Dynamics of Viral Infection – Dr. Steeve Boulant

Molecular Mechanisms of Tumor Invasion – Dr. Björn Tews

Neuropeptides – Dr. Valéry Grinevich

Norovirus Study Group – Dr. Grant Hansman

Proteostasis in Neurodegenerative Disease – Dr. Thomas Jahn
Dynamics of Viral Infection

Goal
The laboratory studies how enteric viruses infect intestinal epithelial cells lining the surface of the gut. In particular, we are interested in how cellular polarity can influence the outcome of a viral infection at mucosal surfaces.

Background
Enteric viruses are a global health concern and represent a major cause of infant mortality in developing countries. These viruses are also a problem in developed countries manifesting themselves as foodborne diseases inducing acute diarrhoea and vomiting. Intestinal epithelial cells (IECs) constitute the primary barrier that enteric pathogens have to face.

To resist the harsh acidic environment in the lumen of the gut, enteric viruses are exclusively non-enveloped. Although non-enveloped viruses were historically intensively used to characterize viruses, remarkably, very little is known about the molecular mechanisms used by non-enveloped viruses to penetrate the plasma membrane of the host cell. Similarly, the mechanism by which innate immunity is regulated in IECs remains unclear. However, it is known that cellular polarity represents a key player, allowing the establishment of a delicate balance that efficiently recognizes pathogens and at the same time does not elicit an immune response against the commensal microbial flora. Inappropriate immune response to the commensal flora is suspected to be responsible for inflammatory bowel diseases.

Research Highlights
Impact of cellular polarity on endocytosis and viral entry
We have previously shown that cellular polarity has direct impact on clathrin-mediated endocytosis (CME). Moreover, we found that polarity can dictate how viruses are endocytosed by cells. We found that clathrin-mediated endocytosis is actin dependent at the apical membrane of polarized cells but actin-independent at the basolateral side. It is known that clathrin-mediated endocytosis can also be actin-dependent at the ventral side of non-polarized cells (Figure 1).

Our goal is to characterize the molecular and biophysical determinants that determine whether a coated structure requires actin or not in order to properly endocytose.
Impact of cellular polarity on membrane penetration by the non-enveloped virus reovirus

All enteric viruses are non-enveloped viruses. The molecular mechanisms used by enveloped viruses to pass the membrane of the host (fusion mechanisms) are very well described. On the contrary, little is known concerning how non-enveloped viruses gain access to the cytosol of the infected cells. In this part, our goal is to characterize the molecular mechanisms used by the non-enveloped virus reovirus to penetrate the endosomal membrane. Our strategy is to combine high spatio-temporal resolution confocal microscopy, super-resolution STORM microscopy and correlative light electron microscopy (CLEM) to shed light on these mechanisms. To detect where within a living cell membrane penetration takes place, we use the Gal3-cherry construct that is recruited at site of endosomal membrane rupture (Figure 2).

Impact of cellular polarity on innate immunity

It is known that cellular polarity represents a key player, allowing the establishment of a delicate balance that efficiently recognizes pathogens and at the same time does not elicit an immune response against the commensal microbial flora. We found that the innate immune response is specifically shaped by cellular polarity in the IECs T84 cells and that type III IFN represents a major player in innate immunity in IECs.

Our current lines of research focus on:

1) How can the cell differentially shape the innate immune response as a function of polarity, and
2) Is there an advantage in IECs to favor signaling via type III IFN and not type I IFN.
Selected Publications

Blaising et al., 2013. Cell Microbiology
Silibinin inhibits hepatitis C virus entry into hepatocytes by hindering clathrin-dependent trafficking.

Boulant et al., 2013. Mol Biol Cell
Similar uptake but different trafficking and escape routes of reovirus virions and infectious subvirion particles imaged in polarized Madin-Darby canine kidney cells.

Cocucci et al., 2012 Cell
The first five seconds in the life of a clathrin coated pit.

Boulant et al., 2011 Nat Cell Biol
Actin dynamics counteract membrane tension during clathrin-mediated endocytosis.

