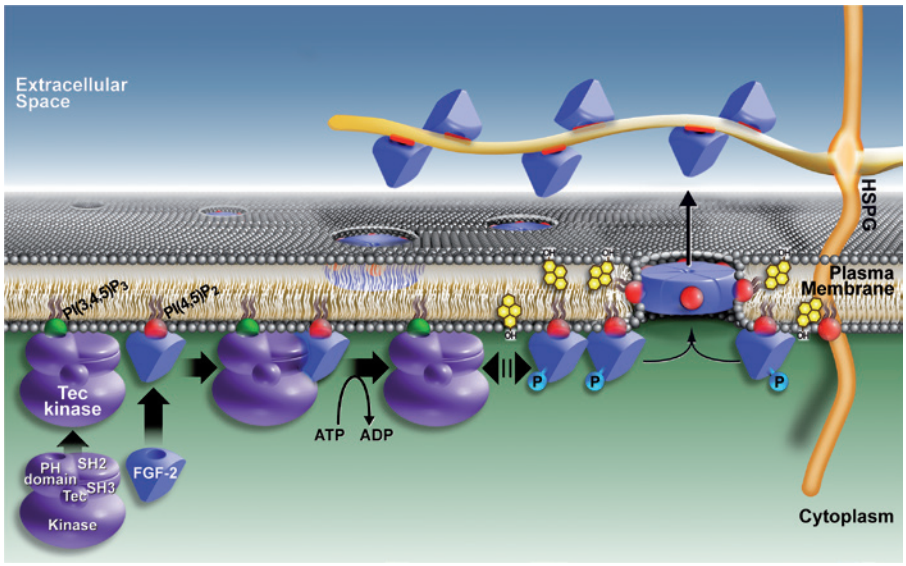


***Fig. 1:*** *Molecular components and mechanisms involved in unconventional secretion of FGF2 from tumor cells.*

membrane lipids. Based on these observations, a toroidal architecture of lipidic membrane pores formed by FGF2 oligomers was proposed.

Membrane insertion of FGF2 oligomers is strongly enhanced by Tec-kinase-mediated tyrosine phosphorylation of FGF2, a potential regulatory mechanism that controls FGF2 secretion. This view is further supported by cell-based assays with Tec kinase being required for efficient FGF2 secretion from cells. Intriguingly, Tec kinase contains a PH domain mediating its recruitment to the inner leaflet of the plasma membrane in a $PI(3,4,5)P_3$ dependent manner. This may in part explain why PI3 kinases, the enzymes that synthesize $PI(3,4,5)P_3$, are up-regulated in many cancers.

Our findings point to a role of membrane-inserted FGF2 oligomers as translocation intermediates in unconventional secretion of FGF2. To translocate FGF2 to the cell surface, membrane-inserted oligomers of FGF2 need to be removed from the membrane, a process that was shown to be mediated by cell surface heparan sulfate proteoglycans that compete against $PI(4,5)P_2$ for binding to FGF2. These findings have been interpreted as an extracellular trapping mechanism resulting in cell surface exposure of FGF2. The overall process of FGF2 secretion therefore relies on sequential interactions of FGF2 with $PI(4,5)P_2$ and Tec kinase at the inner leaflet, membrane pore formation by FGF2 oligomerization and extracellular trapping mediated by cell surface heparan sulfate proteoglycans.

In addition to the molecular characterization of further components of the secretory machinery of FGF2, a major focus of our laboratory is now to identify small molecule inhibitors of FGF2 secretion. In a first attempt, we have identified inhibitors of the interaction of FGF2 and Tec kinase. Intriguingly, such compounds inhibit both phosphorylation of FGF2 and secretion of FGF2 from cells. It is a key aim to expand these efforts to all known components of FGF2 secretion to achieve our long-term goal of the development of anti-cancer drugs that are based on the inhibition of FGF2 release from tumor cells.

## Selected Publications 2011 - 2013

Disanza et al. CDC42 switches IRSp53 from inhibition of actin growth to elongation by clustering of VASP. EMBO J. 2013 Oct 16;32(20):2735-50.

Di Ventura et al. Chromosome segregation by the Escherichia coli Min system. Mol Syst Biol. 2013;9:686.

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Nickel, W. The Unconventional Secretory Machinery of Fibroblast Growth Factor 2. Traffic 2011; 12:799-805.

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| 2004 - 2006 | Ph.D. - University of Granada, Spain |
| 2004 - 2011 | PostDoc - Vlanders Interuniversity Institute for Biotechnology (VIB), Leuven, Belgium (Prof. Peter Carmeliet) |
| since 2011 | Junior Group Leader - BZH |

**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 during development of the central nervous system.

## Background

Despite their distinct functions, the nervous and vascular systems share many more similarities and common principles than previously anticipated. It is striking how similar axons and blood vessels grow. At the end of a growing axon, the *growth cone* is responsible for extending filopodia, sensing the environment and guiding the axon to its final target (Figure 1). Similarly, at the tip of a sprouting blood vessel, a specialized endothelial cell, the *endothelial tip cell*, extends numerous *filopodia* and senses the guidance signals (Figure 1). Both networks also share regulatory molecular mechanisms and guidance cues during the process of pathfinding and growth. These recent observations bring up the new concept of an existing Neurovascular link controlling vascular and neuro-developmental processes. 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 angiogenic factors beyond its normal tissue environment (the vascular system). In addition, nowadays we know that communication between both networks is essential for their precise development and function; yet, the molecular mechanisms of this Neuro-Vascular crosstalk remain still poorly understood.

## Research Highlights

The key angiogenic factor, vascular endothelial growth factor (VEGF-A, termed from hereon VEGF), as well as other members of its family such as VEGF-C and VEGF-D, and their receptors, apart of controlling vascular development, are also expressed in neuronal cells and participate in processes such as neurogenesis, neuronal migration, axon guidance, dendritogenesis and dendrite maintenance. Our previous research showed that VEGF and its receptor VEGFR-2 (also termed Flk1) act as a guidance cue and guidance receptor respectively in neuronal migration during cerebellar development. Moreover, we identified that NMDARs act as co-receptors for VEGFR-2 in migrating cerebellar granule cells (GC). VEGF regulates GC migration by binding to VEGFR-2 and modulating NR2B properties to enhance NMDARs-mediated calcium influx. Additionally, we identified that VEGF can act as a commissural axon guidance cue during spinal cord development. Despite these initial findings, still little is known about the biology of VEGF or of any other angiogenic factors in neurons, the

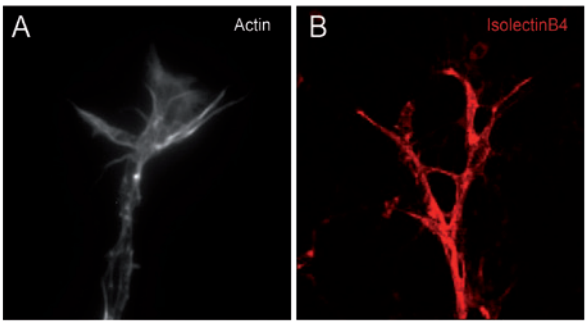


***Fig. 1: Axon growth cone and endothelial tip cell***
***A)*** *Image of a growing growth cone from an isolated hippocampal neuron.* ***B)*** *Image of an endothelial tip cell from a growing blood vessel in the mouse retina.*

signaling pathways that they activate and their functional role in neurodevelopment. Thus, our group is currently focused in further elucidating these processes using as a model systems the developing mouse spinal cord, cerebellum and hippocampus.

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 the CNS angiogenesis.

Here, as model systems we are focused in the developing mouse CNS (brain, spinal cord and retina) (Figure 2). Similar to as above, our experimental approaches also comprise *ex vivo* and *in vivo* methodologies using RNAi technologies and mouse genetics, *in vitro* cell biology and biochemistry and state of the art microscopy.

## Selected Publications 2011 - 2013

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Carmeliet, P, Ruiz de Almodovar, C. VEGF ligands and receptors: implications in neurodevelopment and neurodegeneration. *Cell Mol Life Sci.* 2013, May;70(10):1763-78.

Snuderl M*, Batista A*, Kirkpatrick ND*, Ruiz de Almodovar C*, et al. Targeting placental growth factor/neuropilin 1 pathway inhibits growth and spread of medulloblastoma. *Cell*, 2013 Feb 28;152(5):1065-76.

Meissirel, C*, Ruiz de Almodovar, C* et al. VEGF modulates NMDA receptor activity via a Src-family-kinase-dependent cross-talk. *Proc Natl Acad Sci U S A.* 2011 Aug 16;108(33):13782-7.

Ruiz de Almodovar, C*, Fabre, P* et al. VEGF mediates commissural axon chemoattraction through its receptor Flk1. *Neuron* 2011, June 9 (70):966-78.

(* Equal contribution)

## Awards and Honors

| | |
|---|---|
| 2009 | FEBS Young Investigator Award |
| 2011 | Marie Curie Career Integration Grant |
| 2013 | ERC Starting Grant |

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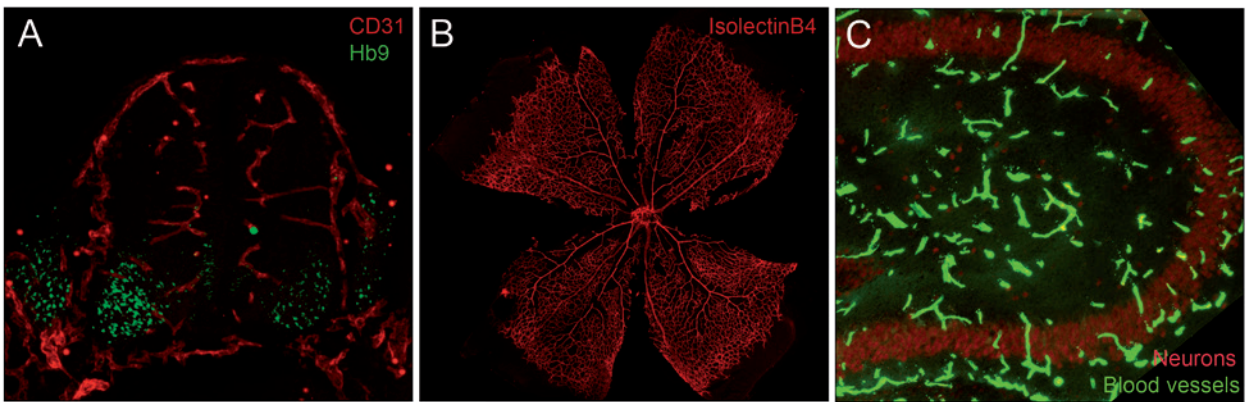


***Fig. 2: Model systems to study neuro-vascular communication during CNS development. A)*** *Image of a spinal cord cross-section from a E11.5 mouse embryo where blood vessels are labeled with the endothelial cell marker CD31 (red) and motor neurons with the HB9 marker (green).* ***B)*** *Whole mount 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).*

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| 1989 | Ph.D. - Ludwig-Maximilians-Universität München, Germany (Max Planck Institute of Biochemistry, Martinsried) |
| 1989 - 1991 | PostDoc - Max Planck Institute for Biophysics, Frankfurt, Germany (Prof. Hartmut Michel) |
| 1991 - 1993 | Scientist - Biomedical Centre, Uppsala, Sweden (Prof. Alwyn Jones) |
| 1994 - 1999 | Group Leader - European Molecular Biology Laboratory (EMBL), Heidelberg, Structural Biology Programme |
| since 2000 | Full Professor - BZH |
| 2006 - 2009 | Director - BZH |
| 2011 - 2013 | President of the GBM |

**Irmgard Sinning**

# Molecular Machines in protein targeting and membrane protein biogenesis

## Goal

**To understand the structure and the functional mechanisms of molecular machines in co- and post-translational protein targeting, and in particular in membrane protein biogenesis.**

## Background

Membrane proteins comprise a major fraction of the cellular proteome and their function depends on insertion into the correct target membrane. Already during synthesis at the ribosome, nascent proteins experience a number of interactions and modifications by targeting factors, chaperones and enzymes (Fig. 1). We aim at a detailed analysis of the structure and function of the involved molecular machines by an integrated structural biochemistry approach – combining protein crystallography with biochemical and biophysical techniques.

The understanding of the universally conserved delivery pathway for membrane proteins by the signal recognition particle (SRP) is one major interest of the lab. The SRP pathway elegantly couples protein synthesis at the ribosome to membrane targeting and insertion. This co-translational mechanism allows to avoid exposure of hydrophobic transmembrane domains. 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. The RNA not only provides a scaffold for SRP assembly, it is actively involved in the regulation of protein targeting by accelerating complex formation between SRP and the membrane bound SRP receptor (SR), and by activating the GTPases present in SRP and SR. Despite its conservation in evolution, the SRP system has been adapted for specific requirements. The post-translational function of SRP in chloroplasts (cpSRP) is a particularly interesting adaptation as it guides nuclear encoded light-harvesting chlorophyll a,b binding proteins (LHCPs) to the thylakoid membrane. We dissect the structure and function of cpSRP43, a unique component of cpSRP, in order to understand its role in LHCP biogenesis. Our data provide structural snapshots of SRP and SR in different functional states and our aim is to finally arrive at a movie of SRP driven membrane protein biogenesis in all kingdoms of life. In particular, we are interested in the role of membrane lipids in the regulation of SR and membrane protein activity.