Dixit et al., 2010 Cell
Peroxisomes and mitochondria cooperate to induce antiviral innate immunity

Lab Members
Megan Stanifer, Post-doc
Anja Rippert, Technician
Pranav Shah, PhD student
Delia Bucher, PhD student
Kalliopi Pervolaraki, PhD student
Marta Fratini, PhD student
Markus Mukenhirn, Master student
Simon Schroeder, Master student
Christian Kischnick, Master student

Awards and Honors
- Post-doctoral fellowship (2011-2012), GlaxoSmithKline, Boston MA, USA
- Pilot and Feasibility award program HDDC, Boston MA, USA (2009-2010)
- Post-doctoral fellowship Marie-Curie IEF, Glasgow, UK (2006-2008)
- Bridging grant, FRM, Lyon, France, (2004-2005)
- EURODOC fellowship, Lyon-Glasgow exchange grant (2001)

Teaching Activities

2012
Heidelberg University, Virology Master Program: Virology tutorial

2013
Heidelberg University Virology Master Program: Virology Practical
Heidelberg University Frontiers of Bioscience: Virology lecture
HBIGS: PhD student course on single virus particle imaging
Heidelberg University, Virology Master Program: Virology tutorial
EUVIRNA Advanced Microscopy Workshop: Imaging Lecture
From Infection to Therapy: Trends in Virology, University of Luebeck: Virology/Imaging seminar

2014
Heidelberg University Virology Master Program: Virology Practical
Heidelberg University Frontiers of Bioscience: Virology lecture
International Graduate School: Pathogen-Host Interactions at Cellular Barriers, University of Muenster: Virology Lecture
2012 – present  Group Leader, University of Heidelberg and German Cancer Research Center (DKFZ)
2007 – 2012  PostDoc, Brain Research Institute, Prof. Martin E. Schwab, ETH Zürich
2006 – 2007  PostDoc, Molecular Genetics, Prof. Peter Lichter, DKFZ
2002 – 2006  PhD Molecular Genetics, Prof. Peter Lichter, DKFZ

Björn Tews

Schaller Research Group
Molecular Mechanisms of Tumor Invasion

Goal
Our goal is to elucidate the molecular mechanisms underlying brain tumor cell invasion.

Background
The main topics of our lab are:

- The role of sphingolipid-receptor signaling for glioma invasion
- Lysophospholipids as a messenger enabling glioma – microglia crosstalk
- Peroxiredoxin 1 (PRDX1) in glioma pathogenesis
- The role of myelin- vs. neuron-derived Nogo-A in regulating synaptic plasticity and cognitive (dys)function

The role of sphingolipid-receptor signaling for glioma invasion

High-grade astrocytoma, such as the most common form glioblastoma, are among the most deadly of all human cancers reflected by a median patient survival of less than 12 months (Barbus and Tews et al., 2011). Invasive growth and early infiltration of the surrounding healthy brain is a hallmark of glioma (Giese, 2003). This invasive nature mainly accounts for their resistance to current treatment modalities. The diffusely infiltrating tumor cells, which evade surgical resection and survive treatment, inevitably give rise to reoccurring tumors. Glioma invasion was found to be associated with distinct anatomic structures following myelinated axons, the basement membranes of blood vessels as well as the subependyma (Giese, 2003). Hence, spreading of tumor cells via myelinated fiber tracts of the corpus callosum as a main trajectory route is a common phenomenon in patients, termed butterfly glioma.

The Rho family of small GTPases comprises important regulators of cell migration and invasion (Kempf and Tews et al., 2013). Increased RhoA/ROCK activation in glioblastoma cells has been linked to impaired cell migration by induction of profound morphological actin cytoskeleton changes. Sphingosine 1-phosphate (S1P) regulates Rho GTPase activities through five different variants of a G-protein coupled receptor family: S1PR1-5 (Pyne and Pyne, 2010). S1P induces RhoA and subsequent stress fiber formation and has been shown to inhibit cell spreading and outgrowth of neurons and non-neuronal cells (Kempf and Tews et al., 2013). Yet, strong S1PR2 expression can be detected in gliomas (Figure 1). We could show that in a subset of glioma cells, S1PR2 signaling rather induces migration and invasion in classical scratch- as well as real-time cell analysis (RTCA)- and cell spreading bioassays on central nervous system (CNS) myelin extractions. Currently, our lab is extensively investigating and characterizing the underlying mechanism in in vitro and in vivo.
Sphingolipids as a messenger enabling glioma – microglia crosstalk

Gliomas contain abundant activated microglia/macrophages. Increasing evidence indicates that the glioma microenvironment converts the glioma-associated microglia/macrophages into glioma-supportive, immunosuppressive cells.

We investigate the role of S1P and its derivatives as a potential messenger enabling communication between microglia and glioma cells. We have established primary mouse microglia extraction protocols using CX3CR1-GFP (Jung et al., 2000) and VM/Dk mice (Serano et al., 1980). Our aim is to coculture these green microglia cells with transgenic mouse glioma cells expressing RFP as well as modulated levels of various S1P-metabolizing enzymes (sphingosine kinase 1/2) and S1P receptors. Selective Plane Illumination Microscopy (SPIM) is applied to study glioma morphology (Figure 2) as well as microglia – glioma interaction and its dependence on S1P in RCAS-driven and xenograft mouse models of glioma in vivo.

Peroxiredoxin 1 (PRDX1) in glioma pathogenesis

After surgery, radio- and chemotherapy is the mainstay of treatment for patients with glioblastoma. Ionizing radiation is known to induce ROS production due to radiolysis of water and direct ionization of target molecules, which results in oxidative damage to critical cellular biomolecules such as nucleic acids, proteins and lipids. PRDX1 functions as a thiol reductase involved in oxidative stress defense mechanisms through its ability to catalyze peroxide reduction of reactive oxygen species (ROS). Recently, we could detect PRDX1 hypermethylation and reduced expression in oligodendrogial tumors and secondary glioblastomas (Tews et al., 2006, Dittmann et al., 2012). We could show that loss of PRDX1 significantly increases apoptosis and reduces cell viability of glioma cells exposed to ionizing irradiation and also TMZ. Currently, we are investigating the molecular mechanisms of PRDX1-dependent increase in cell migration and invasion in vitro and ex vivo.

The role of myelin- vs. neuron-derived Nogo-A in regulating synaptic plasticity and cognitive (dys)function

Since we are interested in myelin inhibitory proteins, we will further address the neurobiological function of Nogo-A. Nogo-A is expressed by oligodendrocytes but also neurons (Schwab, 2010). We could show that neuronal Nogo-A restricts synaptic plasticity and furthermore that depletion leads to schizophrenia-like endophenotypes in a transgenic Nogo-A knockdown rat model (Tews et al., 2013). This transgenic neuronal Nogo-A deficient rat model was investigated in detail for anxiety, motivation and place avoidance in the Carousel maze during the last year, revealing very interesting data on Nogo-A dependent regulation of higher cognitive functions (Enkel et al., 2014; Kristofikova et al., 2013; Petrasek et al., 2014a and 2014b).

Figure 1: S1P receptor mRNA expression in glioma patients (French dataset, n = 284 (left panel) and Hegi dataset, n = 84 (right panel); R²: microarray analysis and visualization platform, http://r2.amc.nl).

Figure 2: Selective Plane Illumination Microscopy (SPIM) brain image of an RCAS/TVA glioma. A high-grade glioma is visible in the left brain hemisphere.
In a collaborative effort with Prof. Martin Schwab (ETH Zürich) and Prof. Dusan Bartsch (ZI Mannheim) we are now investigating the underlying mechanisms and contributions of neuronal vs. oligodendrocytic Nogo-A in different conditional mouse models we have generated (oligodendrocytic KO of Nogo-A: CNP-Cre x Nogo-A; neuronal KO of Nogo-A: CamKII-Cre x Nogo-A). We analyze the animals in classical learning and memory / behavior paradigms as well as more specific tests addressing pathological changes observed in human neuropsychiatric diseases such as Prepulse Inhibition (PPI). Deficits in PPI manifest in the inability to filter out the unnecessary information; they have been linked to abnormalities of sensorimotor gating. These deficits can be observed in patients suffering from neurological diseases such as schizophrenia. Notably, recent evidence suggests that Nogo-A and NgR might constitute candidate genes for schizophrenia susceptibility (Willi and Schwab, 2013).

**Selected Publications**


*contributed equally
Neuropeptides

Goal
To study mechanisms of neuropeptide actions in the brain.