In contrast to the typical SRP clients with an N-terminal signal sequence, tail-anchored (TA) membrane proteins contain a single transmembrane domain at their C-terminus, which excludes

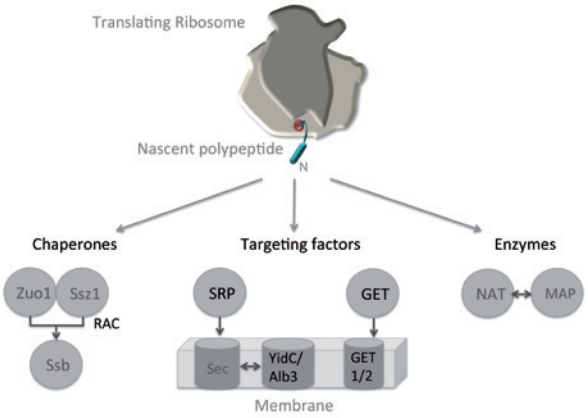


***Fig. 1:*** *Targeting factors, chaperones and enzymes interact with the nascent polypeptide already during translation.*

them from the co-translational SRP pathway. TA proteins play important roles in membrane insertion, membrane fusion and apoptosis, and utilize the so-called Get (guided-entry of tail-anchored membrane proteins) pathway for delivery to the ER. The Get pathway depends on ATP - like other post-translational targeting pathways, but the central Get3 ATPase shares common principles of regulation with the SRP GTPases. We unravel mechanistic details and common principles of regulation of both, the SRP and Get pathway, and we are particularly interested in the membrane-associated steps.

Besides these two targeting pathways, we got attracted by the biogenesis of eukaryotic ribosomes, which involves more than 150 factors. Only little is known about nuclear import of these factors, their role in the assembly, maturation and export of pre-ribosomal subunits, and we aim to contribute by structural and biochemical studies.

## Research Highlights

cpSRP43 is a unique component of chloroplast SRP (cpSRP) and consists of chromodomains and ankyrin repeats. Ankyrin repeats are well established as versatile protein interaction modules, while chromodomains are almost exclusively known for their key role in the regulation of gene expression, reading the so-called histone code. The cpSRP43 ankyrin repeats provide the binding site for an internal signal sequence present in LHCPs, the L18 region between the transmembrane helices 2 and 3, which is recognized with high sequence specificity in an SRP-unlike manner. cpSRP43 acts as an ATP independent membrane protein chaperone and is able even to disaggregate fresh LHCP aggregates. We have shown that the overall structure and charge distribution of cpSRP43 mimicks the SRP RNA which is absent in cpSRP. How the regulatory role of the SRP RNA is replaced in cpSRP is however still not understood. cpSRP54 contains a chloroplast specific C-terminal extension (the 'RRKR' peptide) required for interaction with chromodomain 2 (CD2) of cpSRP43. Chromodomains typically recognize lysine methylation in histone tails by cation-$\pi$ interaction in a so-called aromatic cage. Structure determination of the cpSRP43-RRKR complex showed that CD2 binds an arginine-rich motif in a modified aromatic cage reminiscent of the canonical chromodomain-histone tail interaction (Fig. 2). We have identified arginine-rich motifs also in the C-terminal extension of the Alb3 membrane insertase responsible for LHCP insertion. These motifs are involved in cpSRP43/Alb3 interaction and we expect a similar read-out as in the CD2/RRKR complex. Our studies suggest

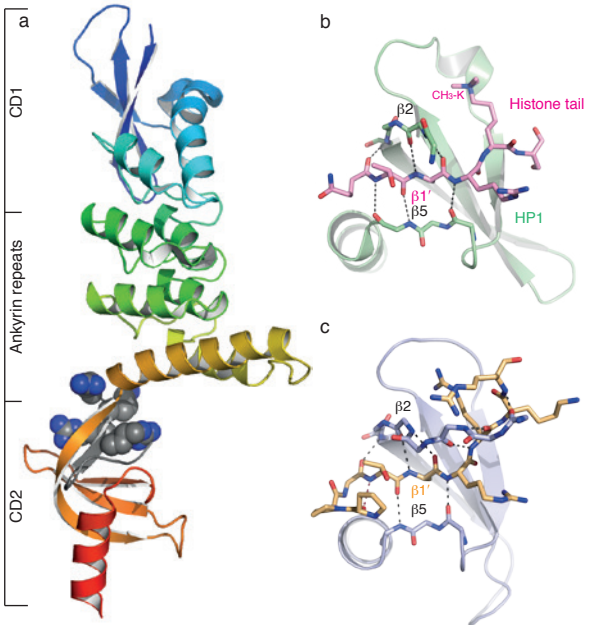


***Fig. 2: Adaptation of the chloroplast SRP system to post-translational targeting.*** ***a)*** *cpSRP54-RRKR motif binds at the interface between cpSRP43 ankyrin repeat 4 and CD2.* ***b)*** *'Canonical' histone tail–chromodomain interaction by β-augmentation, and c) RRKR-CD2 interaction.*

a model for the formation of the transit complex (consisting of cpSRP43/54 and LHCP) and its delivery to the thylakoid membrane by interaction with Alb3. We continue our efforts in characterizing the transit complex and understanding the post-translational adaptation of the SRP system.

The SRP GTPases form a distinct subfamily of the SIMIBI (for Signal recognition particle, MinD, BioD) class of NTP binding proteins with only three members: the SRP core protein SRP54, the SRP receptor protein FtsY (in bacteria; SR$\alpha$ in eukaryotes), and FlhF, a protein involved in the assembly of polar flagella. We have previously identified a conserved membrane targeting sequence (MTS) in FtsY that is required and sufficient for directing the SRP receptor to the plasma membrane. Amide hydrogen-deuterium exchange with mass spectrometry (HX-MS), combined with X-ray crystallography and CD spectrometry allowed us to show that anionic phospholipids trigger a conformational switch necessary for subsequent activation of the FtsY GTPase. This conformational switch presents a crucial checkpoint in SRP mediated protein targeting.

The central component of TA membrane protein biosynthesis, the ATPase Get3, is also a member of the SIMIBI class of NTP binding proteins. Structure determination of Get3 in different nucleotide loaded states and characterization of Get3/TA protein interaction by HX-MS analysis showed that the basic principles of cargo recognition are conserved between the SRP and Get pathways. The Get3/TA protein complex is delivered to the ER by a direct interaction between Get3 and the Get receptor complex consisting of two integral membrane proteins, Get1 and Get2. In collaboration with Vlad Denic and Volker Dötsch we showed, that Get1 and Get2 are both necessary for TA protein insertion, and that they use adjacent, partially overlapping binding sites on the Get3 dimer (Fig. 3). Based on X-ray and NMR data together with detailed mutagenesis and interaction studies, we derived a model for how the Get3 ATPase cycle is linked to TA protein binding and finally release at the ER. We favour the idea that the energy of ATP hydrolysis drives Get3 to an 'open' state, which allows release of ADP-$Mg^{2+}$. Rebinding of ATP at the membrane facilitates dissociation of Get3 from the receptor and allows the next targeting cycle. Related structural transitions have been reported for ATP-binding cassette (ABC) transporter proteins, suggesting how nucleotide dependent changes in the Get3 ATPase could be communicated to the transmembrane segments in the receptor proteins. We continue to unravel the precise mechanism of TA protein insertion.

Additional research activities center on interactions of nascent chains at the ribosome with chaperones and enzymes. The eukaryotic ribo-

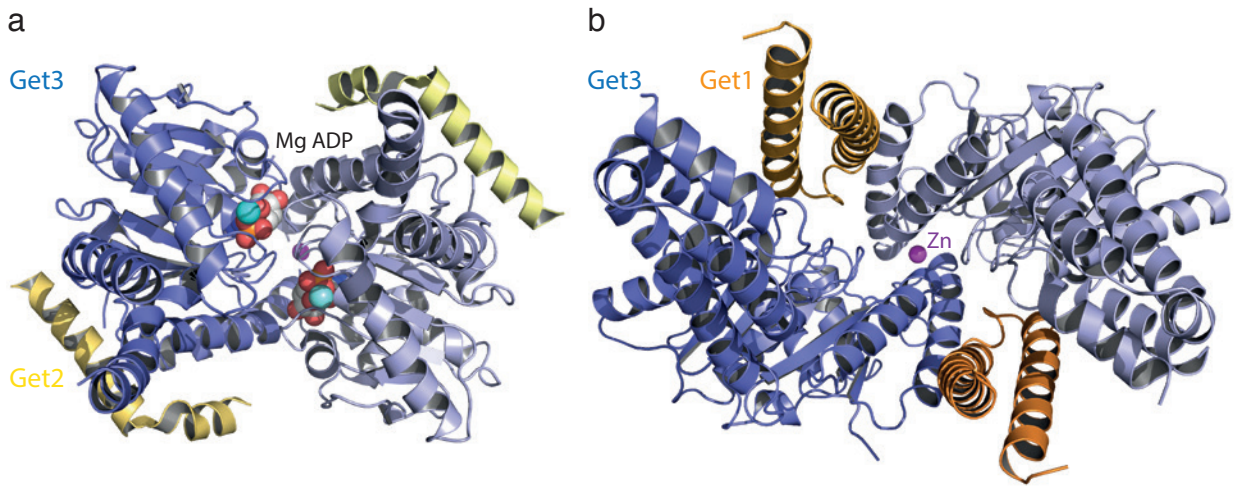


***Fig. 3: The Get3 ATPase interacts with the Get1 and Get2 receptor proteins at the ER. a)*** *Get2 interacts with only one monomer of the Get3 dimer ('closed' state).* ***b)*** *Get1 inserts like a wedge into the Get3 dimer interface ('open', apo state).*

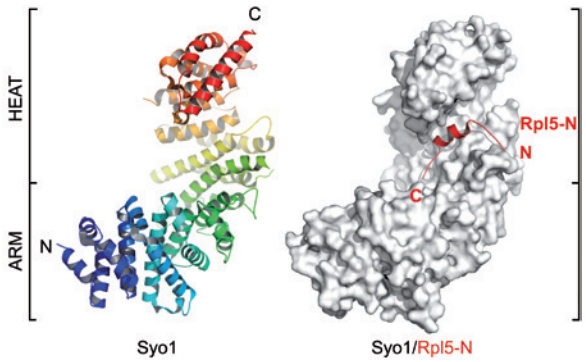


***Fig. 4: Ribosome biogenesis.*** *The nuclear import adaptor Syo1 binds the N-terminus of Rpl5 in an elongated groove.*

some associated complex (RAC) is a chaperone that acts early in protein synthesis. It consists of heat-shock protein 70 (Hsp70) Ssz1 and the Hsp40 Zuo1. Together with Roland Beckmann, we performed structural analyses of RAC alone and bound to the ribosome by electron microscopy, SAXS and X-ray structure determination. RAC crouches over the ribosomal tunnel exit and is stabilized in a distinct conformation by the eukaryote specific expansion segment ES27 of the ribosomal RNA. Our data provide insights into the interplay between RAC and other tunnel exit ligands such as SRP.

For a long time, the ribosome has been in the center of our research as an interaction platform for a whole set of factors acting early on nascent proteins (Fig. 1). More recently, we got interested in the assembly and maturation of eukaryotic ribosomes which involves a myriad of different factors (in collaboration with Ed Hurt). It was thought, that after synthesis in the cytosol each ribosomal protein (r-protein) is individually transported to the nucleus by its import receptor. However, a number of r-proteins form functional clusters or assemble at distinct entry points during ribosome formation – raising the possibility of coordinated nuclear import and assembly. We focused on the assembly of the 5S RNP, consisting of 5S RNA and the two r-proteins L5 and L11, which associates at an early stage with the pre-60S ribosomal subunit. Dieter Kressler identified a transport adaptor, which we named symportin (Syo1). Syo1 facilitates the synchronized co-import of L5 and L11 utilizing the import receptor Kap104 and Ran-GTP. Syo1 is an unusual chimera of ARM and HEAT repeats and binds the N-terminus of L5 similar to the importin-$\alpha$/cargo interaction (Fig. 4). We analyse the role of Syo1 in 5S RNP biogenesis. Synchronous nuclear import of functionally related or topologically linked cargo might be a more general strategy to streamline downstream nuclear processes.

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Bange, G. & Sinning, I. (2013) SIMIBI Twins in protein targeting and localization, Nat. Struct. Mol. Biol. 20: 776-80.

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Kityk, R., Kopp, K., Sinning, I. & Mayer, M.P. (2012) Structure and dynamics of the ATP-bound open conformation of Hsp70 chaperones, Mol Cell 48: 863-74.

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Holdermann, I., Meyer, H., Round, A., Wild, K., Sattler, M. & Sinning, I. (2012) Chromodomains read the arginine code of post-translational targeting, Nat. Struct. Mol. Biol. 19: 260-63.

Bange, G., Kümmerer, N., Grudnik, P., Lindner, R., Petzold, G., Kressler, D., Hurt, E., Wild, K. & Sinning, I. (2011) Structural basis for the molecular evolution of SRP-GTPase activation by protein, Nat. Struct. Mol. Biol. 18: 1376-80.

Lupo, D., Vollmer, C., Deckers, M., Mick, D.U., Tews, I., Sinning, I. & Rehling, P. (2011) Mdm38, is a 14-3-3-like receptor that associates with the protein synthesis machinery at the inner mitochondrial membrane. Traffic 12: 1457-66.

Stefer, S., Reitz, S., Wang, F., Wild, K., Pang, Y.-Y., Schwarz, D., Bomke, J., Hein, C., Löhr, F., Bernhard, F., Denic, V., Dötsch, V. & Sinning, I. (2011) Structural basis for tail-anchored membrane protein biogenesis by the Get3-receptor complex, Science 333: 758-62.

Stjepanovic, G., Kapp, K., Bange, G., Parlitz. R., Graf, C., Wild, K., Mayer, M.P. & Sinning, I. (2011) Lipids trigger a conformational switch regulating SRP-mediated protein targeting, J. Biol. Chem. 286: 23489-97

## Awards and Honors

2010 Member of EMBO

2010 Member of LEOPOLDINA

2010 HMLS Award

2014 Gottfried Wilhelm Leibniz Prize

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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 |

**Thomas Söllner**

# Regulated Membrane Fusion: Molecular Mechanisms and Machinery

## Goal

**To decipher the cascade of reactions that confers regulated exocytosis, revealing the stage-specific molecular mechanisms that each individual component (proteins and lipids) plays along the reaction pathway. Structural alterations in the fusion machinery shall be linked to dynamic architectural changes in the lipid bilayers during membrane merger (fusion pore formation).**

## Background

Neurotransmitter release is the paradigm for regulated exocytosis, characterized by unprecedented temporal and spatial precision. The core machinery mediating intracellular membrane fusion consists of compartment-specific cognate v/t-SNARE pairs. The pairing of v-SNAREs (VAMP2) on a synaptic vesicle with their cognate t-SNAREs (syntaxin1/SNAP-25) on the plasma membrane (SNAREpin formation) is sufficient to drive membrane fusion. Actually, the assembly of cognate SNARE motifs - coiled-coiled regions with 15 layers of hydrophobic amino acids and a central ionic layer - into a four-helix bundle provides the driving force for membrane merger. This protein folding process starts at the membrane distal N-terminal end and sequentially progresses towards the membrane proximal C-terminal end of the SNARE complex thereby bringing the two membranes into close proximity. Remarkably, this assembly process is characterized by inherently built-in breakpoints, which are localized to the ionic layer in the center of the SNARE motif and the transitions to the subsequent linker region and to the transmembrane domain. These natural turning points seem to be superior sites for regulatory components that either slow down or speed up distinct steps. Thereby, defined vesicle pools of distinct sizes can be tailored to the specific needs of a secretory cell. Regulated exocytosis may be relatively slow, for example, by incorporating significant amounts of glucose transporters into the plasma membrane (to regulate the blood glucose level) or occur instantly e.g. releasing a single quantum of neurotransmitters (to propagate signals between neurons in the central nervous system). It has been shown that *in vitro* a single SNARE complex is sufficient to overcome the repulsive forces, which prevent spontaneous membrane fusion. However, in a physiological environment it is more likely that several SNARE complexes form macromolecular assemblies to build fusion pores of a defined size. Fusion pores are well characterized by electrophysiological methods, but their morphological architecture remains obscure

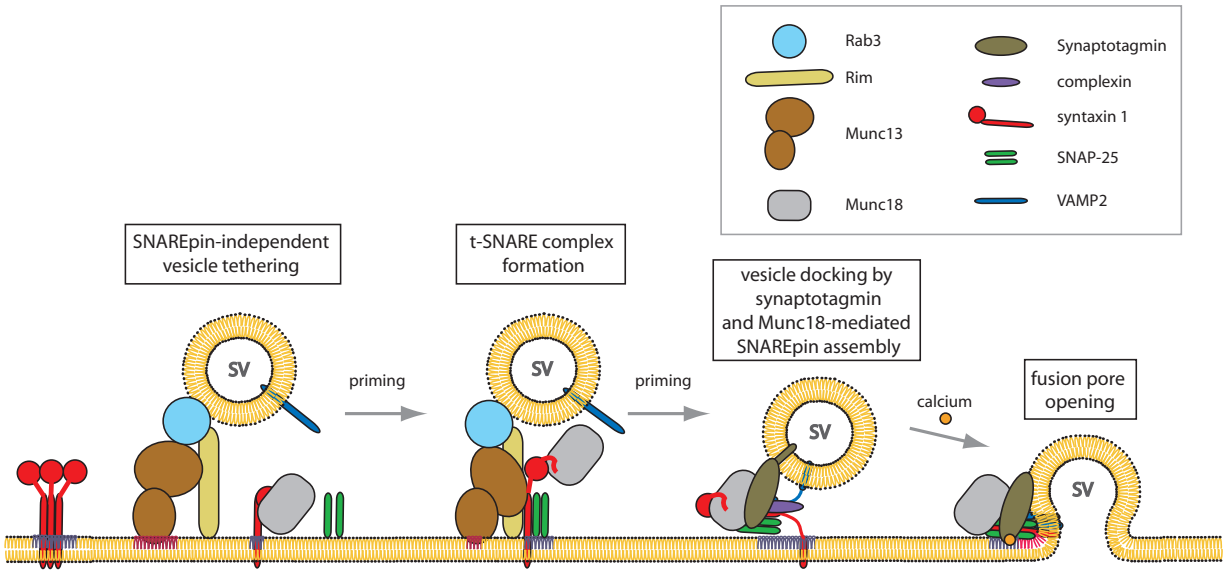


***Fig. 1:*** *Components and reaction steps involved in regulated exocytosis at the neuronal synapse. Please note that only functionally relevant components are shown at the distinct reaction steps. Protein-lipid interactions are depicted by colored lipids.*

and controversial models are highly debated. Overall, neurotransmitter release requires several reaction steps (see Fig. 1). In an initial event synaptic vesicles are selectively *tethered* at the active zone of the nerve terminal. This step seems to occur independently of SNAREpin formation but involves small GTP binding proteins – Rab proteins – like Rab3/Rab27 on the synaptic vesicles and its effectors, like Rim1 at the active zone of the presynaptic plasma membrane. Subsequently, *vesicle priming* mediates t-SNARE complex assembly and initial SNAREpin formation resulting in a pool of *docked vesicles*, which is then converted into a *readily-releasable vesicle pool*. These reactions involve regulatory proteins like Munc13-1, Munc18-1, and the incorporation of the calcium responsive machinery - synaptotagmin 1 and complexin. The *readily-releasable pool* can now instantly respond to a $Ca^{2+}$ trigger, which involves local membrane perturbations mediated by the $Ca^{2+}$-dependent interaction of synaptotagmin 1 with anionic membrane lipids and the release of the complexin clamp. Different types of regulated exocytosis use distinct synaptotagmin isoforms or other putative calcium sensors such as Doc2 and ferlin proteins, which are characterized by the presence of at least two C2 domains. In summary, the orderly interplay of these components with the local lipid environment precisely controls membrane merger.

## Research Highlights

By reconstituting preassembled t-SNAREs into giant unilamellar vesicles (GUVs) and v-SNAREs into small unilamellar vesicles (SUVs) mimicking the plasma membrane and synaptic vesicles, respectively, we have tested which regulatory components are necessary to confer fast $Ca^{2+}$-synchronized membrane fusion *in vitro* (see Fig. 2). A biochemical docking assay revealed that SNAREs when reconstituted at their physiological protein - lipid ratios are not sufficient to confer efficient vesicle docking. In contrast, the presence of membrane-anchored synaptotagmin 1 in SUVs and its interaction with the t-SNARE or $PIP_2$ on the GUV surface mediates potent vesicle docking. Such a mechanism ensures the presence of the calcium sensor and the orderly formation of SNAREpins. Docking occurs calcium-independent, and results in slow $Ca^{2+}$-independent membrane fusion. $Ca^{2+}$ addition confers a fast fusion burst, but of low amplitude (see Fig. 2B). To obtain potent calcium-synchronization, complexin, a small cytosolic protein, needs to be added to the reaction. In the absence of synaptotagmin, complexin weakly stimulates the fusion reaction

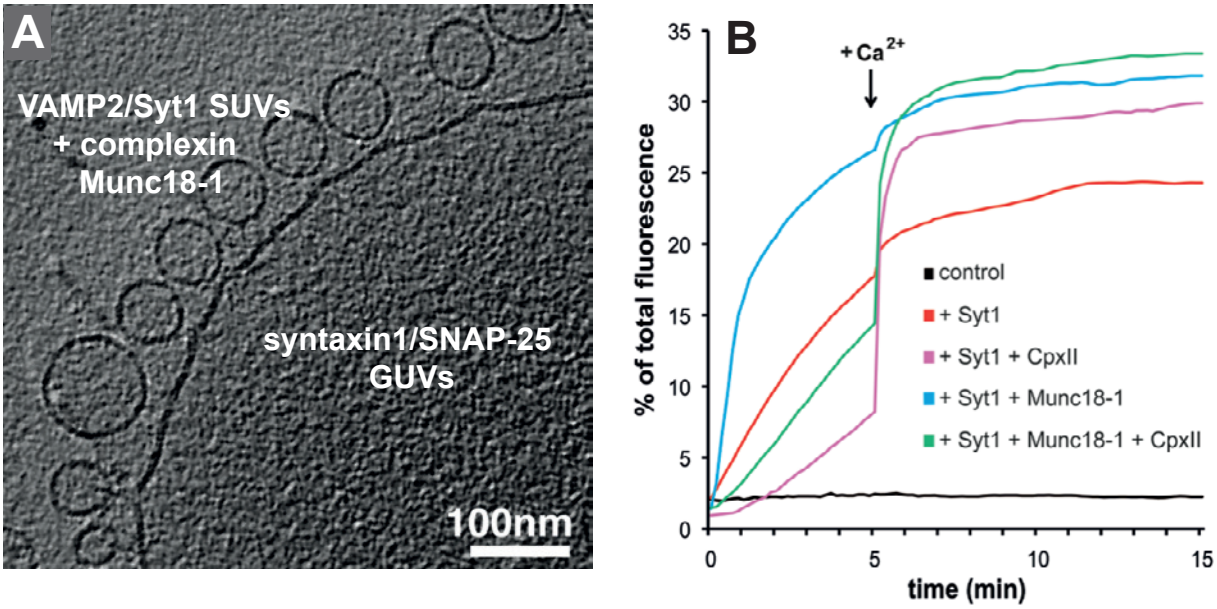


***Fig. 2:*** *A) Cryo-electron micrograph of VAMP2-SUVs bound to syntaxin1/SNAP-25-GUVs by synaptotagmin 1 (Syt1) and arrested by complexin in the presence of Munc18-1, but in absence of $Ca^{2+}$. SNAREpins together with regulatory components cause the formation of local membrane protrusion pointing towards the SUV membrane, likely reducing the activation energy for lipid bilayer merger (EM-studies were conducted together with John Briggs' group at EMBL). B) Membrane fusion measured by lipid mixing. The data show the fusion arrest by complexin, the stimulatory function of Munc18-1 and the calcium synchronization by synaptotagmin 1. Please note that SNAREs by themselves do not confer significant fusion under the conditions employed.*

likely by stabilizing newly formed partially zippered SNARE complexes. However, in the presence of synaptotagmin, complexin suppresses/clamps the $Ca^{2+}$-independent reaction and shifts the reaction towards fast $Ca^{2+}$-synchronization. These different functions could be assigned to distinct protein domains. SNARE binding and the fusion stimulation by complexin require the central helix of complexin. The clamping function of complexin depends on its amino- and carboxy-terminus. A recent SNAREpin-complexin crystal structure indicates that the amino-terminus of complexins competes with VAMP2 for binding to the membrane proximal region of the t-SNARE, thereby blocking SNAREpin zippering. To which degree complexin also interacts with synaptotagmin remains to be shown. $Ca^{2+}$-independent vesicle docking by synaptotagmin requires the polybasic motif of the C2B domain. Membrane fusion at physiological $Ca^{2+}$ concentrations strictly depends on the known $Ca^{2+}$ binding site on the tip of the C2B domain.

By cryo-electron tomography, in collaboration with John Briggs' laboratory at the EMBL, we could show that the clamped pre-fusion state induces a local protrusion in the GUV membrane at the vesicle-docking site (see Fig. 2A). Such a local high curvature protrusion likely generates a metastable state, which lowers the activating energy for membrane fusion and thus allows instant lipid bilayer merger upon $Ca^{2+}$ triggering.

In addition, we could demonstrate that following vesicle docking by synaptotagmin 1, Munc18-1 profoundly accelerates SNARE complex assembly. On a mechanistic level, Munc18-1 binds the membrane proximal region of VAMP2, which facilitates initial SNAREpin formation or/and helps to transduce force generated by SNAREpin zippering to the lipid bilayer. This reaction involves helix 12 exposed on the surface of Munc18-1 and neighboring regions, which seems to function as a folding template for VAMP2/SNAREpin assembly (see Fig. 3). Remarkably, conversion of a proline residue at the amino-terminus of helix 12 into an alanine residue extends helix 12 and switches Munc18-1 into an activated conformation further stimulating membrane fusion. This activated Munc18-1 likely favors an open conformation of syntaxin 1 promoting SNAP-25 binding and thus allowing fast and efficient-SNARE com-

plex assembly. Physiological reactions that would promote such a switch reaction still need to be identified. Thus, domain 3a and helix 12 seem to function as the central hub of Munc18-1 controlling SNARE complex assembly. Overall, the reconstituted reaction pathway may reflect the generation of a readily-releasable pool of synaptic vesicles at the neuronal synapse, which can fuse with the plasma membrane within less than 1 msec.

Future studies shall reveal how structural changes in the membrane fusion machinery or the assembly of the fusion machinery into macromolecular complexes locally affects the lipid bilayer architecture, dictating the subsequent membrane merger reaction. These studies will be conducted in collaboration with John Briggs' group. Having established the function of synaptotagmin and complexin, the molecular mechanisms of other calcium sensors such as Doc2 and ferlins will be analyzed. In addition to these late steps in the fusion reaction, the early vesicle tethering events and the subsequent vesicle priming reactions at the active zone will be reconstituted. These studies will also focus on the interplay of regulatory proteins with distinct lipids using biochemical and microscopic single vesicle docking/fusion assays.