Background
Neuropeptides represent a group of non-canonical neuromodulators, which modify activity of neuronal ensembles resulting in specific changes of behavior. Among ~ 100 of neuropeptides, the most popular and the best-studied neuropeptide is oxytocin. Despite very pronounced effects of various form of behaviors ranging from anxiolytic action to social attachment, the basic questions on how oxytocin exerts its effects is far from understanding. Our team focuses on dissecting neuropeptidergic circuits controlling distinct forms of behavior as well as regulating reproduction, pain processing and metabolism (see Research Highlights).

Research Highlights
We work on identification of specialized neuropeptidergic circuits, executing distinct form of behavior, such as fear vs. cooperative behavior.

We explore interactions between neuropeptidergic systems in regulation of reproduction, pain processing and metabolism.

We monitor neuropeptide trafficking under specific metabolic and behavioral challenges.

Available techniques
Our work is based on using viral-based vectors in combination with other advanced techniques as opto-, pharmacogenetics and functional magnetic resonance imaging. We employ freely moving rodents to monitor their behavior affected by neuropeptides. In a short-while perspective, we also plan to establish the in vitro and in vivo setups to record electrical activity of neuronal ensembles sensitive to neuropeptides.
Figure 1: Novel oxytocin circuit comprising forebrain, brainstem and spinal cord (Knobloch et al., manuscript in preparation)

Selected Publications (since 2012)

Grinevich et al. (in press)*
Master Classes in Molecular Neuroendocrinology, Wiley, Somatic transgenesis (Viral vectors).

Vinnikov et al., 2014*
Journal of Neuroscience
Hypothalamic miR-103 protects from hyperphagic obesity in mice

Knobloch, Grinevich, 2014*
Frontiers in Behavioral Neuroscience
Evolution of central oxytocin pathways in vertebrates

Knobloch et al., 2014*
Springer Protocol Book “Viral Vectors in Neurobiology and Brain Diseases”. Viral vectors for optogenetics of hypothalamic neuropeptides

Silva et al., 2013
Nature Neuroscience
Independent hypothalamic circuits for social and predator fear

Knobloch et al., 2012*
Neuron
Evoked axonal oxytocin release in the central amygdala attenuates fear response.

* Senior/corresponding authorship

Awards and Honors (since 2012)

- Royal Society Award Edinburgh (2013)
- Otto Hahn Medal from the Max Planck Society awarded to Sophie Knobloch for the best PhD thesis in the field of Neurobiology (2012).
- Chica and Heinz Schaller Foundation award for establishing and conductance of the new laboratory affiliated at the Heidelberg University and the German Cancer Research Center (2012-2017).

Lab Members (August 2014)

- Apar Jain, PhD, Postdoctoral Fellow
- Androniki Raftogianni, PhD, Postdoctoral Fellow
- Marina Eliava, PhD, Postdoctoral Fellow
- Miriam Kernert, Graduate (PhD) student
- Ferdinand Althammer, Graduate (PhD) student
- Tim Gruber, Master student
- Lena Heuschmid, Master student
- Judith Müller, Technical assistant
- Heike Böhli, Technical assistant

Teaching Activities (since 2012)

- Lecture for Graduate Program in Oncology, DKFZ (2014)
- Lectures for Interdisciplinary Center of Neuroscience, University of Heidelberg (2010, 2012, 2014)
- Center of Neuroscience summer lecture series for Master students, University of Heidelberg (2012, 2013)
- Practical courses for Bachelor students, University of Heidelberg (2012, 2013, 2014)
- Lecture for PhD students of the Max Planck Institute for Medical Research (2012).
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Norovirus Study Group

Goal
The purpose of my research group is to better understand norovirus capsid flexibility with respect to receptor binding interactions and virus evolution with the ultimate aim of developing norovirus antivirals. We are performing numerous projects that are interconnected in one way or another.

Background
Human noroviruses are the dominant cause of outbreaks of gastroenteritis around the world and infect all age groups. There are no antivirals or vaccines against noroviruses, mainly because these viruses cannot be grown in cell culture. The prevention and treatment of human norovirus are of major public health concerns. Human noroviruses bind to the histo-blood group antigens (HBGAs) and this interaction is thought to be important for norovirus infection. The great genetic diversity of norovirus (genotypes) has permitted the different strains to bind to the HBGAs in diverse ways. Despite the complex binding patterns, the HBGA-binding pocket is a potential site of vulnerability for human norovirus.