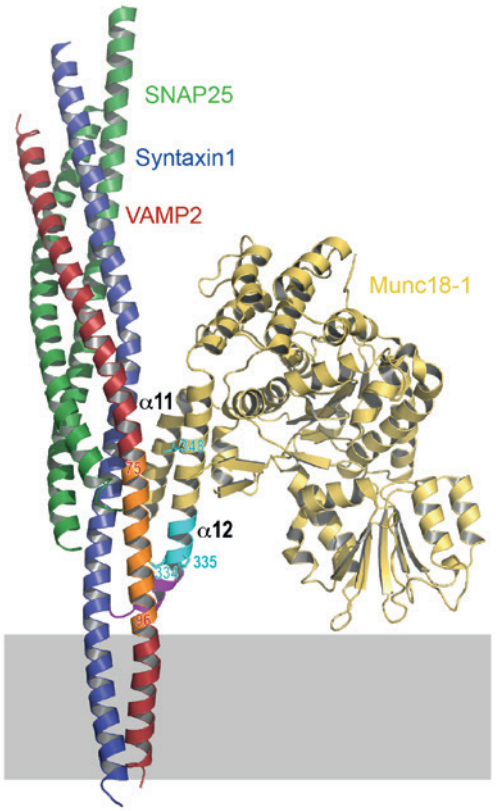


***Fig. 3:*** *Structural model for the interaction of Munc18-1 with a fully assembled post fusion v-/t-SNARE complex. The model is based on published crystal structures of Munc18-1 and the SNARE complex and was developed together with Klemens Wild in Irmi Sinning's laboratory. The model predicts an interaction of domain 3a of Munc18-1 with the membrane proximal region of VAMP2. Such an interaction could initiate v-/t-SNARE complex formation or/and help to transduce the force generated by SNAREpin zippering to the lipid bilayer favoring membrane fusion.*

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| 1995 | Diplom (Chemie), University of Heidelberg, Germany |
| 1995 - 1997 | Ph.D. - Sloan-Kettering Cancer Center, New York, USA (Prof. Franz Ulrich Hartl) |
| 1997 - 1999 | Ph.D. - Max Planck Institute of Biochemistry, Martinsried (Prof. Franz Ulrich Hartl) |
| 1999 - 2003 | PostDoc - The Scripps Research Institute, La Jolla, USA (Prof. Steve Kay) |
| 2003 - 2004 | PostDoc - University of California, San Diego, USA (Prof. Maho Niwa) |
| 2004 - 2013 | Emmy-Noether Group Leader / Junior Group Leader, BZH |

**Frank Weber**

# Circadian Regulation and Biological Timing

## Goal

**We aim to elucidate molecular mechanisms and neuronal networks that facilitate a temporal synchronization of physiology and behaviour. We specifically aim to understand:**

1. **How the activity of transcription factors can be controlled to specific times.**
2. **How the circadian clock is able to sense physiological states in order to control genome wide transcription accordingly.**
3. **How specific groups of neurons assemble a CNS-network that controls a multitude of behavioural and physiological activities to specific times of day.**

## Background

In most organisms the circadian clock synchronizes physiology and behaviour with the environmental cycles of day and night. The circadian clock is assembled by a set of transcription factors that control their own expression as well as genome-wide transcription in a rhythmic fashion, maintaining auto-regulatory 24-hour oscillations. Circadian regulation provides a vital advantage by facilitating a temporal separation and coordination of homeostatic functions, as well as an adaptation to daily environmental cycles.

We investigate the assembly and regulation of the circadian clock in the model organism *Drosophila*, which is highly homologous to the mammalian system. Our goal is to uncover cellular and molecular mechanisms of biological timing. In addition, we aim to elucidate the neuronal network that controls circadian behaviour.

## Research Highlights

**1) The timing of transcription factors**

The core oscillating activity of the circadian clock in *Drosophila* and mammals is formed by the heterodimeric complex of transcription factors CLOCK (CLK) and CYCLE (CYC). We uncovered a post-translational timing mechanism that is based on the sequential and compartment specific modification of the CLK protein, controlling its ~24-hour life cycle (Fig. 1). For example phosphorylation of serine 264 and threonine 268 is important for CLK/CYC-interaction and efficient nuclear import of CLK. In addition, nucleocytoplasmic distribution and stability depend on Casein kinase II (CK II). Within the nucleus CLK is stored in nuclear bodies, a process that involves

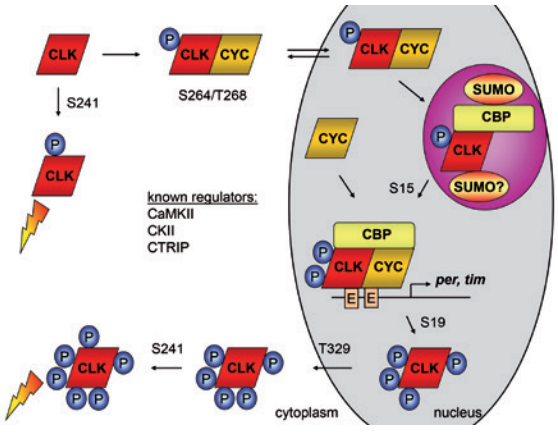


***Fig.1:*** *Post-translational timing of circadian transciption.*

SUMOylation and co-factor interaction. Phosphorylation of serine 15 is important for transcriptional activation, which is accompanied by nuclear re-localization of the CLK protein. Transcriptional inhibition and nuclear export are similarly controlled by specific phosphorylation events. Degradation of CLK eventually involves the E3-ubiquitin ligase CTRIP. Our results indicate that every step of the CLK life cycle is precisely controlled by co-factor interaction and a cascade of specific post-translational modifications including phosphorylation, SUMOylation and ubiquitination. Thereby an accurate timing of CLK accumulation, nucleo-cytoplasmic transport, storage in nuclear bodies, transcriptional activation, inhibition, and finally degradation is achieved (Fig. 1). These results elucidate cell biological and molecular mechanisms that facilitate a precise timing of transcription factors in general and of circadian transcription in particular.

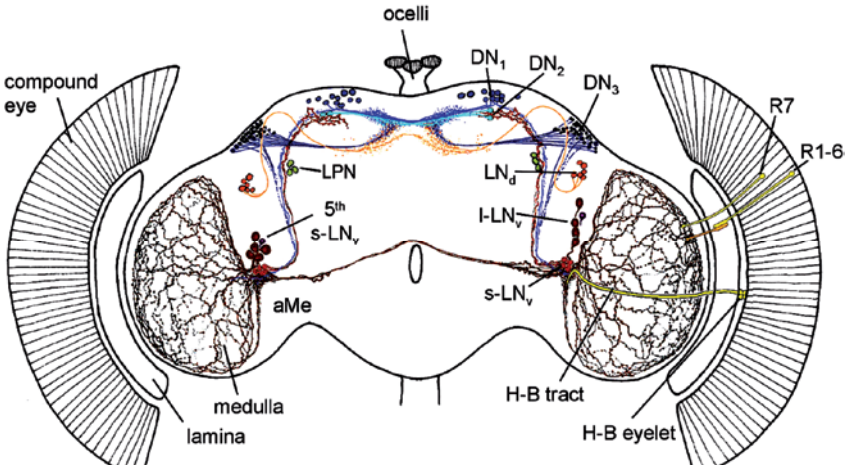


***Fig. 2:*** *The circadian neuronal network (adopted from Helfrich-Förster C et. al. J Comp Neurol. (2007) 500:47-70.).*

**2) Metabolic regulation**

We found that molecular oscillations of the *Drosophila* circadian clock can be synchronized by cycles of 12 hours feeding and 12 hours starvation, indicating that the circadian clock responds to metabolic activity. Cyclic feeding of carbohydrates synchronizes molecular oscillations more efficiently than proteins or lipids. We previously showed that cyclic-nucleotide/PKA, calcium/CaMKII and Ras/MAPK pathways regulate circadian transcription by direct phosphorylation of the CLK protein and through regulation of the CREB-binding protein (CBP), which acts as a transcriptional co-activator for CLK/CYC-dependent transcription. These signalling pathways likely mediate a regulation of the circadian clock by metabolic activity.

**3) Neurobiology of circadian behaviour**

In order to gain insights into neuronal network structures that underlay behaviour we aim to map the circadian neuronal network in *Drosophila*. Several neuronal groups were identified by different labs (Fig. 2) and specific functions were attributed to some of them, e.g. light signalling (l-$LN_v$) or core pacemaker function (s-$LN_v$). Using a new antibody against the CLK protein, we identified additional groups of circadian neurons. We aim to map the physical connections within the circadian neuronal network as well as towards output neurons that control behaviour. In addition, we are interested in neurotransmitter pathways that establish communication between circadian neurons. These studies aim to uncover the neuronal basis of circadian behaviour.

## Selected Publications


A. Szabo, C. Papin, D. Zorn, P. Ponien, F. Weber, T. Raabe and F. Rouyer (2013) The CK2 kinase stabilizes CLOCK and represses its activity in the *Drosophila* circadian oscillator. *PLOS Biol*. 11:e1001645.

F. Weber, D. Zorn, C. Rademacher and H-C. Hung (2011) Post-translational timing mechanisms of the *Drosophila* circadian clock. *FEBS Lett.* 585:1443-1449.

A. Lamaze, A. Lamouroux, C. Vias, H-C. Hung, F. Weber and F. Rouyer (2011) The E3 ubiquitin ligase CTRIP controls CLOCK levels and PERIOD oscillations in *Drosophila*. *EMBO Rep.* 12:549-557.

H-C. Hung, C. Maurer, D. Zorn, W-L. Chang and F. Weber (2009) Sequential and compartment-specific phosphorylation controls the life cycle of the circadian CLOCK protein. *J. Biol. Chem.* 284:23734-23742.

H-C. Hung, S. Kay and F. Weber (2009) HSP90, a capacitor of behavioural variation. *J. Biol. Rhythms*. 24:183-192

C. Maurer, H-C. Hung and F. Weber (2009) Cytoplasmic interaction with CYCLE promotes the post-translational processing of the circadian CLOCK protein. *FEBS Letters* 583:1561-1566.



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| 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 - University of Heidelberg, Germany |
| 1991 - 2003 | Chairman SFB 352 |
| 1997 - 2002 | Director - BZH |
| since 2001 | Managing Editor FEBS Letters |
| 2005 - 2007 | President of the GBM |
| since 2003 | Chairman SFB 638 |

**Felix Wieland**

# Molecular mechanisms of COPI transport

## Goal

**Our research interests comprise two converging fields:**

**i) Molecular mechanisms of coated vesicle formation and uncoating, and**

**ii) Specificity and structural basis for protein - lipid interactions within a bilayer that regulate membrane protein activity.**

**These research interests have led us to develop and maintain**

**iii) A platform for quantitative lipidomics.**

**We are characterizing the components and their coordinate action that allow formation, fission and uncoating of Golgi-derived COPI-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. The mechanism of fusion of vesicles as well as their role in neurotransmission has been recognized by the 2013 Nobel Prize for Physiology and Medicine. 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 clathrin-coated vesicles operating in the late secretory and endocytic pathway. 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 vesicular transport by COPI vesicles. In contrast to COPII and clathrin coats, the heptameric large COPI coat component coatomer is recruited en bloc to the membrane, so that both the inner and outer shell of the vesicle are formed at the same time.

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).

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| Ph.D. - University of Frankfurt, Germany | 1998 |
| PostDoc - Memorial Sloan Kettering Cancer Center, New York, USA (Prof. James E. Rothman) | 1998 - 2000 |
| PostDoc - BZH (Prof. Felix Wieland) | 2000 - 2002 |
| Research Fellow - BZH | since 2002 |
| Habilitation in Biochemistry, University of Heidelberg, Medical Faculty | 2007 |

**Britta Brügger**

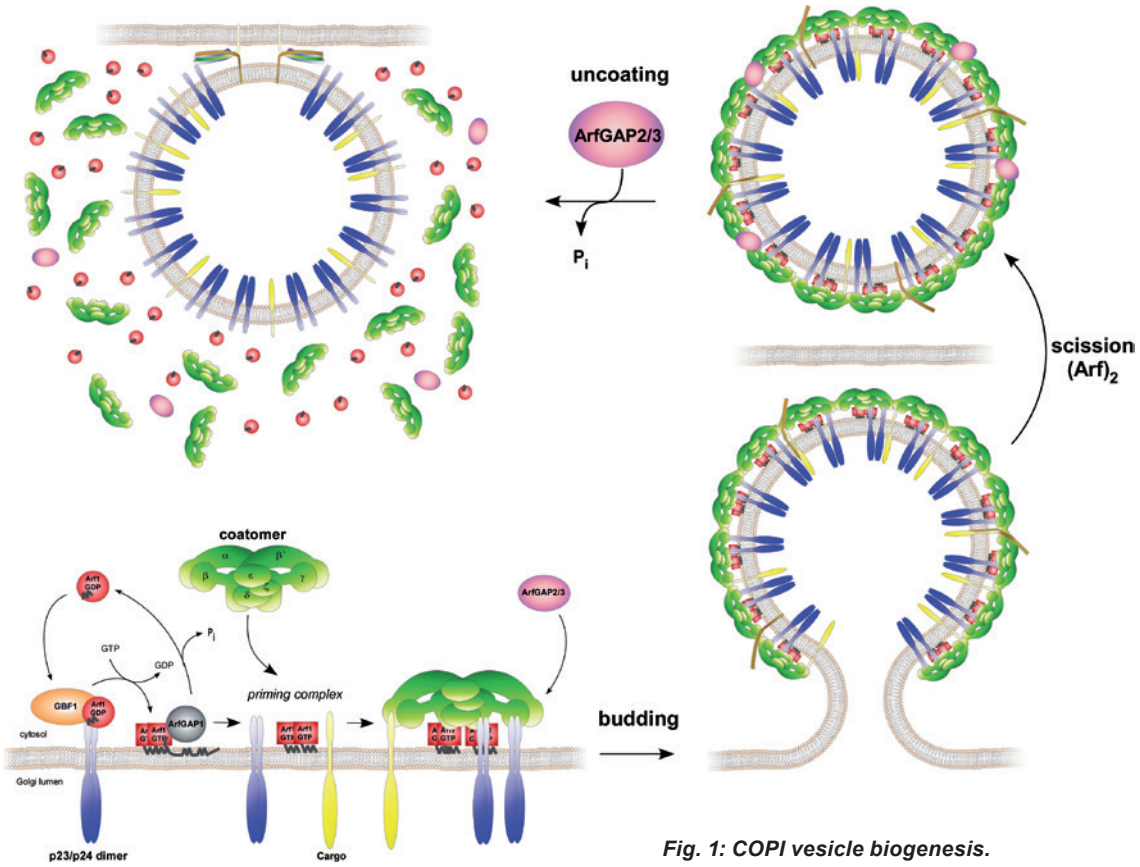


***Fig. 1: COPI vesicle biogenesis.***

A schematic view of individual steps in COPI vesicle biogenesis is given in Fig. 1.

## Research Highlights

**Molecular mechanisms of COPI vesicle biogenesis**

*1) Roles of Arf1 in vesicle formation and fission*

Formation of coated vesicles requires two striking manipulations of the lipid bilayer. First, membrane curvature is induced to drive bud formation. Second, a scission reaction at the bud neck releases the vesicle. Using a reconstituted system for COPI vesicle formation from purified components we find that a non-dimerizing Arf1 mutant, which does not display the ability to modulate membrane curvature in vitro or to drive formation of coated vesicles, is able to recruit coatomer to allow formation of COPI-coated buds, but does not support scission. Chemical cross-linking of this Arf1 mutant restores vesicle release. These studies show that initial curvature of the bud is driven primarily by coatomer, whereas the mem-

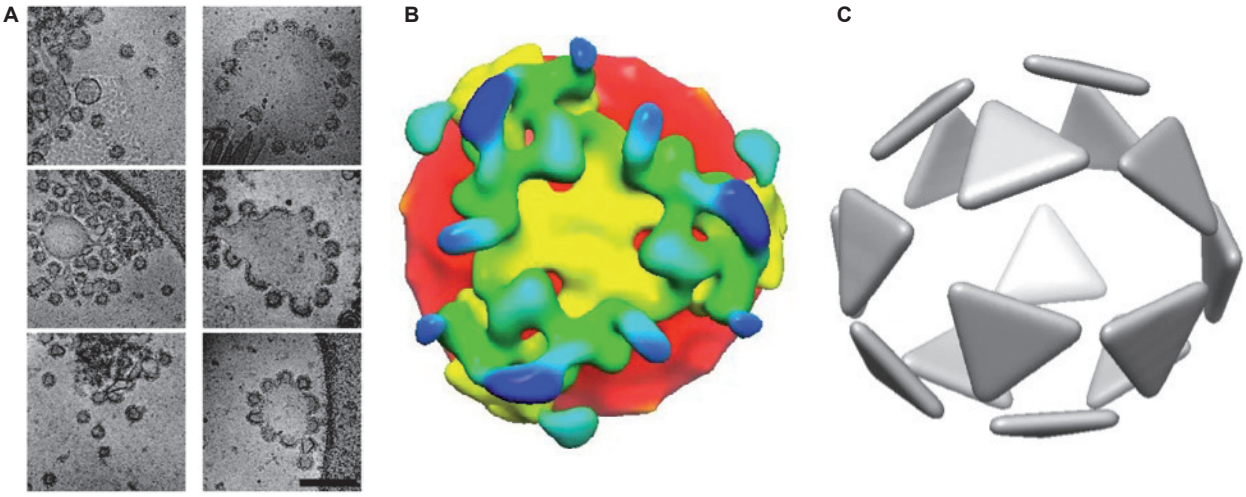


***Fig. 2: COPI vesicle biogenesis. A)*** *Cryo-electron microscopy of COPI-coated vesicles generated with Arf wt (left hand panels) and COPI-coated buds generated with a scission arrest Arf mutant (right hand panels).* **B)** *Three coatomer complexes form a triade, the basic structure of the COPI lattice,* ***C)*** *Arrangement of triads to form the COPI lattice.*

brane curvature potentiating activity of dimeric Arf1 is required for membrane scission. Using a semi-intact cell system we have shown that during the scission reaction Arf does not hydrolyse its bound GTP.

*2) Structures of coatomer and of the COPI coat*

Together with John Briggs' group at the EMBL we investigate the structure of soluble coatomer by single particle electron microscopy, and of the coatomer shell on coated vesicles. With the first data of a coat on a membrane, a structure emerges that is strikingly different from those of the COPII and the clathrin systems as delineated from protein assemblies. The basic unit of the lattice is 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.

*3) Regulation of COPI transport by a unique sphingolipid/cargo-receptor complex*

We have discovered a specific binding of the sphingomyelin molecular species SM 18:0 to the transmembrane domain of one member of the p24 family, p24. SM 18:0 binding favors dimerization of p24. Dimeric p24, in turn, recruits coatomer and triggers a conformational change of the complex resulting in polymerization, initiating COPI bud formation. Thus, a membrane lipid molecular species can serve as a cofactor in controlling vesicle budding. We have defined steps in vesicular transport *in vivo* that depend on this specific interaction.

**Structural principles of transmembrane protein/membrane lipid interactions**

*1) A signature within the p24 transmembrane domain for recognition of a sphingolipid molecular species*

We have discovered a peptide signature for sphingolipid binding within the transmembrane span of p24. When transplanted, the signature confers sphingolipid binding to a non-sphingolipid binding transmembrane domain. Results from a data mining approach indicate that this signature represents a conserved binding site for sphingolipids in several transmembrane proteins.

2) We found that a functional sphingomyelin-binding signature in the influenza neuraminidase is necessary for optimal transport of this protein to the host's plasma membrane.

3) Capitalizing on fluorescent sphingomyelin species containing pentaenyl fatty acids we have developed a simple assay to assess specific lipid-lipid recognition in liposomal membranes.

**Lipidomics platform**

Using our lipidomics platform, we have elucidated lipidoms of organisms and subcellular systems (see lipidomics by Britta Brügger on page 52).

Our investigations are based on a wide range of methods, including live cell imaging (with Rainer Pepperkok; EMBL), bioinformatics (with Gunnar von Heijne and Arne Elofsson, Stockholm), molecular dynamics simulations (with Erik Lindahl, Stockholm), *in vivo* and *in vitro* FRET studies, cryo-electron microscopy (with John Briggs, EMBL), protein chemistry, molecular biology, and quantitative nano-mass spectrometry of lipids, as well as chemical biology approaches.

Our research is supported by the German Research Council (SFB 638: Dynamics of macromolecular complexes in biosynthetic transport, SFB/TRR 83: Molecular architecture and cellular functions of protein/lipid assemblies, GRK 1188: Quantitative analysis of dynamic processes in membrane transport and translocation, and CellNetworks Heidelberg.

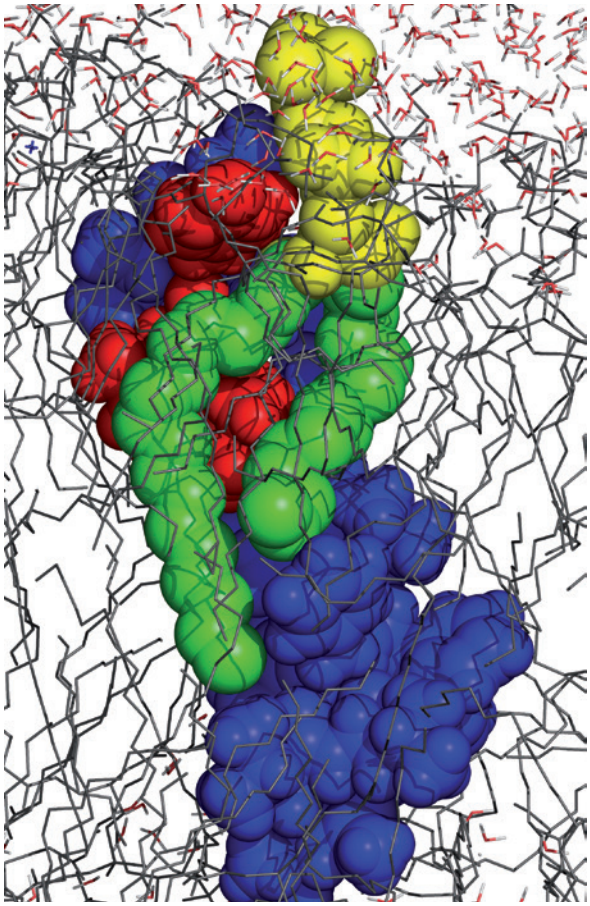

***Fig. 3: Structure of a SM 18-binding motif.*** *Molecular dynamics simulation of p24 TMD (blue, with the motif highlighted in red) and SM 18:0 (green, hydrocarbon chains; yellow, headgroup of SM 18:0) in a POPC bilayer.*

## Selected Publications 2011 - 2013

F. Adolf, A. Herrmann, A. Hellwig, R. Beck, B. Brügger and F. T. Wieland (2013) Scission of COPI and COPII vesicles is independent of GTP hydrolysis. Traffic 14(8):922-32.

A.M. Ernst, S. Zacherl, A. Herrmann, M. Hacke, W. Nickel, F.T. Wieland, B. Brügger (2013) Differential transport of Influenza A neuraminidase signal anchor peptides to the plasma membrane. FEBS Lett. 587(9):1411-7.

M. Faini, R. Beck, F.T. Wieland, J.A. Briggs (2013) Vesicle coats: structure, function, and general principles of assembly. Trends Cell Biol. Feb 12.

J.M. Duran, F. Campelo, J. van Galen, T. Sachsenheimer, J. Sot, M.V. Egorov, C. Rentero, C. Enrich, R.S. Polishchuk, F.M. Goñi, B. Brügger, F. Wieland, V. Malhotra (2012) Sphingomyelin organization is required for vesicle biogenesis at the Golgi complex. EMBO J. 12;31(24):4535-46.

M. Faini, S. Prinz, R. Beck, M. Schorb, J.D. Riches, K. Bacia, B. Brügger, F.T. Wieland* and J.A. Briggs* (shared corresponding authors) (2012) The Structures of COPI-coated vesicles reveal alternate Coatomer Conformations and Interactions. Science 336(6087):1451-4.

F.-X. Contreras, A.M. Ernst, P. Haberkant, P. Björkholm, E. Lindahl, B. Gönen, C. Tischer, A. Elofsson, G. von Heijne, C. Thiele, R. Pepperkok, F. Wieland, and B. Brügger (2012) Molecular recognition of a single sphingolipid species by a protein's transmembrane domain. Nature 481:525-529.

R. Beck, S. Prinz, P. Diestelkötter-Bachert, S. Röhling, F. Adolf, K. Hoehner, S. Welsch, P. Ronchi, B. Brügger, J.A. Briggs, and F. Wieland (2011) Coatomer and dimeric ADP ribosylation factor 1 (Arf1) promote distinct steps in membrane scission. J Cell Biol. 194(5):765-77.

F.X. Contreras, A.M. Ernst, F. Wieland and B. Brügger (2011). Specificity of intramembrane protein-lipid interactions. Cold Spring Harb Perspect Biol. Jun 1;3(6)

M.C. Sahlmüller, J.R. Strating, R. Beck, V. Popoff, M. Haag, A. Hellwig, I. Berger, B. Brügger, and F.T. Wieland (2011) Recombinant heptameric coatomer complexes: novel tools to study isoform-specific functions. Traffic 12(6):682-692.

## Awards and Honors Felix Wieland

| | |
|---|---|
| 1993 | Honorary Member of Charité, Medical Faculty of the Humboldt University, Berlin |
| since 2000 | Elected EMBO Member |
| 2001 | Heinrich-Wieland Award |
| since 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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**Britta Brügger**
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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, University of Heidelberg / BZH |
| 1999 | Habilitation in Biochemistry, University of Heidelberg, 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

**We provide training in biochemistry for students from three different faculties (Medicine, Biosciences, Chemistry & Geosciences) at the undergraduate and graduate level. Furthermore, we engage in the development of new curricula and learning formats in order to continuously adjust our programs to the demands on state-of-the-art biochemical education.**

## The teaching unit

The teaching unit provides services and infrastructure for high-end education in modern biochemistry. Responsibilities include the development and organization of courses and examinations in collaboration with the lecturers and the dean's offices, services for students and teaching staff, maintenance of the electronic learning platform and the teaching laboratories as well as set up the practicals. Lab space can house up to 120 students with about half of the benches equipped with basic instruments for biochemical analysis. For advanced courses, a cell culture lab, a cold room, a dark room and equipment for large scale preparations, like centrifuges and incubators, are available. In a computer room with 14 workstations students can use special software or online tools, like databases for gene and protein analysis or virtual patients. Outside the BZH-course hours, the entire infrastructure of the teaching unit can be used by other groups on the campus.

## Undergraduate Program

Approximately 800 medical students, 190 biology students and 120 chemistry students participate in biochemistry courses each year. In addition, 25 students are trained in a selective biochemistry study program which is described in a separate section of this report. All students attend obligatory basic 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.

### Medical students' education

The medical students' courses extend from the second throughout the fourth semester. They are systematically structured from the basics of biomolecules to complex metabolic pathways and cellular functions. The preclinical core curriculum at Heidelberg University is unique in Germany since all topics are taught interdisciplinary with cell biology, anatomy and physiology. Our curriculum is very well accepted and led to better results in internal as well as national examinations: In the last 3 years Heidelberg ranked among the top three German medical faculties (out of 32) in the national state examinations. In addition to the obligatory courses, we offer the possibility to perform a practical in one of the research groups or to attend a seminar on a selected topic. With the aim to foster self-directed learning and to

integrate clinical aspects into the preclinical curriculum, we - in collaboration with clinicians and computer scientists - developed virtual cases in an electronic format.