Norovirus interaction with receptors
The human HBGAs have been identified as potential co-factors for norovirus. The HBGAs are complex carbohydrates linked to proteins or lipids present on epithelial cells and other cells in the body or found as free antigens. At least nine different HBGAs have been described that can bind to norovirus. Noroviruses bind to HBGAs at the outer surface of the capsid. The interaction is coordinated by both hydrophobic and hydrophilic interactions, but the binding is different among the genetically diverse norovirus strains.

Our aim is to describe the structural basis of norovirus interaction with HBGAs. A panel of different norovirus strains will be produced in E.coli and insect cells.

Our aim is to identify potent compounds that can bind to the norovirus capsid protein and then test the compound inhibition using virus-like particles (VLPs) and cultured cells. Compound libraries will be screened using X-ray crystallography and Biacore. A method that can track norovirus VLP entry to cells will be developed. The best candidate compounds will be examined for their ability to block VLP entry.

Norovirus drug discovery
Recently, we analyzed the interaction of citrate with noroviruses using X-ray crystallography and saturation transfer difference (STD) NMR. We found that the citrate interaction was coordinated with an almost identical set of capsid interactions as involved in recognizing the norovirus receptor. We showed norovirus had weak affinity for citrate, but could compete against the soluble receptors.

Protein complexes of norovirus and HBGAs will be produced and X-ray crystallography data will be collected at Grenoble, France. Structures will be solved and refined using different software. Binding interactions will also be examined using Biacore and ELISA.
**Norovirus capsid flexibility and antibody binding**

Human norovirus cannot be grown in cell culture, but expression of the VP1 in a baculovirus expression system leads to the self-assembly of VLPs that are morphologically and antigenically similar to the native virion. The X-ray crystal structure of the prototype norovirus VLP identified two domains, shell (S) and protruding (P) domains.

We recently determined the cryo-EM structure of a human norovirus VLPs and showed that the P domain was raised off the S domain by ~15 Å. The exposed regions on the lower side of the P domain ultimately allowed monoclonal antibodies to bind at occluded sites on the VLPs. It was interesting to note that we found the human genogroup VLP was more similar to an infectious murine norovirus virion than to the prototype human norovirus VLP, which had a P domain resting on the S domain.

Our aim is to identify sites of vulnerability on the capsid, to untangle the importance of the flexible P domain, and to answer questions of antigenicity. This will be carried out using X-ray crystallography and/or cryo-EM. Structures of VLPs in complex with monoclonal antibodies and nanobodies will be determined.

**Development of a novel reverse genetics system for human noroviruses**

The human norovirus genome is divided into three open reading frames (ORFs), where ORF1 encodes the non-structural proteins including the RNA dependent RNA polymerase (RdRp), ORF2 encodes the capsid protein (VP1), and ORF3 encodes a small structural protein. Human noroviruses are uncultivable in cell culture, but reverse genetic systems have shown that virus-like particles (VLPs) can be generated, but these did not infect neighboring cells.

Our aim is to develop a novel reverse genetics system using advanced technologies. Strategies to study particle attachment and entry will also be developed. Different norovirus strains will be tested and chimeric human-murine norovirus constructs will also be made, including a construct with a switched ORF1 and a construct with switched capsid S and P domains.

**Selected Publications**


**Lab Members**

Balendu Avvaru, Post-doc
Sylvie Dörflinger, PhD student
Anna Koromyslova, PhD student
Mila Leuthold, PhD student
Bishal Kumar Singh, PhD student
Cletus Wezena, PhD student
Thomas Jahn

Schaller Research Group
Proteostasis in Neurodegenerative Disease

Goal

The goal of our work is the mechanistic understanding of events triggering protein misfolding and the subsequent impact of aggregated protein species on cellular homeostasis.

Background

As the human population ages the incidence of a wide range of disorders, particularly those associated with abnormal protein folding and aggregation, will increase dramatically. The burden of suffering caused by these disorders translates into an enormous cost to society. At present there is no cure for neurodegenerative diseases such as Alzheimer’s disease available, however this disappointing summary belies an enormous growth in the understanding of the molecular pathogenesis. One line of neurodegeneration research has proven to be particularly productive. This line of research approaches the question of pathogenesis by investigating the molecular behavior of the peptides and proteins that aggregate in the brains of patients.