***Medical students dealing with a virtual patient in our CIP pool.***

### Education of students of biosciences

For students of biosciences, we offer a program which starts in the first semester with an introductory seminar “On current topics of the life sciences” and continues longitudinally to the master program with selected topics related to ongoing research at the BZH. Basic modules for bachelor students as well as advanced modules of the major “Molecular and Cellular Biology (MCB)” for students of the international master program “Molecular Biosciences” are arranged in collaboration with the coordinators and teaching staff from other subjects, like molecular biology and cell biology. The course concept aims to provide a solid and modern education in biochemistry from basic knowledge to high-end methods, like tandem affinity purification or lipid analyses. 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 students’ education

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 students gain basic knowledge and are trained to handle basic lab equipment. In the elective module students get insight into more sophisticated techniques, like protein crystallography and structural analyses. Master students perform a lab rotation in a research group and participate in the groups’ research seminars. Our electives are in great demand which gives us the privilege to choose the most talented students for a bachelor thesis and afterwards for the master program.

***Students of the master program “Molecular Biosciences” in a MCB practical.***

## Graduate program

Our 65-70 graduate students receive an intense and professional training in our internal BZH graduate program or in one of the other graduate programs on campus, like the international graduate program Hartmut Hofmann-Berling international graduate school of molecular and cellular biology (HBIGS) or the DFG-funded research training group GRK 1188. Graduate students’ education includes supervision by a thesis advisory committee, reports about the progress in the BZH department seminar and participation in a course program which offers a vast diversity 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 BZH lab day, which are organized by our board of PhD students. In addition, opportunity is given to discuss science issues in guest speakers’ seminars.

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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**

# Biochemistry Bachelor

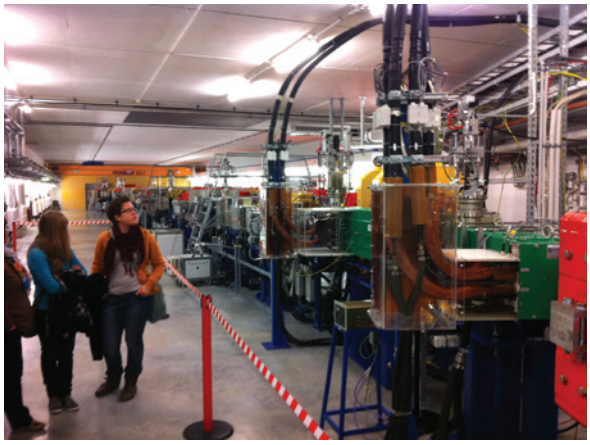
**Since winter 2012, the Faculty of Biosciences and the Faculty of Chemistry and Geosciences, offer a joint study program Biochemistry Bachelor. The goal is to prepare excellent students for a career in applied research in Life Sciences. Starting winter 2015, the consecutive study program Biochemistry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

*Dr. R. Beck (BZH) and Dr. M. Schmidt (NCT).*

## Capacities and Selection of Applicants

Biochemistry Bachelor in Heidelberg is a very selective study program. In 2013 over 1100 applicants competed 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 in national and international 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.

## The Bachelor Program

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:

*Biochemistry Practical A.*

We designed a new lecture series Biochemistry I-III specific for this study program. Our goal is to offer our students unique lectures (and tutorials), where we can take the time to thoroughly explain and, together with the 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.

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*Lecture Biochemistry I.*

# Facilities and Platforms

## Fluorescence Activated Cell Sorting (FACS)

BZH and ZMBH have established a common FACS facility, which is operated by a scientist funded by the DFG collaborative research center 638.

A Becton-Dickinson FACSAria cell sorter is available for cell sorting experiments that has been funded by the Dietmar Hopp foundation and was upgraded in 2012 by means of BZH, ZMBH, and SFB 638. The FACSAria III now is equipped with 4 lasers for excitation and can detect 2 scatter-light and 11 fluorescence parameters simultaneously. Furthermore a new fluidics system has been installed what has highly improved the quality and sterility of cell sorting.

For analytical measurements the ZMBH has contributed a Becton-Dickinson FACSCanto flow cytometer in 2009, which has 3 lasers for excitation and detects 2 scatter-light and 8 fluorescence parameters at the same time.

Additionally, for simple analytical flow cytometry experiments a 2-laser Becton-Dickinson FACSCalibur system is available at BZH and a 1-laser Becton-Dickinson FACScan machine at the ZMBH.

Due to the implementation of a new user fee system in 2013 the facility is able to cover the running costs and service contract fees of the instruments very well.

The FACS facility is available to all scientists of the University of Heidelberg. As a result of some advertising effort the facility could welcome many new clients this year and the utilization of the instruments has remarkably increased.

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## 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.

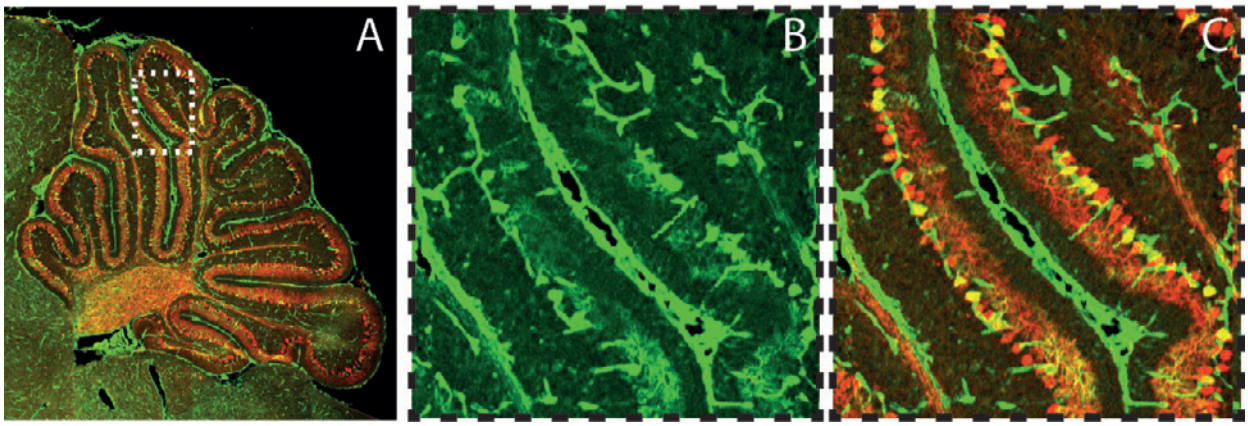


***Fig. 1: Blood vessels and neurons in the cerebellum. A)*** *Immunostaining for Calbindin (red), expressed in Purkinje cells, and for a protein expressed in blood vessels and in Purkinje cells (green) in a postnatal day 7 (P7) mouse cerebellum. B,C) higher magnification of the inset from A.* ***B)*** *green channel.* ***C)*** *overlay of green and red channels. Imaging performed using the Zeiss LSM 510 META confocal microscope and the motorized stage. (Image courtesy of R. Luck & C. Ruiz de Almodovar).*

**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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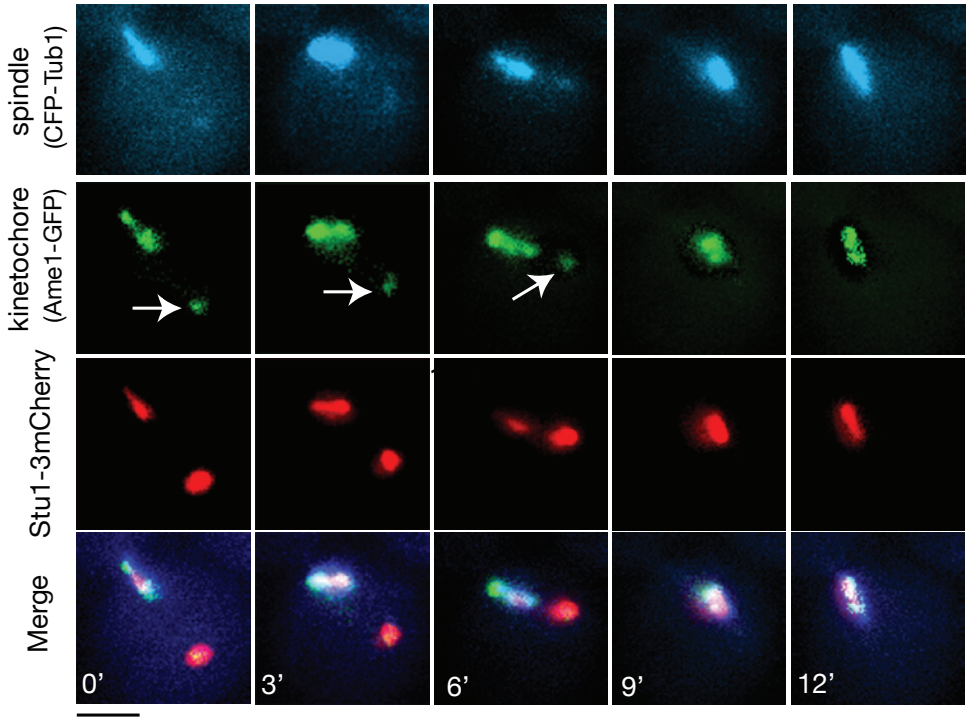


***Fig. 2: Stu1/CLASP is recruited to unattached kinetochore and facilitates their capture to the mitotic spindle.*** *The unattached chromosome state to the attached state is shown in the budding yeast with time lapse fluorescence imaging performed on an Olympus CellR Imaging Station. Kinetochores are 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).*

## Electron Microscopy (EM)

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

Information on the size, shape, homogeneity, and plasticity of macromolecular complexes or single proteins (≥ 200 kDa) can be achieved of samples that exist in multiple identical copies. From such defined and reproducible arrangements a 3D structure at 1-3 nm resolution can be calculated. This allows the mapping of individual subunits or the modeling of high-resolution structures leading to a basis for functional understanding (for an overview of the experimental steps see Böttcher and Hipp, 2010, "Single-particle applications at intermediate resolution").

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

In close cooperation with the Electron Microscopy Core Facility (EMCF) at the Bioquant, we have unlimited access to microtomes, a freeze-substitution machine, high-end transmission EMs and a scanning EM as well as expertise in tomography and cryo-EM. This enables the groups of the BZH to answer a variety of question from cell- to structural biology.

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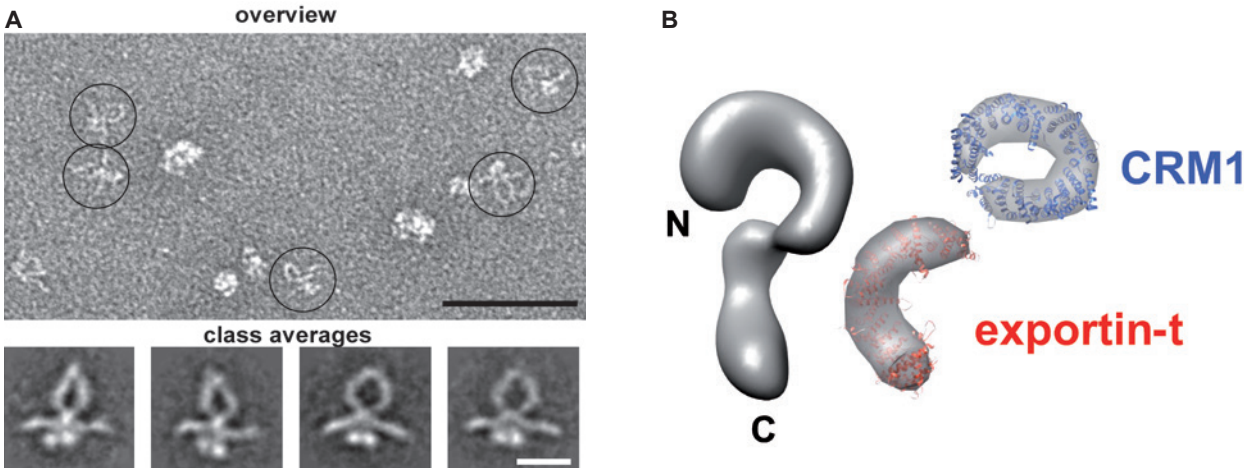


***Fig.: (A)*** *Overview of an electron micrograph and class averages showing the dimerization of a reconstituted complex in a side-to-side arrangement.* ***(B)*** *Three-dimensional reconstruction of a nucleoporin revealing the structural similarity to karyopherins.*

## 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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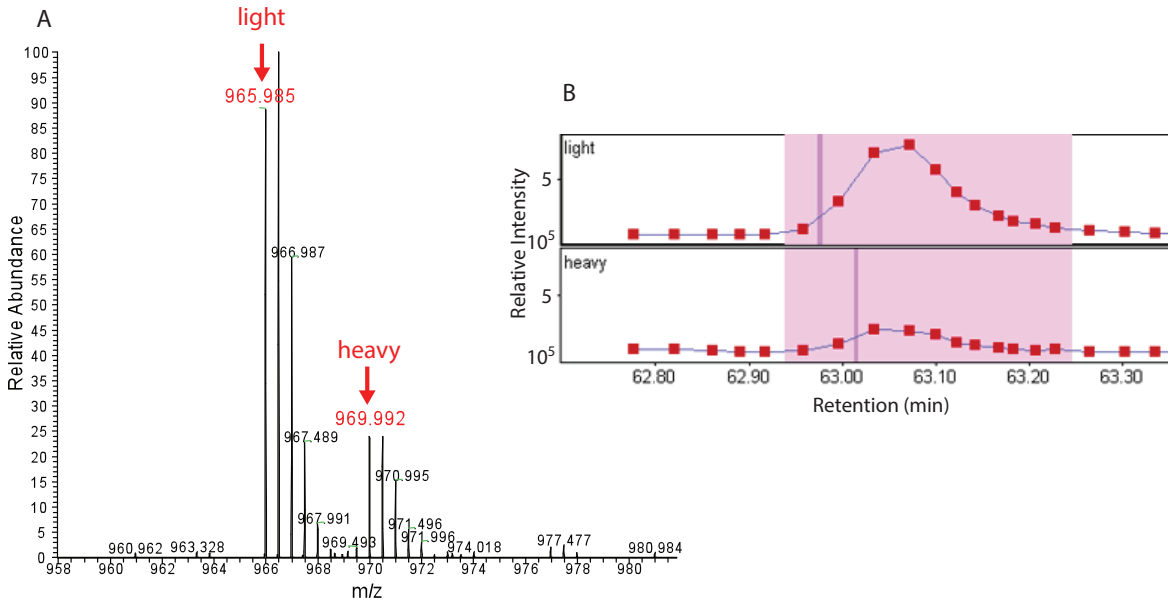


***Fig.: Quantitative Mass Spectrometry with SILAC. A:*** *Survey scan revealing a peptide pair (z=2) with one light or heavy lysine respectively.* ***B:*** *Ion intensities of the light and heavy peptide extracted from the chromatogram.*

## Protein Crystallization Platform

In 2008, the Cluster of Excellence CellNetworks and Prof. Irmgard Sinning have established a state-of-the-art high-throughput crystallization platform for biological macromolecules. Dr. Jürgen Kopp is running the facility assisted by Claudia Siegmann and Gabriela Müller. The platform is equipped with a Phoenix nano-liter dispensing robot which allows screening of 1000 crystallization conditions with as little as 100 microliters of protein sample. The crystallization trials are imaged automatically and stored under strict temperature control in two Rigaku Minstrel incubators with a total capacity of 800 crystallization plates. Images can be viewed and analyzed on the web. A Formulatrix UV microscope allows distinguishing between protein and salt crystals at

a very early stage by detecting the fluorescence signal of tryptophan residues. Standard and user-defined crystallization screens are available for soluble proteins, RNA-protein and other complexes as well as for membrane proteins. In 2013, the infrastructure of the platform was enhanced with a Gryphon dispensing robot for handling highly viscous solutions at nano-liter scale and lipidic cubic phase crystallization of membrane proteins.

For more information, please visit the platform homepage at http://xtals.bzh.uni-heidelberg.de.

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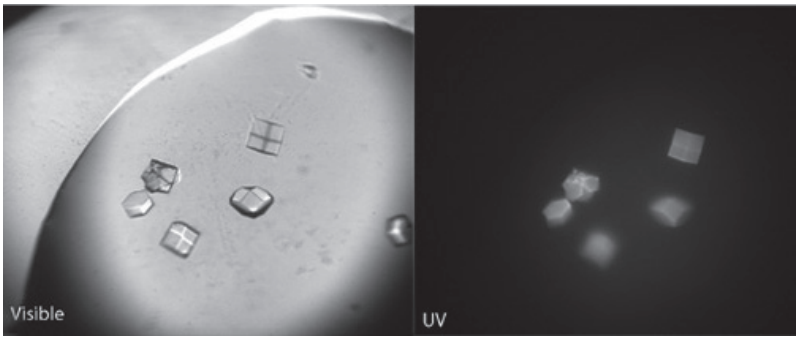


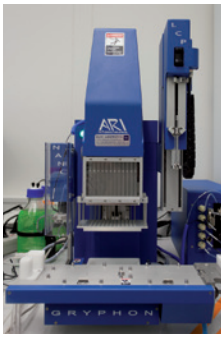

## Lipidomics platform

Lipidomics aims to analyze cellular lipid metabolic pathways and signaling networks by quantitatively and comprehensively defining the lipidomes of biological sources ranging from organelles via cells to whole organisms. 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 successfully addressed in collaborations a multitude of fundamental cell biological questions, including characterisation of the lipidomes of divers subcellular membrane systems and of viral particles. Based on an interdisciplinary approach, where we combined *in vivo*, *in vitro* and *in silico* studies, lipidomics allowed us to define a novel paradigm of regulation of membrane protein activity by direct and specific interaction of

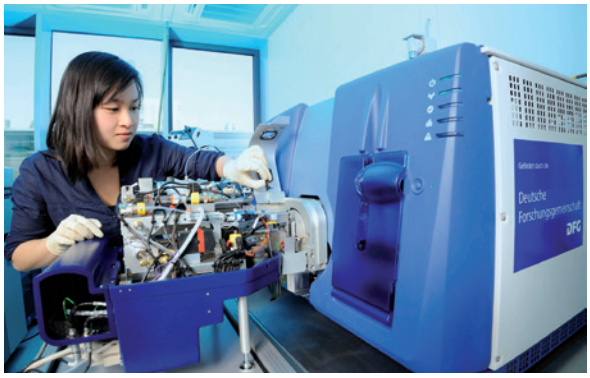

***Fig. 1: A hybrid triple quadrupole-linear ion trap mass spectrometer.***

transmembrane domains with single lipid species within the membrane. The lipidomics platform is available for groups on Heidelberg Campus and beyond.

**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, four complementary nano-platforms are available: a hybrid quadrupole-Orbitrap mass spectrometer, a hybrid triple quadrupole linear ion trap mass spectrometer, a hybrid quadrupol time-of-flight mass spectrometer, and a triple quadrupole mass spectrometer. All hybrid mass spectrometers operate assisted by automated Nanomate devices, either in direct injection or in LC-coupling mode.

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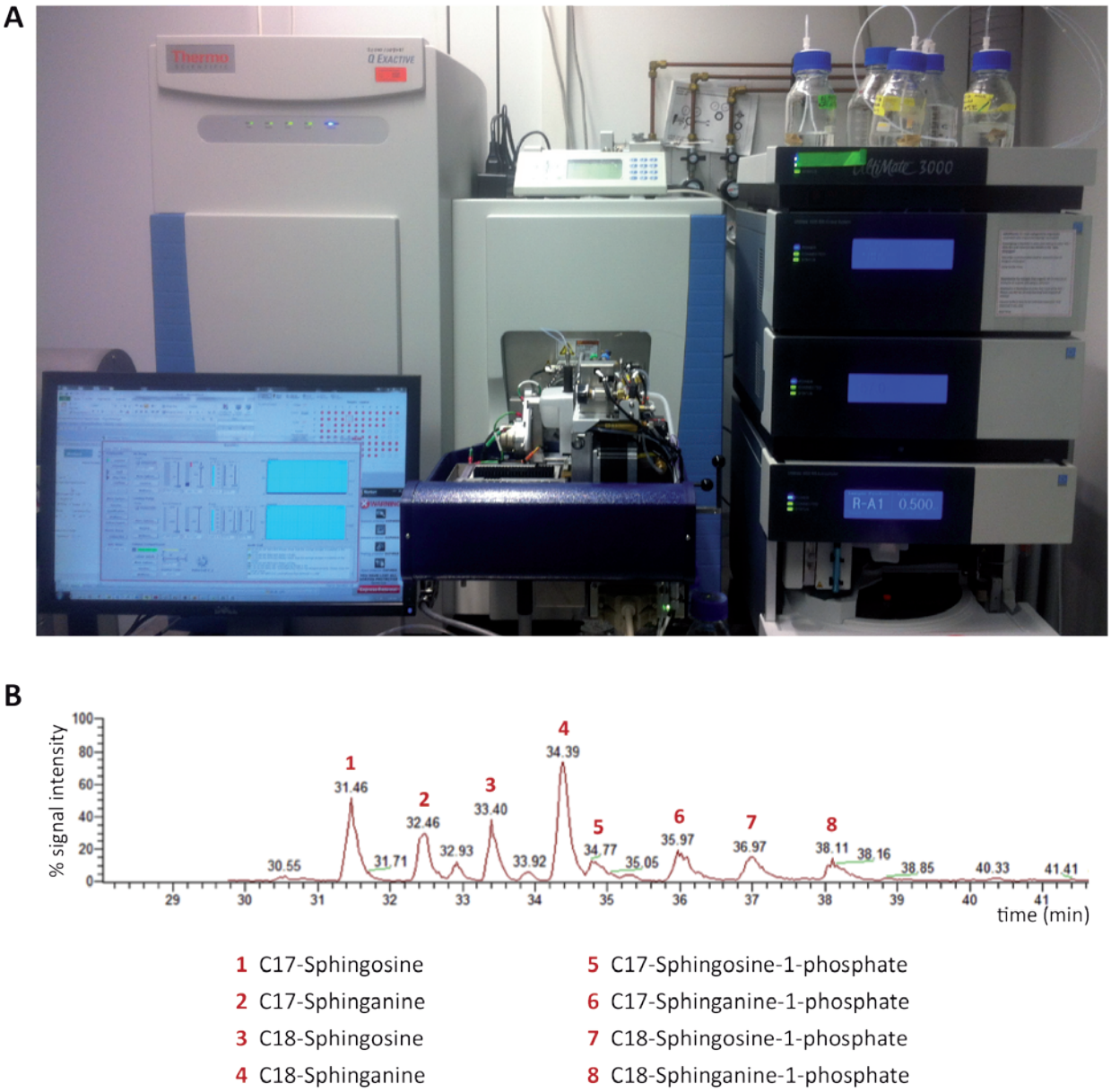



***Fig. 2: Mass spectrometric analysis of lipids on a high resolution mass spectrometer. A:*** *A QExactive coupled to a nano-UPLC system with automated NanoMate-assisted sample application.* ***B:*** *LC separation of intermediates in sphingolipid metabolism.*

# Funding

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

Coordinator: Felix Wieland, Biochemie-Zentrum der Universität Heidelberg (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. 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 combine the single parts to functional assemblies; once an assembly is defined functionally, such assemblies are combined to even higher aggregates at a next layer of complexity, again functionally 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 the final goal, we believe that many important lessons can be learned during this journey. The expertise existing in Heidelberg has led us to focus our research on biosynthetic transport. In this context we use the term dynamics at two levels: I) dynamics of macromolecular complexes (e.g. their conformational change, or their assembly and disassembly), and ii) the dynamics of the interplay of macromolecular complexes during their further assembly or disassembly to form functional subcellular structures (e.g. formation and transport of a pre-ribosomal assembly through the nuclear pore, the formation or disassembly of a coated membrane carrier, or the formation and transport of a virus particle). Biosynthetic transport is a cellular housekeeping function of special interest with respect to medical research, because many congenital diseases are caused by defects in transport machinery and biosynthetic transport is exploited at various steps by pathogenic viruses for productive infection and synthesis of viral progeny. Thus, our SFB brings together research groups using structural, cell biological, biochemical, molecular biological and virological methods and analysing various model organisms, from bacteria via yeasts to mammalian cells. Our collaborative approach allows integration of colleagues coming from different fields in the life sciences, driven by their common research interest. As a result, exchange between the groups of a wide range of knowledge and methodology is achieved naturally, and this, combined with the common interest, fosters creativity and at the same time strengthens a competent and critical view to evaluate results.

## Sonderforschungsberreich (SFB) / Transregio (TRR) 83: Molecular architecture and cellular functions of lipid/protein assemblies

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

Biological membranes with their two key constituents - proteins and lipids - mediate an astonishing array of functions ranging from simple barriers to complex signal transduction platforms. In contrast to an aqueous solution, where protein interactions are controlled by a single solvent - water -, lipid bilayers contain hundreds of different acyl chains and head groups providing distinct amphipathic interfaces and thus open another dimension to create architectural and functional diversity. 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 joins scientists 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 the highly specific interaction of a single sphingolipid species with a vesicular membrane protein. These and other functionalized lipids are now used by many researchers world-wide. Similarly, the lipidomics platforms have made significant progress in the analysis of critical, but hard to detect lipid species, and have established comprehensive and quantitative lipid analysis ranging from distinct protein/lipid assemblies to entire organisms. The TRR 83 joins experts in fields as different as e.g. synthetic organic chemistry, biochemistry, cell biology, virology, and immunology (as well as many others) and provides them with the opportunity to address challenging questions, which could not be solved by individual approaches. Using new functionalized analogues of single lipid species that can be introduced into living cells, their fates and functions can be determined by physicochemical and imaging methods. Together with continuously developing analytical mass spectrometric approaches these tools are used to elucidate a contribution of specific lipid environments to the translocation of proteins across membranes, to the intracellular formation of virus particles, lipid droplets, and endosomes, and to understand the complex events that allow the controlled fusion of membranes, as e.g. in neurotransmission. 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.

After a successful evaluation, the DFG now funds the TRR 83 in its second term (January 2014 – December 2017). The TRR 83 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 the administration of the TRR 83 are located at the BZH.