The efforts of our group are focused on the molecular mechanisms of neuronal degeneration linked to the accumulation and propagation of aggregated protein species. We combine biochemical and molecular biological studies with cellular and Drosophila models of human disease processes to tackle questions such as:

- What are the characteristics of aggregated protein species accumulating in vivo?
- Why and how are specific species toxic to neurons?
- Which cellular mechanisms can be modified to counteract the toxicity of these species?

Extensive research activity in the past has addressed the fundamental mechanism of protein folding and misfolding through a combination of in vitro and in silico studies, and we now have considerable understanding at a molecular level of the fundamental principles underlying this complex process. It is clear that protein homeostasis is closely coupled to many other biological processes ranging from the trafficking of molecules to specific cellular locations to the regulation of the growth and differentiation of cells. In addition, only correctly folded proteins have the ability to remain soluble in crowded biological environments and to interact selectively with their natural partners. It is not surprising, therefore, that the failure of protein homeostasis mechanisms is the origin of a wide variety of pathological conditions.
**Research Highlights**

*Drosophila* has become a widely used invertebrate *in vivo* model. We have previously shown that there are many reasons why *Drosophila* is a potentially powerful model to study human diseases (Jahn et al. 2011a). Probably the most important feature of this model is the number of genetic tools available to modify the *Drosophila* genome.

Furthermore, *Drosophila* is characterised by complex learning and locomotor behavior, and therefore has functionally relevant neuroanatomy for mammalian conditions. These characteristics have encouraged us to perform detailed mechanistic studies in *Drosophila* models of neurodegeneration, based on the aggregation and toxicity of specific proteins.

We have developed various *Drosophila* models of Alzheimer’s disease based on the toxicity of the Amyloid-beta (Aβ) peptide and the tau protein. Importantly, we find that a number of quantitative phenotypes, such as longevity and locomotor deficits, correlate well with the aggregation propensity of these peptides and the observed pathology in the human case (Fig. 1).

![Figure 1. Accumulation of Alzheimer’s disease associated Aβ plaques in the Drosophila brain dramatically impact fly survival.](image)

Using a series of protein engineering techniques, we were recently able to develop *Drosophila* models that capture soluble oligomeric aggregates and suggest them as culprit species resulting in severe neurodegeneration (Speretta et al. 2012).

Aβ variants can be modified to be fast aggregating, however, these variants show no significant neurotoxic effects despite their dramatic accumulation as insoluble plaques. In contrast, we were able to show that specific soluble aggregate species, transient intermediates on the Aβ assembly pathway, are the neurotoxic species. We are currently trying to specifically trap these species *ex vivo* to describe their characteristics in more detail.

An important tool developed in our lab to examine early neuronal decline on an organismal level is an automated 3D tracking system for fly locomotion. Using this setup, we can quantitatively assess fly locomotion (Jahn et al. 2011b).

We have recently shown that significant differences in neuronal function are apparent well before Aβ deposition into insoluble plaques, again pointing towards a role of smaller soluble aggregate species in neurotoxicity. This setup is currently explored in RNAi-based modifier screens and by performing a series of biochemical and immunohistochemical protocols to visualise and quantify *in vivo* protein aggregation.

Future studies aim at establishing novel experimental tools to characterize the spatial and temporal distribution of protein aggregation species in the *Drosophila* brain, which include, for example, the characterisation of protein-protein interactions via the exploitation of fluorescently-tagged protein constructs for multiparametric fluorescence microscopy techniques. The knowledge and tools generated during this work will hopefully provide a rational basis for the detection of disease-associated mechanisms and the development of biomarkers/diagnostics that will enable us to detect the disease in its earliest stages when only biochemical changes on the protein level are apparent.

**Selected Publications**


**Lab Members**

Nina Dräger, PhD Student
Taxisarchis Katsinelos, PhD Student
Ramona Sowade, PhD Student
Zeenna Stapper, PhD Student
Tairi Aljand, PhD Student
Eliana Nachman, PhD Student
Ronny Heidasch, Master Student
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