## 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" is funded by the German Research Foundation (DFG) since October 1, 2005 and is currently in its second funding that started on April 1, 2010. Modern cell biology is currently integrating 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. This development is facilitated by novel key technologies that enable us to understand the dynamics of molecules in living cells. Employing state-of-the-art screening technology it is now possible to identify those protein or lipid components that specifically govern a given biological process out of the enormous complexity of the eukaryotic cell. This 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 benefits from these technical advances. The growing complexity of biologically relevant information derived from such studies demands from the experimentalist an increasingly sophisticated and interdisciplinary knowledge. The mission of GRK 1188 is 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 is on specific methodology that the fellows will directly apply to their own research. These qualification activities are complemented by measures to promote soft skills and scientific independence of the fellows. In order to promote the international exposure of GRK 1188, the program collaborates with its international partner, the University of Manchester. GRK 1188 integrates 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). Their general research interests are in the areas of

1.) Insertion of proteins or protein complexes into membranes
2.) Translocation of proteins and protein complexes across membranes
3.) Dynamic properties of membranes including vesicular transport

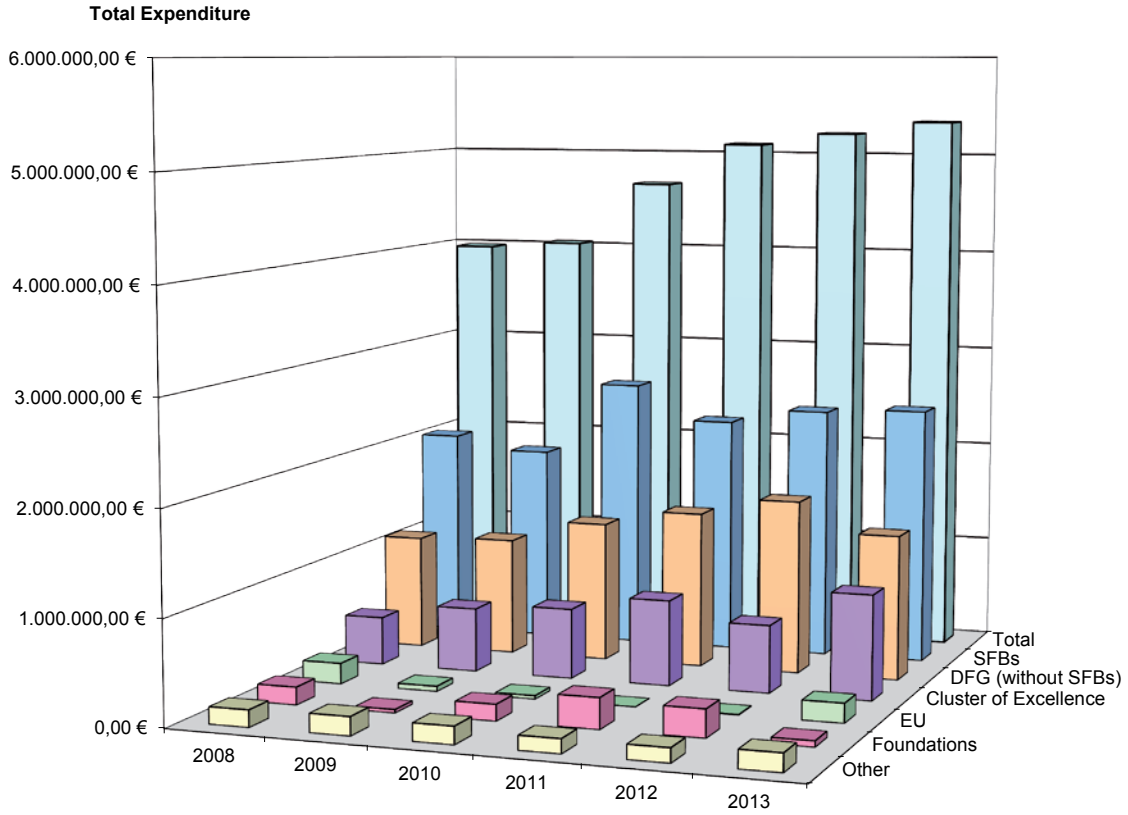
## Cluster of Excellence, Cellular Networks:
## A quantitative view of complex cellular processes

Coordinator: Hans-Georg Kräußlich; Vice-coordinators: Thomas Holstein and Frauke Melchior

CellNetworks is a research cluster funded by the German Excellence Initiative. The aim of CellNetworks is to develop a systemic understanding of the regulation of complex biological networks. This question is addressed at various levels of complexity by scientists from different disciplines. Leading research groups of the DKFZ, EMBL, Max-Planck-Institute for Medical Research and Heidelberg University cooperate in CellNetworks. In the first funding phase CellNetworks has established an elaborate network of core facilities that provide state-of-the-art technology. Researchers of the BZH contribute to three of these core facilities a protein

crystallization platform at the BZH is headed by Irmi Sinning and Jürgen Kopp. The facility for mass spectrometry of lipids, headed by Britta Brügger at the BZH, is also supported by CellNetworks. Furthermore, Michael Brunner (BZH) and Jochen Wittbrodt (COS) are in the steering committee of the deep sequencing facility that has been established by CellNetworks in Bioquant. In addition CellNetworks supports the BZH junior groups of Martin Koš (first funding phase) and Julien Béthune (second funding phase). One of the five emerging collaborative topics that are supported by CellNetworks in the second funding phase is coordinated by Irmi Sinning and Ed Hurt of the BZH: the program EcTop1 focuses on the structural characterization of molecular machines of the thermophile fungus *Chaetomium thermophilum.*

## External Funding BZH 2008 - 2013




| | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 |
|---|---|---|---|---|---|---|
| **SFBs** | 2.046.501,31 € | 1.920.863,50 € | 2.652.768,20 € | 2.317.649,28 € | 2.460.113,04 € | 2.504.700,00 € |
| **DFG (without SFBs)** | 1.110.847,03 € | 1.143.187,06 € | 1.362.110,46 € | 1.519.314,46 € | 1.692.638,98 € | 1.408.174,71 € |
| **Cluster of Excellence** | 467.100,15 € | 619.281,22 € | 678.848,92 € | 825.684,95 € | 651.296,71 € | 1.007.941,32 € |
| **EU** | 200.292,68 € | 50.643,42 € | 34.101,48 € | 806,72 € | 5.237,62 € | 187.302,50 € |
| **Foundations** | 169.875,43 € | 33.664,63 € | 158.160,96 € | 294.815,47 € | 267.347,07 € | 57.958,97 € |
| **Other** | 163.322,88 € | 178.678,21 € | 169.562,22 € | 137.014,79 € | 137.360,79 € | 173.711,18 € |
| **Total** | **3.975.333,61 €** | **4.031.446,44 €** | **4.673.109,41 €** | **5.095.285,67 €** | **5.213.994,20 €** | **5.339.788,68 €** |

Expenses according to SAP (01.01. - 31.12.)
only SFBs: according to grants

# Theses 2011 - 2013

## 2011

**Michael Diechtierow,** Funktionelle Charakterisierung der *T. brucei* Tryparedoxin-Peroxidase.
Group Leader: Krauth-Siegel

**Sebastian Falk,** cpSRP43 is a specific membrane protein chaperone and targeting factor.
Group Leader: Sinning

**Caroline Funk,** Beschreibung der Funktion von am Kinetochor lokalisierten Stu1 (Mitglied der CLASP Familie).
Group Leader: Lechner

**Gabriela Guedez-Rodriguez,** Assembly and Activation of the Plasmodial Pyridoxal 5'-Phosphate Synthase Complex.
Group Leader: Sinning

**Laure Johann,** Redox balance and drug development in Schistosoma mansoni : Synthesis of inhibitors of Schistosoma mansoni thioredoxin-glutathione reductase.
Group Leader: Davioud-Charvet

**Marisa Kirchenbauer,** A cortically-anchored nuclear envelope domain facilitates spindle positioning in *S. cerevisiae.*
Group Leader: Liakopoulos

**Ines Kock,** Analyse des Rhodopsin Transports in Invertebraten am Beispiel transgener *Drosophila melanogaster*.
Group Leader: Sinning

**Erik Malzahn,** Photoadaption in *Neurospora crassa*.
Group Leader: Brunner

**Simone Paulsen,** The role of diacylglycerol and the double C2 protein alpha in SNARE-mediated membrane fusion: in vitro analysis.
Group Leader: Söllner

**Patricia Rusu,** The Role of V-ATPase Subunit a in Vesicle Fusion.
Group Leader: Söllner

**Gencer Sancar,** Tightly coupled hierarchical tranciption cascades generate multi-phasic circadian gene expression in *Neurospora crassa*.
Group Leader: Brunner

**Phillip Sarges,** Functional analysis of two structural nucleoporins and their role the formation of higher order assemblies within the NPC.
Group Leader: Hurt

**Yvette Schollmeier,** Munc18-1 und seine duale Funktion in der SNARE-vermittelten Membranfusion.
Group Leader: Söllner

## 2012

**Przemyslaw Grudnik,** Structural and functional characterization of prokaryotic SRP systems.
Group Leader: Sinning

**Katrin Höhner,** Zur Rolle des kleinen GTP-bindenden Proteins Arf1 bei der Biogenese von Transport-Vesikeln.
Group Leader: Wieland

**Iris Valerie Holdermann,** Structural and biochemical characterization of the chloroplast signal recognition partical.
Group Leader: Sinning

**Wilfried Klug,** The protein network around the intracellular domain of APP with focus on LRP1 and the neuronal adaptor protein Fe65.
Group Leader: Sinning

**Nico Kümmerer,** Biochemische Charakterisierung von FLHF und seinen Interaktionspartnern.
Group Leader: Sinning

**Christoph Rademacher,** Kasein Kinase I kontrolliert den nuklearen Import und die Aktivität des CLOCK Proteins.
Group Leader: Weber

**Angela Roldán Sánchez,** Rolle des Flavoenzyms Liponamiddehydrogenase in Afrikanischen Trypanosomen.
Group Leader: Krauth-Siegel

**Ana Stelkic,** Characterization of the protein-protein interaction network within the central domain of the *S. cerevisiae* kinetochore.
Group Leader: Lechner

**Julia Steringer,** The Role of PI(4, 5)P2-dependent Fibroblast Growth Factor (FGF2) Oligomerization in Unconventional Secretion.
Group Leader: Nickel

**Goran Stjepanovic,** Biophysical and biochemical characterisation of membrane interactions of the *Escherichia coli* signal recognition particle FtsY.
Group Leader: Sinning

**Tao Wang,** Functional Characterization of Gene Products Mediating SH4-Domain-Dependent Protein Targeting.
Group Leader: Nickel

**Daniela Zorn,** Analysis of functional post-translational modifications of the circadian CLOCK protein for the regulation of circadian transcription.
Group Leader: Weber

## 2013

**Frank Adolf,** Molecular Mechanism of COPII and COPI Vesicle Formation - Studies on Cargo Sorting and Vesicle Membrane Scission.
Group Leader: Wieland

**Stilianos Ciprianidis,** Rhythmic and light dependent binding of the white collar complex to the promotor of the clock gene frequency in *Neurospora crassa*.
Group Leader: Brunner

**Andreas Max Ernst,** Complexity in the hydrophobic core of biological membranes.
Group Leader: Wieland

**Mareike Laußmann,** Molecular Analysis of Phosphorylation-dependent Secretion of FGF2.
Group Leader: Nickel

**Basak Özata,** Functional and kinetic studies of COP machinery.
Group Leader: Wieland

**Julia Stefanski,** Binding of Circadian Transcription Regulators and Rhythmic Gene Expression in Human Cells.
Group Leader: Brunner

**Lea Stevermann,** Dissecting the Regulation of Kar9 Stability and Turnover in *Saccharomyces cerevisiae*.
Group Leader: Liakopoulos

**Ann Na Tan ,** Cytoplasmic microtubule regulation and spindle positioning in budding yeast, *Saccharomyces cerevisiae*.
Group Leader: Liakopoulos

**Karsten Thierbach,** Investigating the interaction network and structure of the Nup84 complex from *S. cerevisiae* and C. thermophilum.
Group Leader: Hurt

**Paulina Turcza,** Diacylated SH4 domain proteins: studies on their lipid and protein environment at the plasma membrane and intracellular transport.
Group Leader: Nickel

**Volker Windeisen**, Assemblierung und Reaktionsmechanismen von eukaryotischen PLP-Synthasen.
Group Leader: Sinning

# Publications 2011 - 2013

## 2011


Sancar G, Sancar C, Brügger B, Ha N, Sachsenheimer T, Gin E, Wdowik S, Lohmann I, Wieland F, Höfer T, Diernfellner A, Brunner M. A global circadian repressor controls antiphasic expression of metabolic genes in *Neurospora*. Mol Cell. 2011; 44(5):687-97.

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In order to maintain the highest standard of research, the BZH uses a process of review and feedback: The Scientific Advisory Board, composed of internationally recognized scientists, meets every three years at the BZH. We thank the present and past members of the SAB for support and guidance, for advice and for their precious time.

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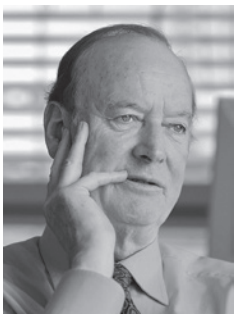

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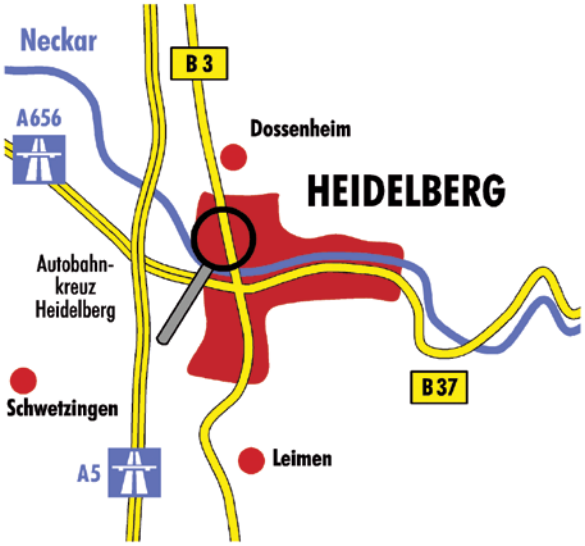


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